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SCHOOL OF HEALTH SCIENCE AND EDUCATION

DEPARTMENT OF NUTRITION AND DIETETICS

"Assessment of genetic variants and lifestyle determinants in obesity-related traits modifications"

Doctoral Thesis

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ΧΑΡΟΚΟΠΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ

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«Αξιολόγηση γενετικών δεικτών και χαρακτηριστικών του τρόπου ζωής στη διαμόρφωση δεικτών που σχετίζονται με την παχυσαρκία»

Διδακτορική Διατριβή

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“There are two ways to live: you can live as if nothing is a miracle; you can live as if everything is a miracle.”

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“In all things of nature there is something of the marvelous.”
“For the activity of the mind is life”
— Aristotle

“Genetics is where we come from. It’s deeply natural to want to know.”
— Ellen Ullman

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ii. **Kafyra, M.,**Kalafati, I.P., Gavra, I., Ntalla, I., Siest, S., Dedoussis, G.V. Associations of VEGF-A-related variants with adolescent cardiometabolic and dietary parameters. Nutrients 2023; 15(8):1884. doi.org/10.3390/nu15081884 (**PubMed IF: 6.706)**

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v. **Kafyra, M**., Kalafati, I. P., Katsareli, E. A., Lambrinou, S., Varlamis, I., Kaliora, A. C., & Dedoussis, G. V.The iMPROVE Study; Design, Dietary Patterns, and Development of a Lifestyle Index in Overweight and Obese Greek Adults. *Nutrients*. 2021;1*3*(10): 3495. Published 2021 Oct 3. doi.org/10.3390/nu13103495. (**PubMed IF: 6,706)**

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Abstract in Greek

Η ύπαρξη υπερβάλλοντος βάρος αποτελεί βασικό βοηθητικό παράγοντα για την εκδήλωση καρδιομεταβολικών νοσημάτων. Η δραματική αύξηση της παγκόσμιας συχνότητας της παχυσαρκίας αποδίδεται τόσο στην ύπαρξη ευνοϊκών εξωτερικών ερεθισμάτων όσο και στην αλληλεπίδρασή τους με ευνοϊκούς προδιαθεσικούς γενετικούς παράγοντες. Η αποτρέψιμη φύση της διαταραχής κάνει την ανάγκη για αποτελεσματική πρόληψη και αντιμετώπισή της προτεραιότητα στην προσπάθεια αντιμετώπισης και άλλων μη μεταδοτικών ασθενειών. Σε μια προσπάθεια εμβάθυνσης της κατανόησης των μηχανισμών της πολυπαραγοντικής αιτιολογίας της παχυσαρκίας, η παρούσα εργασία διερεύνησε αλληλεπιδράσεις γονιδίων και παραγόντων τρόπου ζωής και διατροφής στη διαμόρφωση χαρακτηριστικών που σχετίζονται με αυτή.

Η μελέτη χρησιμοποιήσε δεδομένα από 202 συμμετέχοντες της μελέτης iMPROVE για να εξετάσει το αντίκτυπο μιας υποθερμιδικής δίαιτας διαφορετικού μακροθρεπτικού περιεχομένου σε ανθρωπομετρικούς δείκτες και δείκτες του τρόπου ζωής Ελλήνων ενηλίκων. Παρόμοιες παράμετροι εξετάστηκαν και σε εφηβικούς πληθυσμούς, χρησιμοποιώντας δεδομένα από 766 και 287 συμμετέχοντες των μελετών TEENAGE και STANISLAS. Τέλος, ένα πολυγονιδιακό σκορ κινδύνου (ΠΣΚ) για το Δείκτη Μάζας Σώματος (ΔΜΣ) ενηλίκων δημιουργήθηκε χρησιμοποιώντας δεδομένα από τις μελέτες NAFLD, THISEAS και OSTEOS.

Το διαφορετικό μακροθρεπτικό περιεχόμενο δεν επηρέασε τις αλλαγές που παρατηρήθηκαν στους συμμετέχοντες της μελέτης iMPROVE. Φορείς των αλληλομόρφων των παραλλαγών FTO-rs1421085 και MC4R-rs17782313 που συσχετίζονται με το ΔΜΣ έδειξαν μειωμένη απώλεια βάρους μετά την παρέμβαση.

Στους εφηβικούς οληθυσμούς, ένα γενετικό σκορ κινδύνου για αυξημένα επίπεδα VEGF-A συσχετίστηκε με αυξημένο ΔΜΣ, συστολική πίεση και μειωμένη χοληστερόλη HDL. Τέλος, το ΠΣΚ για το ΔΜΣ ενηλίκων παρουσίασε περαιτέρω επεξήγηση του δείκτη κατά 2.3% στο δείγμα των τριών μελετών. Η αξιολόγηση διατροφογενετικών συσχετίσεων οδήγησε στη διαμόρφωση ενδιαφέροντων αποτελεσμάτων σε πληθυσμούς ενηλίκων και εφήβων, θέτοντας τις βάσεις για περαίτερω μελλοντική έρευνα στο πεδίο.

Λέξεις-κλειδιά: Γενετική, Παχυσαρκία, Διατροφογενετική, Τρόπος Ζωής, Διαχείριση Σωματικού Βάρους

Abstract in English

Excess weight is seen as sine qua non for the manifestation of cardiometabolic comorbidities. The dramatic increase in obesity prevalence is attributed both to the existence of favorable external stimuli and to their interactions with favorable genetic makeup. The preventable nature of the disorder renders its successful prevention and treatment a priority in the effort to tackle non-communicable disease (NCD) prevalence. In an effort to deepen the understanding of the modus operandi of the multifactorial obesity aetiology, the present thesis sought to investigate gene-lifestyle and -diet interactions on the formation and modification of obesity-related traits.

The study used data from 202 participants of the iMPROVE cohort to investigate a hypocaloric diet of different macronutrient composition in anthropometric and lifestyle indices of Greek adults. Similar parameters were explored in adolescent populations, using data from 766 and 287 participants of the TEENAGE and STANISLAS Studies. Lastly, a Polygenic Risk Score (PRS) for adult Body Mass Index (BMI) was created using data from the NAFLD, THISEAS and OSTEOS studies.

Different macronutrient content did not affect changes in the iMPROVE participants. Carriers of the BMI alleles for the FTO-rs1421085 and MC4R-rs17782313 variants showed reduced rates of weight loss. In the adolescent populations, a VEGF-A unweighted genetic risk score was associated with increased levels of BMI, systolic blood pressure (SBP) and reduced high-density cholesterol (HDL-C). Lastly, the constructed PRS for BMI accounted for an overall 2.3% of the observed BMI variance in the three studies.

Assessment of the nutrigenetic associations displayed interesting results in both adult and adolescent populations of European ancestry, laying the ground for future work in the field.

Keywords: Genetics, Obesity, Nutrigenetics, Lifestyle, Body Weight Management

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Abbreviations

Abbreviation	Meaning
Acb	Nucleus Accumbens
ACC	Anterior cingulate cortex
ACTH	Adrenocotricotropic hormone
AgRP	Agouti-related protein-expressing neurons
AHEI	Alternate Healthy Eating Index
AHO	Albright Hereditary Osteodystrophy
AIS	Athens Insomnia Scale
α-MSH	α-Melanocyte-stimulating hormone
ARC	Arcuate Nucleus
BAT	Brown Adipose Tissue
BIA	Bioelectrical Impendence Analysis
BBB	Brain-blood barrier
BBS	Bardet-Bield Syndrome
BCAA	Branched chain amino acids
BDNF	Brain-derived Neurotrophic Factor
BMC	Bone Mineral Content
BMI	Body Mass Index
BMR	Basic Metabolic Rate
CART	Cocaine and amphetamine regulated transcript
CCK	Cholecystokinin
CESD-R-10	Centre for Epidemiologic Studies Depression Score
CRP	C-reactive Protein
CT	Computerized tomography
CVD	Cardiovascular Disease
DASH	Dietary Approach to Stop Hypertension
DBP	Diastolic Blood Pressure
DEE	Died-induced Energy Expenditure
DEXA	Dual-energy X-ray absorptiometry
DIT	Diet-induced Thermogenesis
DOHaD	Developmental Origins of Health and Disease
DPP	Diabetes Prevention Program
DQI	Diet Quality Index
EASO	European Association for the Study of Obesity
EECs	Enteroendocrine cells
EI	Energy Intake
ENS	Enteric Nervous System
FFQ	Food Frequency Questionnaire
FTO	Fat mass and obesity- associated gene
BGA	Gut-brain axis
GABA	Gamma-aminobutyric acid
GEI	Gene-Environment Interactions

GHSR	Growth hormone secretagogue receptors 1a
GIANT	Genetic Investigation of Anthropometric Traits
GLB	Group Lifestyle Balance
GLP-1	Glucagon-like peptide-1
GRS	Genetic Risk Score
GWAS	Genome-Wide Association Study(-ies)
HC	Hip Circumference
HDL-C	High Density Lipoprotein Cholesterol
HICs	High-Income Countries
HPA	Hypothalamus-Pituitary-Adrenal axis
HWE	Hardy-Weinberg Equilibrium
ICHI	Ideal Cardiovascular Health Index
IL-6	Interleukin-6
IQR	Interquartile Range
LCFA	Long-chain Fatty Acids
LD	Linkage Disequilibrium
LEP	Leptin
LepR	Leptin receptor
LI	Lifestyle Index
LDL-C	Low Density Lipoprotein Cholesterol
LMICs	Low- Middle- Income Countries
LPS	Lipopolysaccharides
MC3R, MC4R	Melanocortin 3 and 4 Receptors
MD	Mediterranean Diet
MedDietScore	Mediterranean Diet Score
MetS	Metabolic Syndrome
MHO	Metabolically Healthy Obese
MONW	Metabolically Obese Normal Weight
MRI	Magnetic Resonance Imaging
MUFA	Mono-unsaturated Fatty Acids
MUO	Metabolically Unhealthy Obese
NAFLD	Non-alcoholic Fatty Liver Disease
NCDs	Non-Communicable Diseases
NKs	Natural Killer cells
NTRK2	Neurotrophic receptor tyrosine kinase 2
NTS	Nucleus Tractus Solitarii
NPY	Neuropeptide Y
PA	Physical Activity
PAL	Physical Activity Level
PCA	Principal Component Analysis
PCC	Posterior cingulate cortex
PCSK1	Proprotein Convertase Subtilisin/Kexin Type 1
POMC	Proopiomelanocortin

PP	Pulse Pressure / Pancreatic Peptide
PUFA	Poly-unsaturated Fatty Acids
PVN	Paravetricular Nucleus
PWS	Prader-Willi Syndrome
PYY	YY peptide
RMR	Resting Metabolic Rate
SBP	Systolic Blood Pressure
SCFA	Short-chain Fatty Acid
SD	Standard Deviation
SDGs	Sustainable Development Goals
SE	Standard Error
SES	Socioeconomic Status
SFA	Saturated Fatty Acids
SF-PCS-12	Physical component of the SF-12 short questionnaire
SF-MCS-12	Mental component of the SF-12 short questionnaire
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SIM1	Single-minded homolog 1
SNPs	Single Nucleotide Polymorphism(s)
STANISLAS	Suivi Temporaire Annuel Non Invasif de la Sante des Lorrains Assures Sociaux
TBW	Total Body Water
TC	Total Cholesterol
TEF	Thermic Effect of Food
TEENAGE	Teens of Attica: Genes and Environment
TG	Triglycerides
TMAO	Trimethylamine N-oxide
TNF-α	Tumor Necrosis Factor-α
T2D	Type 2 Diabetes
uGRS	unweighted Genetic Risk Score
VEGF-A	Vascular Endothelial Growth Factor-A
VLDL	Very low density lipoprotein
VMN	Ventromedial hypothalamic nucleus
WAGR	Wilms Tumor Aniridia, Genitourinary abnormalities, mental retardation syndrome
WAT	White Adipose Tissue
WC	Waist Circumference
WD	Western Diet
WHO	World Health Organization
WHR	Waist-to-hip ratio
wGRS	weighted Genetic Risk Score
2-C model	Model: 2-component model of body composition
3-C model	Model: 3-component model of body composition
4-C model	Model: 4-component model of body composition

Glossary

- **Genetic/Polygenic Risk Score (GRS, PGS):** The aggravated score calculated by the summation of multiple genetic variants and used to assess the heritable risk for a particular phenotype in question.
    - **Unweighted Genetic Risk Score (uGRS):** The sum of the number of risk alleles related to a particular phenotype.
    - **Weighted Genetic Risk Score (wGRS) or Polygenic Risk Score:** The sum of the number of risk alleles related to a particular phenotype, each multiplied by their estimated weight coefficient for said phenotype.
- **Genotype:** The sum of an organism's genes and their genetic variations.
- **Nutrigenetics:** The scientific field investigating the impact of single nucleotide polymorphisms (SNPs) on gene nutrient interaction and their role in metabolic pathways.
- **Nutrigenomics:** The scientific field investigating the effect of nutrients on gene expression.
- **Phenotype:** A noticeable characteristic attributed to gene expression.
- **Pleiotropy:** The phenomenon during which expression of a single gene can simultaneously affect more than one unrelated phenotypes.

1. Introduction

1.1. Overweight and Obesity

1.1.1. Epidemiological Data

The classic definition of overweight and obesity comes from the World Health Organization (WHO), who traditionally defines their existence as *"abnormal or excessive fat accumulation that presents a risk to health"* [1]. Prevalence of overweight or obesity has increased steadily in the last decades, with the latter being currently regarded as a chronic disease [2]. The dramatic rise in obesity rates during the past decades has led WHO to reshape our perception of the disorder in a global epidemic, currently even using the term "globesity" to describe it [3]. According to data up to 2016, 39% of the global, adult population were presented either overweight or obesity, with 13% solely presenting obesity . As of March 2023, the World Obesity Atlas suggested that about half of the global population will present either overweight or obesity by the end of 2015 [4].

Augmenting rates are partly attributed to the vast global increase of the double burden of malnutrition, as obesity also currently presents higher rates than underweight in specific developing

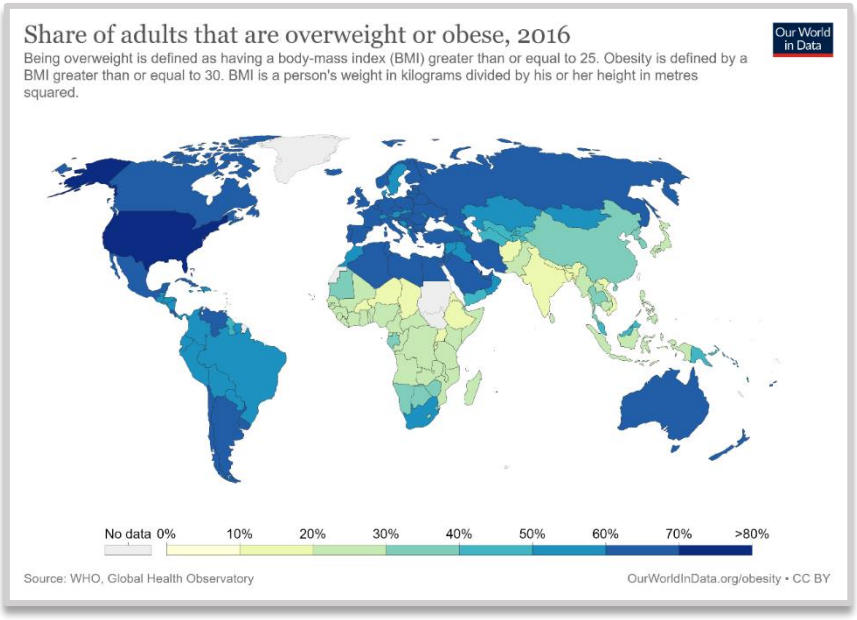


Figure 1. Worldwide prevalence of adult overweight and obesity for 2016 [6].

countries (sub-Saharan Africa and Asia). Presence of overweight appears dominating across the world, with regions such as the majority of Europe, the United States of America (USA), Russia, Australia and North Africa to be presenting a respective prevalence of above 40%. Similarly, obesity maintains a corresponding augmented prevalence of more than 25% in the USA, South America, Europe, Russia, Australia and Saudi Arabia [6]. More specifically, data deriving from 2016 (Figure 1) estimates show that region-specific prevalence percentages of adult overweight or obesity were at 70.2% for the United States, 40 to 60% for the European Region, 50-60% for the region of Latin America, 70-80% for Asia and 60 to 70% for Australia [7]. According to

Eurostat, in Greece, up to the year 2014, about 48% and 17% of adult men presented overweight and obesity, respectively, while the rates for adult women reached a respective 65% and 19% [8]. Current projections for Greece dictate rising rates for all men, women, boys and girls by 2035, with a projected 2035 obesity prevalence of 39%. Annual rates appear elevating for both adult and childhood obesity, with a respective 2% and 2.4% rise until the year 2035 [4]. Indicatively, when referring to children and adolescents, from the period 1988-1994 up to the period 2013-2014, prevalence of obesity appeared to be increasing, with about 21% of female adolescents in the United States classified as obese in the year 2014 [9]. According to WHO, the prevalence of overweight of obesity in children and adolescents increased from 4% in 1975 to 18% in 2016 [1].

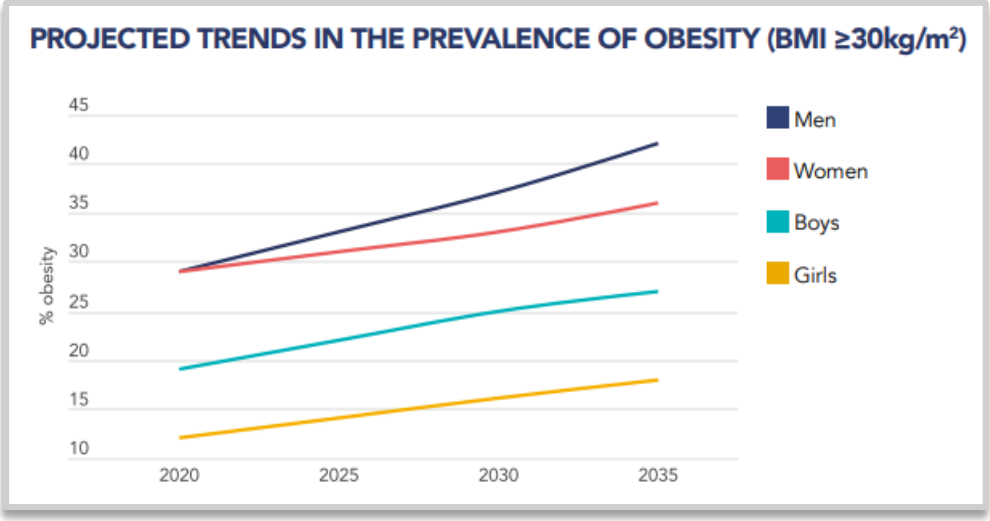


Figure 2. Projected trends in obesity prevalence in Greece for 2035 [4].

Excessive weight directly increases the risk for a variety of other cardiometabolic-related, non-communicable diseases (NCDs), such as cardiovascular disease (CVD), type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD) and cancer [10]. Its continuous and propound contributions to increasing multimorbidity render obesity an ongoing public health emergency. Indicatively, about 44% of T2D and a range of 7-41% of various types of cancer cases are met in persons with overweight or obesity [8,11]. Subsequently, according to Our World in Data, obesity was the cause for 8% of global deaths in 2017. This percentage varied across regions, with high-income countries (HICs) to note a range of 8-10%, middle-income countries (MICs) to reach up to 15% and low- and middle-income countries (LMICs) to show a percentage lower than 5% [6]. In the region of Greece, relevant deaths showed a 10.6% prevalence in 2019 and presented an increase from 1990 up to 2012, with the reported rates steadily remaining above 10% ever since 1997. A region high was observed in 2012 where the rates touched 12.77%, whereas a respective low of 9.64% was noted in 1990 (Figure 3) [6].

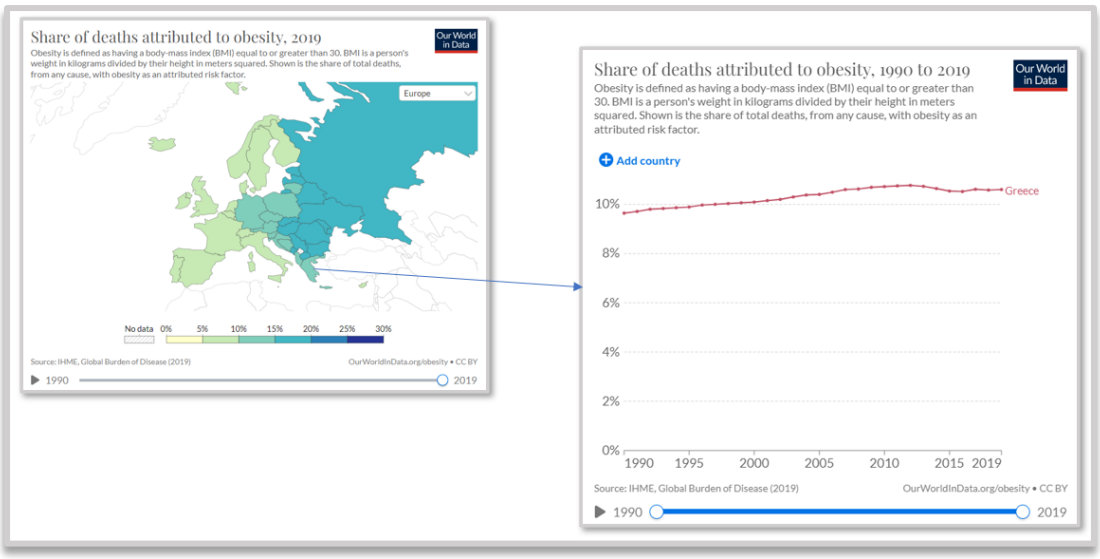


Figure 3. Percentage of obesity-attributed deaths in Europe and trend of obesity-attributed deaths in Greece for the time period 1990-2019 and Projected Trends in the Prevalence of Obesity in Greece up to 2035 [6].

Due to its widespread impact but preventable nature, the need for effective obesity prevention, prognosis and treatment strategies and policies is currently highlighted. It is in this context that within their framework of Sustainable Development Goals (SDGs) for 2030, the United Nations have set the achievement of good health and well-being in the third place of their seventeen targets [12].

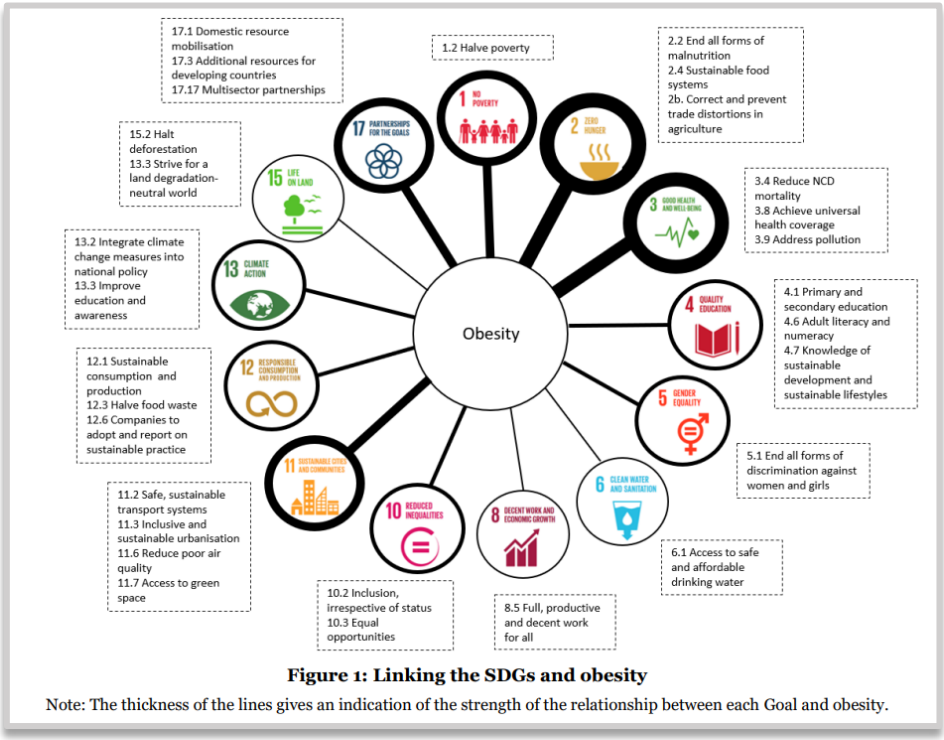


Figure 4 Associations between obesity with the 17 United Nations SDGs [12].

A massive focal point aims at the reduction of NCD-related mortality via successful prevention and treatment, essentially putting obesity-tacking strategies at the centre of current and future efforts (Figure 4) [13]. In this spectrum, a WHO resolution for obesity was shaped as of 2020, aiming at recognizing the severity of the disease. Its effective monitoring and creation of prevention and treatment strategies has also been brought to the forefront with system-based approaches, such as the ones outlined in the context of the ROOTS framework [12]. Naturally, nutrition lies at the center of these efforts, with WHO focusing on the importance of nutrition such strategies [14].

As it will be further analysed below, these increasing trends appear associated to the increased food availability and accessibility observed in the developed countries [11,15]. Another factor lies concerns the increased adherence to dietary regimes with high consumption of energy-dense foods [11,15] (such as the western diet-WD) in various parts of the world. The etiology for overweight and/or obesity development is multifactorial with causes referring to genetic predisposition, ethnicity, family and medical history, environmental factors, such as food availability and accessibility and lifestyle habits, such as low levels of physical activity (PA) [1].This need for efficient prognosis, diagnosis and treatment of overweight and obesity is currently dictating the development of effective methodologies for disease handling in the daily practice. These demand the use of all helpful information available, such as the integration of genetic information in the form of Genetic and Polygenic Risk Scores (GRS and PRSs).

1.1.2. Body Weight and Composition Assessment Methods

Although current perceptions on overweight and obesity reflect their role as chronic diseases pertaining to long-term effects in cardiometabolic status, stigma surrounding increased body weight remains prevalent in modern societies. In an effort to enhance the notion of overweight and obesity as established disorders of multifactorial etiology, rather than lifestyle choices of reduced willpower, the use of a weight-neutral terminology is now strongly suggested as a means to promote references on weight assessment, as well as well-being interventions, without strengthening weight stigma. Indeed, the European Association for the Study of Obesity (EASO) recommends the use of *"people-first language"* (eg use of terms such as individuals with overweight or obesity in the place of overweight or obese individuals) as a means to highlight the role of the latter as significant diseases. Accordingly, the present study will henceforward use the corresponding terminology to refer to relevant study populations, characteristics and findings [16].

A variety of methods implemented in the assessment of body weight and body composition contribute to the diagnosis of overweight and/or obesity. To date, the most widely used approach to assessing body weight status remain anthropometric measurements, with the primary one being the calculation of Body Mass Index (BMI). BMI is calculated when dividing an individual's weight in kilos to their respective squared height in squared meters [Weight (kg)/ Heigh^{t2} (m^2)]. According to the majority of global organizations, i.e. WHO, the Centre for Disease Prevention (CDC), World Obesity Federation (WOF), the classification in the rankings of overweight or obesity categories takes place according to the following cut-offs:

- An individual with BMI< 18.5kg/m^2 is considered to present underweight, with mild thickness if in the range 17-18.49 kg/m^2, moderate thickness if in the

values of 16-16.99 kg/m^2 and severe thickness if their BMI is below 16.00 kg/m^2; [6]

- An individual with a BMI of 18.5 to 25 kg/m^2 is considered to present normal weight;
- An individual with 25 <BMI ≤29.99 kg/m^2 is considered to present overweight;
- An individual with 30 ≤BMI ≤34.99 kg/m^2 is considered to present obesity (class 1 obesity);
- An individual with 35 ≤BMI ≤39.99 kg/m^2 is considered to present obesity (class 2 obesity); and
- An individual with BMI≥40.00 kg/m^2 is considered to present obesity (class 3 obesity) [17]

Nowadays, BMI cut-off values for the identification of overweight and/or obesity presence are not universally the same. As will be further analyzed below, use of analytic body composition methods has revealed differences in the body composition of individuals with similar BMI, but different ethnic backgrounds, which can be potentially attributed to a variety of genetic, epigenetic and environment factors. Multiple studies demonstrate variations in the BMI proposed categories and corresponding body fat percentages among populations of different ethnicities, highlighting the potential effect in the corresponding risk for weight-related, cardiometabolic comorbidities [17]. One of the most prominent differences lie in the populations of Asians, who have reportedly demonstrated smaller body structure and, thus, smaller BMI values, when compared to individuals of Caucasian or African-American descent. Although lower levels of body fat and a lower risk to overweight/obesity and related comorbidities would be expected for Asian populations due to their lower BMI levels, in reality they tend to paradoxically display higher levels of body fat percentage for same BMI values when compared to Caucasian populations. More specifically, Asians display a steady 3-5% increased body fat levels, when compared to Europeans of the same BMI [18,19]. Similarly, it has also been shown that for a given percentage body fat, Asian individuals presented a 3–4 unit lower BMI [19]. As such, Chinese and other Asian populations present higher risk for abdominal obesity and, thus, higher prevalence of other obesity-related disorders, such as type 2 diabetes and cardiovascular disease, at lower BMIs [18, 19] Additionally, another contrast is found in African-American populations who present lower body fat and higher muscle mass percentages, when compared to Caucasians of the same BMI [18,19].

Although the WHO first declined to adjust BMI cut-offs for the different populations, appearance of multiple scientific studies has provided the evidence needed to allow a variety of groups to set different metrics for the different populations, as well as prompt WHO to set cut-off values for obesity and weight-related disorders' screening and onset of corresponding interventions. In this context, WHO suggests initiating interventions among increased and high-risk Asians with a BMI of 23kg/m^2 and 25 kg/m^2, respectively [20,21]. For Asian and south Asian populations, same cut-offs are used for overweight or obesity diagnosis [2]. Similarly, presence of overweight and obesity is set at a BMI of 24 kg/m^2 and 28 kg/m^2, respectively, for Chinese and Japanese populations. Moreover, thresholds for populations of Indian or Pacific Island descent are set at 23 kg/m^2 and 26 kg/m^{2,} as well as 27 kg/m^2 and 32 kg/m^2 for overweight and obesity presence, respectively. A

summary of the population-adjusted cut-off values for BMI categories is presented in Table 1.

Table 1. BMI cut-offs for overweight and obesity presence in adult populations of different ethnicities.

	BMI values (kg/m^2)				
	Caucasian populations	**Asian- South Asian populations**	**Chinese-Japanese populations**	**Indian populations**	**Pacific Islander populations**
Overweight	25≤BMI<30	23≤BMI<35	24≤BMI<28	23≤BMI<26	27≤BMI<32
Obesity	≥30	≥25	≥28	≥26	≥32

Although BMI is known for not taking body composition into account, and thus not being the most reliable tool in representing body status, it continues to acquire the title of the gold standard method, mainly due to its character as a non-invasive, rapid, easy and inexpensive measurement. One of the greatest advantages of BMI use is the fact that its calculation only requires information on weight and height, which are usually easy to acquire across a variety of different settings and environments. Furthermore, the widespread use of BMI allows for universal comparisons between populations [22].

However, as BMI is more of a measure representing excess weight rather than increased fat accumulation [22], it may lead to underestimation of the presence of overweight and/or obesity in individuals with a BMI that falls into the normal weight range but who present increased percentage of body fat. In this context, BMI also does not provide information on the type of body fat presented in the individual (eg visceral, subcutaneous, etc). Similarly, assessment of BMI alone may lead to overestimation of overweight/obesity risk in individuals with a high percentage of body muscle mass which contributes to their reported, increased body weight. More considerations in BMI use include the fact that older adults and women tend to present higher levels of body fat when compared to younger adults and men, respectively, who present an equivalent BMI [22].

The importance of body fat percentage is crucial in determining the relatively attributed risk for cardiometabolic disorders. In this spectrum and as body fat is deemed essential in determining adiposity, further anthropometric measurements come to complement BMI in assessing body weight status, with special attention to the presence of central/abdominal obesity. Correspondingly, Waist Circumference (WC) and Waist-to-Hip ratio (WHR) are widely used to assess obesity and risk of cardiometabolic health risks. Sex specific cut-off values for the latter refer to a ratio of 0.85 for women and 1.00 for men being the indicative threshold above which lies increased risk for cardiometabolic reverse outcomes.

When referring to WC measurements, a generally accepted rule of thumb remains with the suggestion that an individual's WC ought not to exceed the half of their respective height measurement [19]. The generalized recommendation of the ATP III criteria for WC measurements includes a threshold of 88cm and 102cm for males and females, respectively. However, in line with the current considerations for the role of ethnicity in the formation of BMI levels and the key part of abdominal obesity in subsequent cardiometabolic risk, the International Diabetes Federation has proceeded to the formation of recommended cut-off points for abdominal obesity

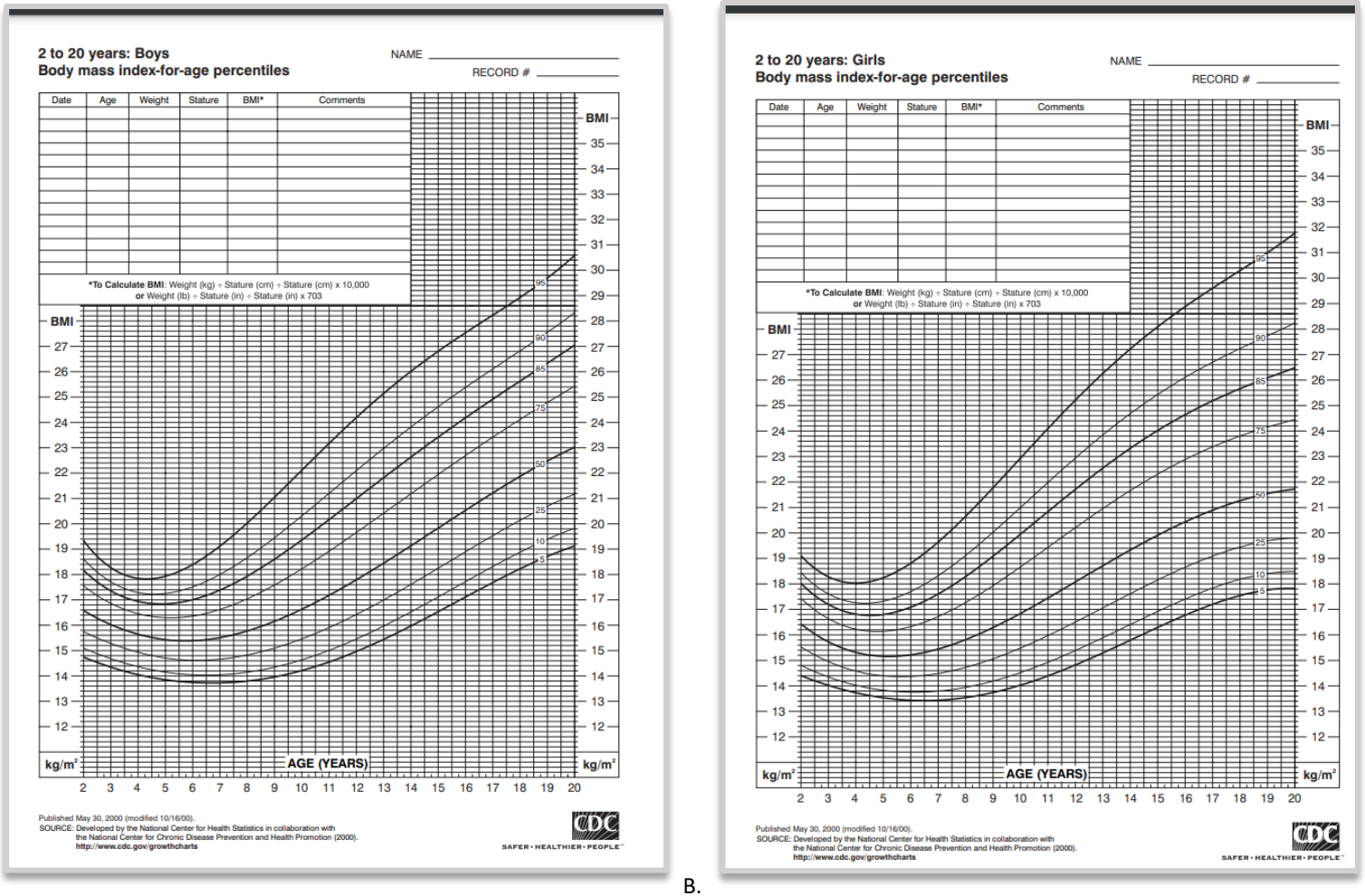
based on different ethnic groups. As depicted in Table 2, the existing ATP III recommendation can still be used for clinical reasons, however a lower threshold for both sexes is uniformly suggested for Europid, South Asian, Chinese and Japanese populations.

Table 2. WC cut-offs in adult populations of different ethnicities [18].

	WC (cm)				
	European, North American, Sub-Saharan African, Eastern Mediterranean, Middle East, populations		**South Asian, Ethnic South and Central American populations**	**Chinese populations**	**Japanese populations**
Men	≥102 (clin. purposes),	≥94	≥90	≥90	≥90
Women	≥98 (clin. purposes),	≥80	≥80	≥80	≥80

To boot, assessment of overweight and/or obesity existence varies not only based on ethnicity characteristics, but also differs when taking age into consideration. In children and adolescent populations, existence of overweight and/or obesity is primarily determined by BMI values specifically adjusted for age and sex and referred to as *BMI-for-age* [23]. Use of the adjusted value of BMI for age and sex, otherwise known as *BMI z score or zBMI*, is also widely used to assess body weight in populations below the age of 18. As shown in Figure 5, for the ages 2 to 20 years old, BMI is classified using percentile ranking, as follows [1]:

- An individual with a ranking under the 5^{th} percentile is considered to present underweight;
- An individual with a 5^{th} percentile < ranking < 85^{th} percentile is considered of normal weight;
- An individual 85^{th} percentile ≤ ranking ≤ 95^{th} percentile is considered to present overweight;
- An individual with ranking ≥ 95^{th} percentile is considered to present obesity [6,9].



A. B.

Figure 5. BMI-for age percentiles for A. 2 to 20 years in Boys; and B. b.2 to 20 years in Girls. Assessment of body weight and classification in the categories of underweight, presenting overweight or obesity for children and adolescents from 2 to 20 years old takes place via calculation of their BMI to the corresponding percentile [24].

In the same context, a differentiated concept concerns the existence of metabolically health obesity (MHO). This term is used to describe obesity existence with a BMI over 30 kg/m^2, accompanied by the absence metabolic dysfunction usually observed in the obese state, such as altered glucose or lipid metabolism, increased blood pressure and/or inflammatory or cardiorespiratory indices [22]. Prevalence of MHO ranges from 10 to 30% of adults with obesity, depending on factors of age and sex. On the contrary, the term metabolically unhealthy obesity (MUO) concerns people with obesity presenting multiple cardiometabolic risk factors and frequently elevated risk for related disorders like cardiovascular disease [22].

The simultaneous use of metabolic health and obesity in the MHO definition has been controversial, as the obese state is by nature considered a circumstance of altered metabolic profile. The need for the creation of a specified definition led to the international effort conducted in the nodes of the BioShare-EU Healthy Obese project, which set out to specify the definition criteria for MHO. The initiative concluded that MHO was defined in the absence of increased blood pressure, blood glucose of over 110 mg/dL, high density cholesterol (HDL-C) lower that 40 mg/dL, triglyceride (TG) levels above 150mg/dL and the existence of CVD [23]. Lavie et al attempted to set stricter criteria by defining MHO as all the aforementioned but with a lower threshold for fasting glucose (<100mg/dL) and the absence of receiving glucose-related or anti-hypertensive treatment [24]. A different definition by Zembic et al focused solely on blood pressure and anthropometrics by stating MHO as the existence of systolic blood pressure levels lower than 130 mmHg and a WHR of less than 1.03 in men and 0.95 in women [22, 25, 26]. Overall, as MHO definitions are mainly focused on cardiometabolic contributors, the need for including more metabolic-related indices such as indicators of inflammation, liver or immune function in future revisiting of the criteria is also underlined [27]. Existence of the state has also been observed in children, where MHO prevalence ranges from 3-87% according to factors of ancestry and puberty level [27]. An internationally accepted definition for MHO in children was provided in 2018 by Damanhoury et al. who included the standardized BMI values of the WHO growth charts, the percentile cut-offs for blood pressure (≤90mmHg) and the suggested cut-offs for glucose, HDL-C and TG levels (≤ 100, >40 and ≤150mg/dL, respectively) [27, 28].

Although at increased risk for cardiometabolic-related disease manifestation than persons with normal weight, individuals with MHO present lower risk for such disorders when compared to people with MUO [22]. It appears that maintenance of satisfactory levels of physical activity and cardio-respiratory condition in MHO can allow for the reduction of CVD risk [22,24]. In children, MHO is linked to lower BMI, WC and body fat measurements promoting better outcomes of insulin sensitivity and inflammatory status [27]. The growing aspect accompanying this age group is also taken into account where visceral fat can be seen reducing due to its observed expansion [27] due to both weight gain, but also developmental growth. Similar to the case in the adults, data from the GENOBOX study also showed that children with MHO and greater physical activity showed better status than their peers with MUO [29]. Roberge et al further demonstrated that children with obesity who adhered to healthier diet (i.e. increased consumption of fruit and vegetables and lower intake of fats and sugars) had lower changes of developing MUO than children with MHO and adherence to an unbalanced diet.

It is worth mentioning that similar discrepancies are also detected among individuals with increased weight when compared to persons with normal weight. The notion described as the “obesity paradox” is used to summarize findings indicating better cardiometabolic or mortality outcomes or prognosis in individuals with increased weight than people with a BMI below 25kg/m^2 [23, 30]. Indeed, patients with overweight and other disease have presented lower all-cause mortality that patients with normal BMI [31, 32].

Although several factors can be considered in promoting this phenomenon [eg increased metabolic reserve, muscle mass and strength, use of pharmaceutical medication and less cachexia in the overweight or obesity state [31], it is mostly possible that cardio-related positive outcomes are accredited to the effect of other factors indirectly related to obesity like physical activity, respiratory and liver function, the smooth maintenance of which probably positively affects the preservation of a good cardiometabolic profile, rather than obesity presence in itself. Additionally, a different phenotype described as metabolic obese normal weight (MONW) is also used for individuals with a BMI in the normal weight range but who present increased disrupted cardiometabolic profile when compared to people with overweight or obesity [33]. Therefore, the advantage noticed in the case of overweight or obesity could also be attributed to the existence of altered cardiometabolic status in individuals with normal weight. This is why more studies are needed to specify the definition criteria for each phenotype as well as how each might prevail to the others.

Such findings underline the need for the proper acquisition of all information related to weight and body composition by emphasizing that individual evaluation should consider detailed both from BMI calculation, as well as the use of a body composition analysis method with information on body tissue content and ratios. In the case of overweight or obesity, special attention is given to the evaluation of body fat using a variety of methods deriving from different principal bases. While using skinfold measurements to assess body fat is a relatively straightforward and easy-to-apply method, employment of methods which assess body composition using different compartment sections is more widely used in current practice.

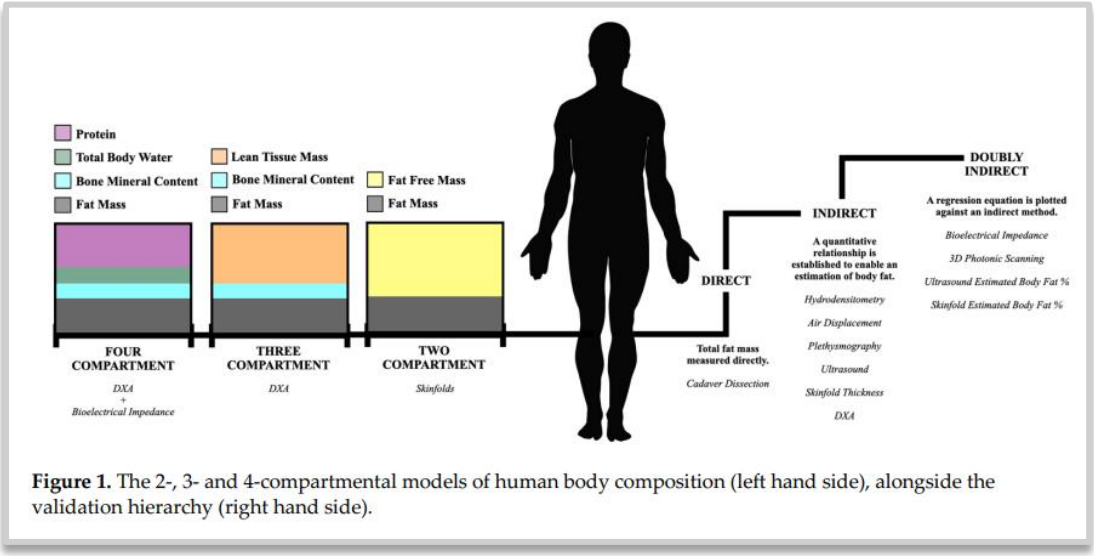


Figure 1. The 2-, 3- and 4-compartmental models of human body composition (left hand side), alongside the validation hierarchy (right hand side).

Figure 6. Body component models and respective body composition methods [34]. Kasper et al. describe the compartment models of body composition and further

categorize assessment methods based on invasiveness. The only direct method refers to cadaveric dissection, while the remaining methods are separated to indirect (skinfold thickness measurements, hydrodensitometry, air displacement, plethysmography, ultrasounds and DEXA) and double indirect methods, where predictive equations are additionally used to estimate tissue levels (BIA, photonic scanning and body fat percentage estimated via ultrasound or skinfolds) [34, 35].

Use of the latter methods is based on the models dividing body composition in distinct components, according to which: a) the two-component (2-C) model focuses on fat distribution by referring to a fat-mass and fat-free mass component; b) the three-component (3-C) model describes the body at fat-mass, fat-free mass and bone mineral content (BMC) levels and; c) the four-component (4-C) model further divides the body at fat mass, metabolic tissue, BMC and total body water (TBW) separate compartments (Figure 6) [34]. Different, multi-component models further separate body compartments, a) by highlighting the existence of essential and non-essential lipid, total body water, protein, bone mineral, soft-tissue mineral and glycogen components or b) by referring to atomic, molecular, cellular, tissue-system and whole-body levels [35].

Indicatively, methods to evaluate body composition based on the 2-C model include: a) hydrodensitometry, which is considered the most reliable and, thus, the gold-standard standard method. Based on the Archimedes' principle on water displacement, in hydrodensitometry an individual's weight is calculated both outside and inside water and the difference between the two values reflects the power of buoyancy to determine body density; b) plethysmography, which is based on Boyle's law of perfect gases and where body density is the ratio of body mass to body volume; and c) calculation of radio-isotopes, which estimates fat and fat-free mass via measurement of total body water or potassium isotopes [36]. Despite their increased accuracy and validity, the aforementioned do not represent the go-to assessment methodology due to their invasive nature, extensive need for appropriate machinery and increased cost.

Use of methods based on more component models is widely met both in current practice and research, primarily due to their increased accessibility and relatively increased accuracy of quick results. Bioelectrical impedance analysis (BIA) constitutes a widely implemented approach where a weak electrical current is applied to the individual, structured on the principle that water, as opposed to fat tissue, is a good conductor of electricity. Therefore, fat, muscle and body water densities are measured according to the observed resistance to the current's flow. A different approach refers to the implementation of the Dual-energy X-ray absorptiometry (DEXA) method. Through using low-power x-ray beams, DEXA measures body fat and fat-free mass, in addition to bone mineral density. Given its increased predictive value, DEXA is extensively used in cases focusing on bone density assessment [34-36].

Similarly based to multi-component principles as in the case of DEXA and primarily used in the evaluation of abdominal obesity, use of computerized tomography (CT) or magnetic resonance imaging (MRI) can increase the individual's detailed assessment due to their ability to differentiate between visceral and subcutaneous fat [34-36].

1.1.3. Body Weight Regulation

Body weight is mainly dependent on the hormonal regulation of energy homeostasis. Homeostatic mechanisms surrounding metabolism heavily involve the reciprocal cooperation of multiple organ systems, namely the nervous, the endocrine and the gastrointestinal ones. Multiple hormonal peptides included in key processes (i.e. cortisol, ghrelin and leptin) are further subject to circadian regulation, i.e. the 24-hour periodic variation of substances involved in numerous human biological procedures [37]. Pathways regulating systemic metabolism operate under the control of the autonomous nervous system via involvement of the vagus nerve, as well as afferent and non-afferent nerves, responsible for receiving endocrine and paracrine signals and subsequently regulating brain-controlled metabolic responses [36,37]. Via both mobilizing and responding to hormonal signals related to food intake, energy homeostasis is dependent on brain-controlled metabolic pathways mainly located on the hypothalamus' arcuate nucleus (ARC), the paraventricular nucleus (PVN), the brainstem's nucleus tractus solitarii (NTS) and nucleus accumbens (Acb). This approach to energy regulation involving multiple organ systems under control of the brain is summarized in what is known as "Homeostatic Control/Mechanism of Energy Balance" [36,37].

Energy control centers around the anorexigenic effect of the proopiomelanocortin (POMC) neurons and the opposite orexigenic influence of the neuropeptide Y (NPY) and agouti-related protein expressing (AgRP) neurons. The latter stimulate the production for the anorexigenic a-melanocyte-stimulating hormone (α-MSH), which promotes catabolic processes and satiety via binding to the corresponding melanocortin receptors located throughout the nervous system (Melanocortin 3 and Melanocortin 4 receptors – MC3R, MC4R) [36]. In a similar manner, a different hormone, the Brain-derived Neurotrophic Factor (BDNF), also promotes MC4R activation and subsequent anorexigenic signaling by responding to peripheral hormone production such as leptin. Similarly, expression of the cocaine-and amphetamine regulated transcript (CART) in the brain's ARC, PVN and Acb, has also been shown to associate with anorexigenic effects, while presenting co-storage in regions with high POMC neuron content [38]. On the contrary, the same MC4R receptor antagonist agouti protein, which is produced by NPY and AgPR, promotes food consumption by increasing orexigenic stimuli [36,39] (Figure 7).

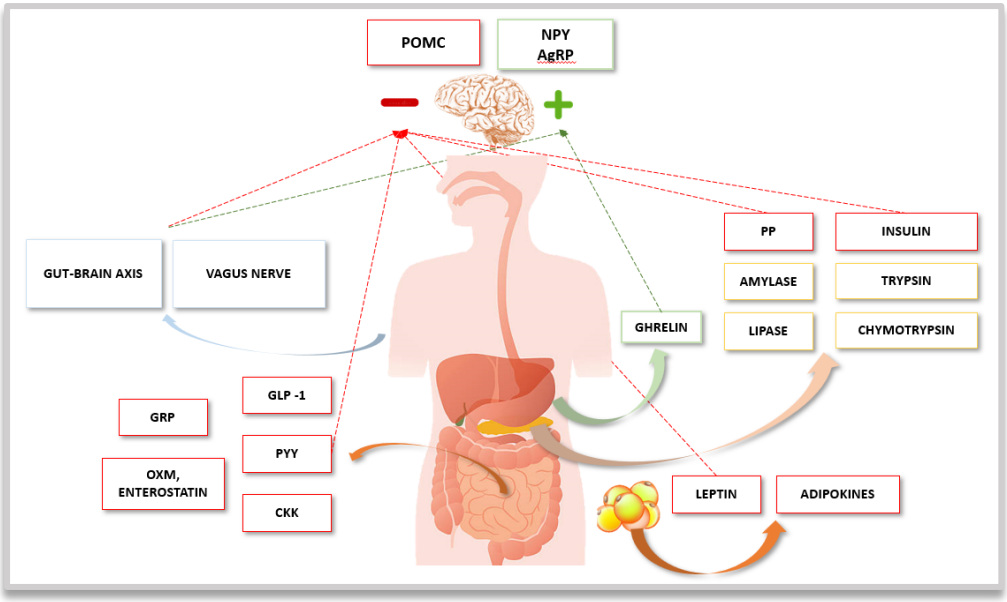


Figure 7. Hormonal regulation of energy metabolism [36].

Energy intake is a multi-sectoral process, where the interaction between hormones secreted throughout the gastrointestinal tract and the vagus nerve instrument collaborative procedures in promoting food consumption or satiety and dictate macronutrient storage or breakdown. Key substances involved in energy regulation follow the order of the digestive system (Figure 37), where more specifically:

- oxyntic glands in the stomach produce ghrelin in response to absence of food, which prompts orexigenic signaling to promote food consumption via binding to vagus nerve receptors. Ghrelin binding to the (Growth Hormone Secretagogue Receptors 1a- (GHSR) affects the vagus nerve projections to the ventromedial hypothalamic nucleus (VMN) and the dorsal vagal complex of the brain (DVC), where orexigenic NPY and AgPR neurons are stimulated;
- small intestine I-cells secrete cholecystokinin (CCK) which stimulates the production of amylase, lipase, bile and other pancreatic enzymes and responsively binds to vagus nerve receptors to inhibit further food intake;
- enteroendocrine L-cells in the small intestine and colon produce the glucagon-like peptide-1 enzyme (GLP-1), promoting glucose and lipid metabolism while simultaneously acting as an anorexigenic agent;
- enteroendocrine L-cells in the ileum and colon of the large intestine cells produce the YY peptide (PYY) and oxyntomodulin prompting satiety via binding to the vagus nerve.

A dual role for both the digestive and the endocrine system is found in the function of pancreas, where secretion of the pancreatic peptide (PP) by the pancreatic polypeptide cells stimulates bile release and vagovagal afferent signals in promoting anabolic processes, while production of insulin in response to glucose presence promotes glucose metabolism and storage [36]. Moreover, large contribution is attributed to adipose tissue-derived hormones called adipokines, such as leptin and adiponectin. Production and binding of the former to the leptin receptors (LepR) in the brain induces satiety and energy expenditure, while secretion of the latter

promotes insulin-controlled glucose anabolism and lipid metabolism [36,40]. More specifically, leptin is produced by adipose tissue cells, crosses the blood-brain barrier and enters the hypothalamus, where it binds to the leptin receptor and contributes to: i) signalling the suppression of energy intake; ii) inhibiting the expression of the orexigenic Neuropeptide Y (NPY); iii) inducing the expression of further anorexigenic peptides, such as proopiomelanocortin (POMC) and α-Melanocyte-stimulating hormone (α-MSH) (under the control of the MC3R and MC4R genes). On the contrary, ghrelin is an orexigenic hormone produced by the stomach, which increases the expression of other such molecules, like NPY. Although the levels of direct ghrelin transport to the brain are potentially quite low, ghrelin is one of the hormones which can cross the brain-blood barrier (BBB) and directly induce brain responses to food intake [41]. Another orexigenic peptide is the melanin-concentrating hormone (MCH), which contributes to appetite and the beginnings of eating episodes [42,43] (Figure 8).

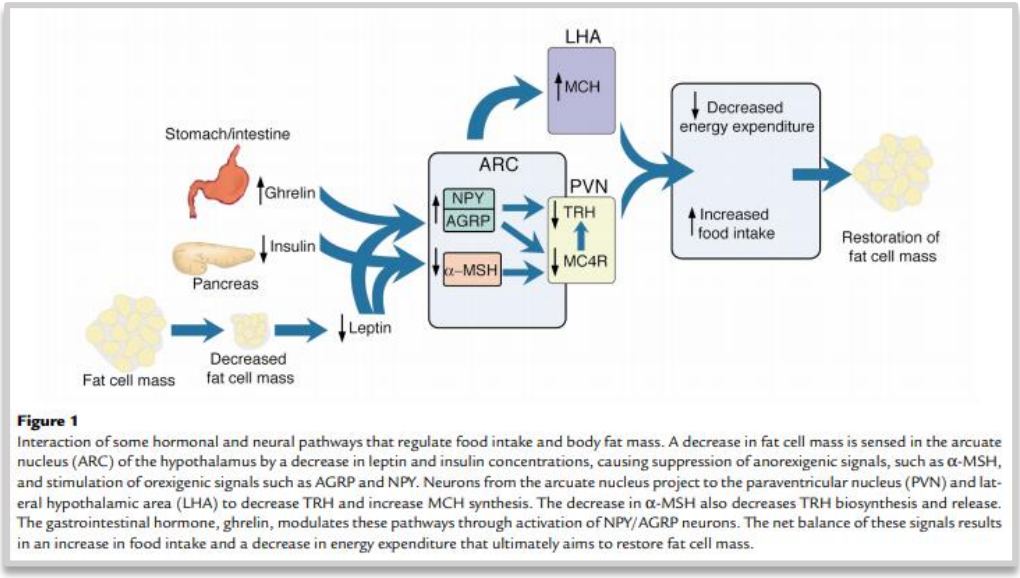


Figure 1
Interaction of some hormonal and neural pathways that regulate food intake and body fat mass. A decrease in fat cell mass is sensed in the arcuate nucleus (ARC) of the hypothalamus by a decrease in leptin and insulin concentrations, causing suppression of anorexigenic signals, such as α-MSH, and stimulation of orexigenic signals such as AGRP and NPY. Neurons from the arcuate nucleus project to the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) to decrease TRH and increase MCH synthesis. The decrease in α-MSH also decreases TRH biosynthesis and release. The gastrointestinal hormone, ghrelin, modulates these pathways through activation of NPY/AGRP neurons. The net balance of these signals results in an increase in food intake and a decrease in energy expenditure that ultimately aims to restore fat cell mass.

Figure 8. Stages of hormonal control of energy homeostasis in the different human organs [42].

Another pillar affecting metabolic regulation and energy homeostasis refers to implications involving the gut. Energy homeostatic mechanisms involve the activation of the bidirectional gut-brain axis (GBA) leading to a cascade of events affecting metabolic pathways, macronutrient synthesis and breakdown and, even, inflammatory response. The secretion of various hormones from endocrine cells throughout the gastrointestinal tract as a response to nutrient influx, acts as a stimulus to responses of the enteric (ENS) and subsequently the autonomous and central nervous systems, via endocrine or paracrine functions from enteroendocrine cells (EECs) to vagal afferents of the GI epithelium and simultaneous activation of pathways in multiple visceral organs [41, 44]. The latter extend to brain regions such as the NTS which, in turn, affect hypothalamus, ARC and PVN, essentially uniting the two systems in regulating metabolic responses based on the existing food availability [45].

Hormonal activation of brainstem-derived neurons of vagal afferents spread throughout the gastrointestinal tract (GI tract) is central in the stimulation of the gut-brain axis. One of the greatest examples lies in the activities of the intestine-secreted,

anorexigenic CCK. With its levels rising after meal intake, the latter promotes satiety and suppresses further food consumption both directly via increase in the peripheral regulation which ultimately creates responses reaching the CCK receptors located in the brain, and indirectly through activation of vagal afferents connected to the gut and ultimately reaching the NTS. Through its role in the activation of the neural-gut connections, CCK is further implicated in more mechanisms responding to food intake, such as the post-prandial suppression of the production of glucose in the liver [41, 46]. A different mechanism refers to the anorexigenic actions of the GLP-1 peptide, the secretion of which appears to be stimulated by neural signals even in the early stages of the nutrient influx in the GI tract. GLP-1 binds to the GLP-1 receptors located in neurons of vagal afferents throughout the GI tract and brain regions, directly stimulating the responses to food presence and regulating the glycemic metabolic responses. Lastly, although studies remain inconclusive on a definite role of PYY on the activation of the axis, the current notion supports that the intestine-produced peptide YY can act in promoting long-term satiety by activating vagal afferents leading to stimulation of POMC neurons through inhibition of NPY [41, 46] . It is worth mentioning that the pathways via which the stimulation of hormonal secretion occurs can be differentiated based on the different macronutrients metabolized. For example, glucose-stimulated release of CCK or GLP-1 from the intestinal cells takes place via different pathways than the one initiated by long-chain fatty acids (LCFA) [41]. However, as food intake predominantly involves the simultaneous metabolism of the different quantities of all macronutrients, it is possible that all candidate pathways are activated at the same time in an effort to promote the systemic responses needed to promote satiety and suppress further food intake.

Furthermore, essential to the role of the nutrient-stimulated hormones is the function of gut-microbiome affected-chemoreceptors located throughout the GI tract. The symbiotic association between the host and gut microbiota creates a bilateral environment, where nutrient influx affects microbiome development and microbiota subsequently affect metabolic and immune pathways [41]. The crucial role of gut microbiota in the activation of the gut-brain axis is summarized in the following actions, the up- or down-regulation of each one can directly impact the regulation of all others. More specifically, GBA is involved in: i) the modulation or production of multiple metabolites, such as SCFA, tryptophan, gamma-aminobutyric acid (GABA), catecholamines and bile acids), which further affect metabolic and physiological pathways; ii) the modulation of 5-hydrotryptamine -or serotonin- production from intestinal cells, which is positively influenced by other gut metabolites (i.e. bile acids and SCFA) and, in turn, impacts the activation of enteric nervous system (ENS) or vagal afferents [41]; iii) the impairment in the function of ENS through production of lipopolysaccharides (LPS) by Gram-negative gut bacteria [41,45]; and iv) the indirect activation of the hypothalamus-pituitary-adrenal (HPA) axis through production of multiple metabolites.

Modulation and production of metabolites is key in the gut-associated activation of metabolic cascades. BAs can bind in corresponding receptors in the ECCs or even increase their transcription factors (G protein-coupled bile acid receptor-1), promoting the production of GLP-1 [41]. Production of SCFAs such as butyrate, acetate and propionic acids resulting from the breakdown of complex carbohydrates, can also bind to ECC and vagal receptors [41] and help regulate glucose metabolism and the

enteral stimulation of production of metabolic hormones such as the GLP-1 or PYY [45]. Propionate is used in gluconeogenesis, while acetate passes to the peripheral circulation and can even cross the BBB and directly affect energy homeostatic pathways [46]. Moreover, gut-derived neurotransmitters, such as GABA, which is produced by bacterial strains like Bifidobacterium and Lactobacillus [41, 45], can also cross the BBB and directly enter the brain affecting multiple metabolic- and other type- metabolic responses [45]. To boot, microbial byproducts like the LPS are recognized by the ENS and lead to the activation of a cascade of signals increasing cytokines and immune system activation [45]. As it will be further analyzed below, these functions are most apparent in the existence of external stressors -such as stress and high fat diets- and can be further associated with activation of the HPA via increase in stress-related hormones like the adrenocorticotropic hormone (ACTH) [45].

Weight gain occurs during a steady state of positive energy balance, where the individual's EI is greater than their energy expenditure (i.e. the sum of Basal Metabolic Rate-BMR, Diet-Induced Thermogenesis-DIT and the Thermic Effect of Food-TEF). The latter leads to the creation of energy surplus to be stored in the form of glycogen (muscle tissue) or triglycerides (muscle and adipose tissues) [47] (Figure 9). Increased EI and storage inevitably lead to the augmentation of fat cells in the respective different kinds of adipose tissues, subsequently increasing the production of adipose tissue-related hormones (Figure 10).

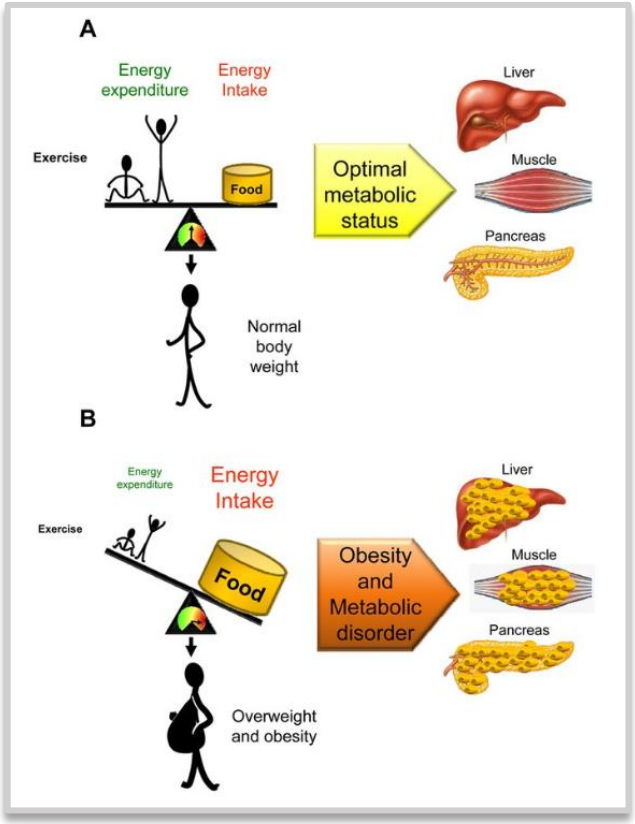


Figure 9. Energy Balance during A. Optimal metabolic Status and B. During increased energy intake [47]

Both the white and the brown adipose tissues (WAT and BAT) contribute to energy metabolism through their ability to store fat cells and participate in fat oxidation and thermogenesis, respectively. For that reason and given the increased endocrine and paracrine metabolic action observed throughout its entirety, adipose tissue is nowadays viewed as an active endocrine gland. Behaviors surrounding food intake entail multiple individual factors, such as genetic predisposition or health status, but also involve contextual determinants, like environmental factors or socioeconomic, family and education status. Originally, research focused on the role of CNS in the pathogenesis of obesity due to its central part in maintaining energy homeostasis and

dictating switches on food consumption. In an effort to unravel the neural parameter of food intake, various patterns have been used to describe the human eating tendencies, namely: i) the model of "reflexive eating", which emphasizes on the human proclivity to overeat as a means to prepare for potential future circumstances of food scarcity and has been shown to implicate brain region like the ARC and the brainstem; and ii) the model of "reflective" eating, which further takes into account cognitive parameters on health and social status related to body shape [48].

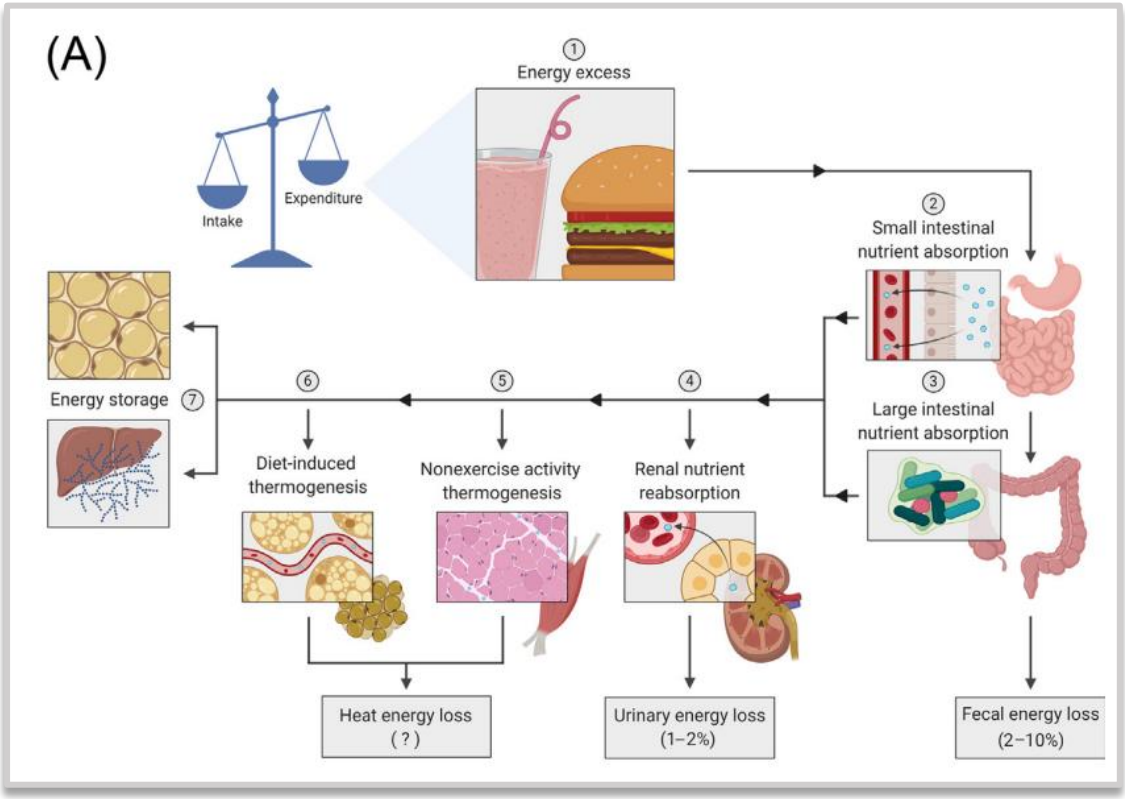


Figure 10. Effect of energy surplus on energy metabolism [49].

The role of the human factor in ultimately shaping metabolic processes was further highlighted in the context of the "Hedonic Mechanism of Energy Balance", which differentiates itself from the aforementioned Homeostatic Mechanism, by underlining the importance of complementary brain regions, such as the hippocampus and amygdala, in dictating food behavior by shaping the will and not the need for food consumption [50]. Thusly, various studies have focused on the potential impact of key brain regions directly or indirectly involved in the regulation of metabolic pathways such multiple brain cortices, which are also implicated in biological processes affected or connected to emotional response (e.g. sadness or reward seeking behavior). In this context, the central role of the brain's anterior and posterior cingulate cortices (ACC and PCC) in emotional regulation is mainly attributed to their ability to receive information and extend projections to the hypothalamus [51], ACC's ability to control reward-related actions and outcomes, as well as PCC's capacity as an action-coordinating domain [17]. Davidson et al. first focused on the potential interaction between altered brain activity and energy homeostasis [52]. Indeed, hippocampal activity was variously related to food intake, namely due to the negative effect of increased fat intake on BDNF expression, the existence of receptors for insulin and

leptin, and its sensitivity to satiety stimuli. The latter promote brain-gut-axis activity via gut-hippocampal signaling and subsequent interactions between the hippocampus and other brain regions involved in energy regulation [51]. Studies in rats further supported that altered hippocampal activity was associated to energy regulation, potentially due to the disrupted function of learning processes that, in turn, affect appetite and consumption [53,54]. Similarly, the amygdala region is also shown to interact with energy-related metabolites. Pineda et al highlighted the anorexigenic effect of the POMC neurons that are projected from ARC to the amygdala as well as the potential effect of amygdala-located NPY receptors. Additionally, α-MSH binding to MC4Rs expressed in the amygdala, also demonstrated a potential effect on the regulation of feeding behavior [55]. A different study in mice demonstrated the positive association between activation of amygdala-located neurons that implicated in neurotensin expression with preference for increased-energy consumption and hedonic eating [56].

Another connective link between emotional state and energy metabolism might refer to the role of the cingulate cortex, due to the ACC's association to food-related stimuli, emotional processing and subsequent decision-making, and PCC's projections to the hippocampus [57]. Indeed, individuals with higher BMI, obesity or binge-eating disorder have repeatedly shown disrupted brain and ACC activity [58-60]. Whiting et al demonstrated that patients with anorexia showed increased ACC activity in response to food images and hypermetabolic activity in the hippocampus and amygdala [61]. A different study in men of normal weight showed an association between ACC activation and ad libidum food consumption [62] and other studies have directly looked at the effect of emotions on BMI levels or presented a partly physiologically-attributed, positive relationship between overeating and BMI in individuals with depression. Furthermore, sums of reactions associated to emotional stimuli are now regarded as part of corresponding "systems" entailing relative cascades of physiological and metabolic responses, such as the PANIC/GRIEF and the SEEKING systems. Activation of the PANIC/GRIEF system occurs in emotional states of sadness and can provoke hormonal adaptations, which can, in turn, affect metabolic pathways related to energy homeostasis. By principle, increased corticotropin releasing factor (CRF) levels (noticed during PANIC/GRIEF system stimulation) lead to decreased feelings of appetite and prolactin, which are associated with lower food intake. At the same time frame but on a contradictory action, the decreased levels of oxytocin observed during the PANIC/GRIEF activity might increase the need for food consumption [63]. Complementing the action of the PANIC/GRIEF system, the arousal of the SEEKING system during the first stages of feeling emotions of despair might increase the drive for food-seeking behavior and thus elevate food consumption. Such notions can be further supported by the hypothalamic and dopamine-regions' imbalance observed in orexin during depression [64].

Presence of obesity directly leads to hormonal imbalances, especially in hormones of the adipose tissue. Individuals with excessive weight show a progressive decline in post-prandial ghrelin levels which results in prolonged tolerance and promotion of its orexigenic signal [36]. Although leptin levels are elevated due to the increase in the number and size of adipocytes, as fat tissue expands the intensity of the hormone's anorexigenic effect appear to grow weaker [36] (i.e. leptin resistance). The observed increase in leptin levels shows presents a dual metabolic disadvantage, as it not only

negatively influences appetite suppression but also leads to establishment of low-grade inflammation. As a consequence to elevated leptin, T-cells, Natural Killer (NK) cells, macrophage proliferate and cytokine production grows (eg Tumor Necrosis Factor α -TNF-α, Interleukin 6 -IL-6), aiding the establishment of oxidative stress and a low-grade, but continuous inflammatory state [65].

Another pillar in excessive weight presence refers to its reciprocal relationship with misaligned circadian rhythms. In the case of humans, the circadian clock refers to the mammal's innate capacity to regulate the stable secretion of various hormones according to external environmental stimuli, such as light presence or absence, during the 24-hour-day period. On the one hand, disrupted circadian rhythms due to factors unrelated to weight at first reading, such as sleep disturbances, can indirectly lead to increase of weight due to the observed metabolic imbalances of circadian hormones [66]. Conversely, the existence of obesity and the cascade of metabolic dysregulation that come with it and aggravate circadian disturbances and lead to further homeostatic misalignment [67].

Lastly, another major connective point with weight imbalances refers to disruptions in the gut-brain axis leading to alterations in gut microbiome [68]. In a reciprocal manner similar to the ones previously analyzed, excessive weight also affects the gut microbiota-host relationship disrupting the latter's part in macronutrient metabolism [69]. Indicatively, gut microbiota are responsible for carbohydrate fermentation and the production of metabolic byproducts rendering them indispensable in the maintenance of gastrointestinal tract homeostasis [69]. Changes in the intake of dietary compounds such as carbohydrates, fiber and fat directly affects microbiome metabolic procedures and subsequent glucose and fat homeostasis by increase or decrease of produced substances, such as the fiber-enhanced production of SCFA [70,71]. Presence of obesity has been connected to altered microbiome profile, with literature dictating increases in strains such as the Firmicutes/Bacteroidetes ratio [72]. These can, in turn, affect metabolic and inflammatory pathways aiding in further metabolic dysregulation. Figure 11 presents the transition from the interaction between gut macronutrient metabolism in normal weight versus in the presence of obesity.

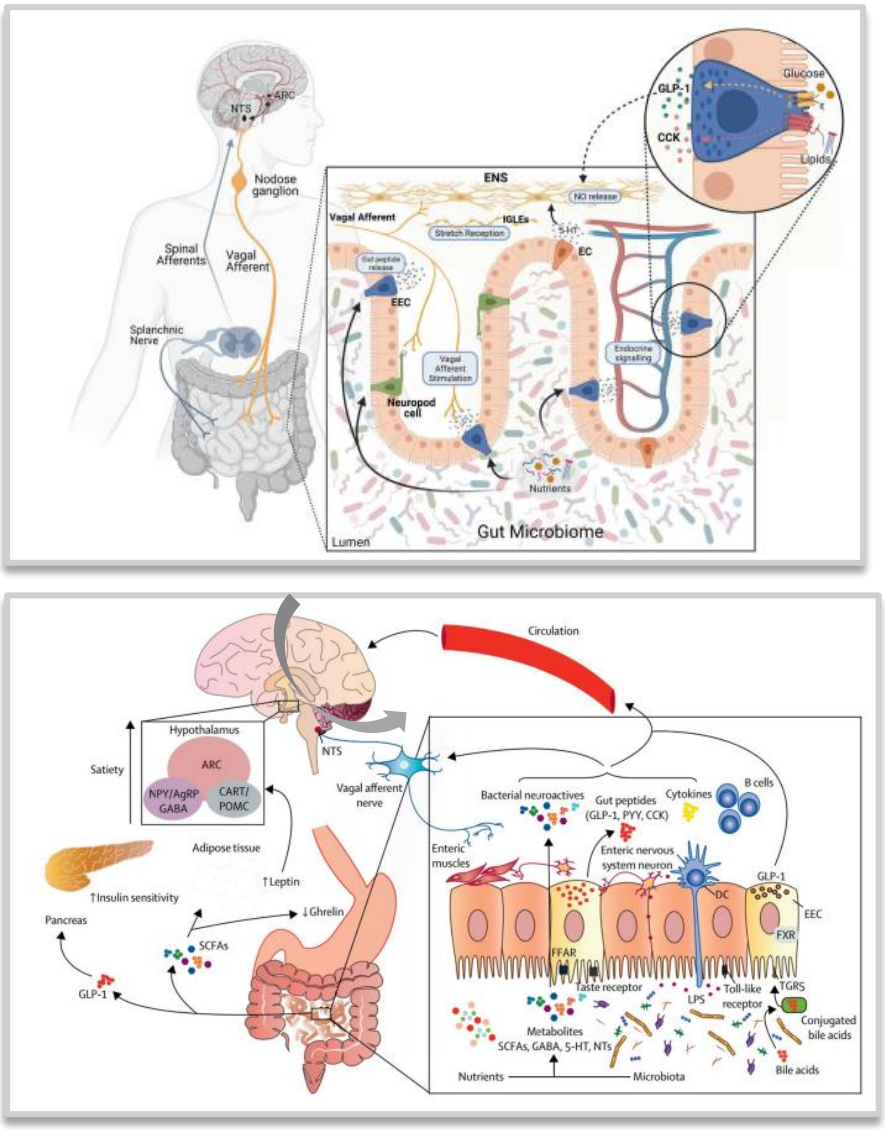


Figure 11. Gut-brain axis in energy metabolism in general versus [41] versus B. gut-brain axis in energy metabolism in the presence of obesity [45].

1.1.4. Genetics and Epigenetics of Overweight and Obesity

Obesity can be a result of multidisciplinary etiology. Different types of obesity presence concern: i) obesity associated with the existence of a specific syndrome-syndromic obesity; ii) obesity attributed to the effect of a single gene-monogenic obesity; and obesity related to the simultaneous and reciprocal influence of multiple genes-polygenic obesity.

Syndromic Obesity

Regarding the first category, syndromic diseases are usually inheritable in autosomal or sex-linked dominant or recessive manners and the severity of their symptoms can vary according to the degree of genetic penetrance and expressivity. The term syndromic obesity refers to the presence of obesity as a phenotype in the context of the manifestation of a diagnosed syndrome along with other signs and symptoms usually involving disabilities, organ abnormalities and/or developmental delay and mental disability [73]. This type of obesity can result from changes in the number or structure of chromosomes [36] or the pleiotropic action of one or multiple genes [36, 73].

One of the most well-studied cases of syndromic obesity concerns the Prader-Willi Syndrome (PWS). The disorder is attributed to the loss of gene function of chromosome 15 due to paternal genomic imprinting. This affects hypothalamic function, resulting in reduced expression of growth hormone and disruptions in metabolic pathways related to energy homeostasis and food consumption [36]. Children with PWS present high rates of morbid obesity due to excessive appetite and overeating appearing after the age of 4, as well as developmental delay, growth abnormalities and learning and behavioral difficulties [74]. In an attempt to further understand the genetic aspects and interactions involved in the disorder, in 2021, the Foundation for Prader-Willi Research announced the PWS Genome Project; the first attempt to conduct whole genome sequencing in PWS patients [36].

A similar case of syndromic obesity is encountered in the presence of the Bardet-Biel Syndrome (BBS) which is caused by multiple mutations in the BBS1-BBS21 chromosomic region and is inherited in an autosomal recessive manner [36]. The disorder is characterized by disrupted vision, cardiac and kidney functions, central obesity and hypogonadism [36]. Due to the observed disruption in energy homeostasis and the high penetrance of genetic mutations related to insulin resistance, patients with BBS present a high risk for type 2 diabetes [36].

Other disorders where the obesity phenotype is manifested as part of syndromic genetic disruption include: i) the Albright Hereditary Osteodystrophy (AHO) syndrome which is inherited in an autosomal dominant manner due to maternal genomic imprinting and includes disruptions in the metabolism of parathormone and growth hormone; ii) the Alstrom Syndrome (ALMS) inherited in an autosomal recessive way and related to disrupted vision, hearing and glucose metabolism; iii) the fragile X syndrome, which is attributed to repetitions of the CGG triplet in the FMR1 gene, related to mental disability and associated with the presence of a Prader-Willi-like obesity phenotype; iv) the Wilms Tumor Aniridia, Genitourinary abnormalities, mental retardation syndrome (WAGR) which is inherited in an autosomal recessive manner and accompanies disruptions in BDNF expression due to loss of genes in the

chromosome 11; v) the Angelman syndrome caused by maternal genomic imprinting in chromosome 15 and associated with mental and developmental retardation; and vi) the Smith-Magenis syndrome, which might occur either randomly or via autosomal dominant inheritance and entails deletions and mutations in chromosome 11 [36].

Monogenic Obesity

Moving on from syndromic obesity, increased weight can further be a result of the disruptive function of a single key-gene. The term monogenic obesity is used to describe the existence of the disorder being attributed to the altered function of one single gene, usually involved in key metabolic pathways. Most forms of monogenic obesity are associated with mutations in genes implicated in the leptin-melanocortin pathway, such the leptin (LEP), leptin receptor (LEPR), POMC, the Proprotein Convertase Subtilisin/Kexin Type 1 (PCSK1), BDNF, neurotrophic receptor tyrosine kinase 2 (NTRK2), the Single-minded homolog 1 (SIM1), and MC4R genes (Table 3) [75,76]. Given the fact that most such mutations are inherited in a recessive manner, homozygote status is a-priori associated with heavy phenotypic manifestations and increased symptom severity. However, cases of heterozygotes may also present "intermediate" phenotypes, where symptom gravity can resemble the one attributed to susceptibility to common obesity [75].

The most well-studied genes related to this rare form of obesity concern the presence of mutations in genes involved in leptin transcription and expression pathways, namely the LEP and LEPR genes. Congenital leptin deficiency is a characteristic example resulting in monogenic obesity, where individuals do not produce sufficient amounts of the hormone due to disruptions in LEP expression [77]. LEP mutations are inherited in an autosomal recessive manner and are further responsible for the presence of hyperphagia in the early stages of life, leading to severe obesity, as well as hyperinsulinemia leading to an increased risk for T2D development [78]. Their presence can also lead to accompanying phenotypes like hypogonadism and late puberty onset attributed to general disruptions in hormones of the HPA axis such as the follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone and growth hormone-releasing hormone [36]. Additionally, deficiency of the hormone can also generate disruptions in immune responses due to reduction in inflammatory cell and cell regulators' inhibitors protecting tissue damage (e.g. serine protease inhibitor a1-antitrypsin). Simultaneously, accumulation of inflammatory cell regulators triggers further metabolic disruptions leading to an increased state of inflammation, such as insulin resistance or hepatic steatosis [78]. In reviewing cases of leptin deficiency, Rodrigues Salum et al underlined the potential effect of the different kinds of mutations in differentiating the severity of the observed manifestations, highlighting the association between mutation homozygote status and increased expression of weight-related altered phenotypes. As of 2021, the study reported a total of 17 different mutations in 67 individuals across the globe [78].

Also inherited in an autosomal recessive manner, mutations in the LEPR additionally promote the onset and establishment of monogenic obesity by inhibiting hormone binding and subsequent signaling. Compared to leptin deficiency alone, reduction in the receptor expression has been associated with the expression of more severe neuroendocrine disruptions (eg more severe hypogonadotropic hypogonadism), potentially due to the loss of its ligand-independent activity [77].

Mutations in LEPR are fairly uncommon, with less than 100 cases having been reported worldwide [36,76].

POMC is a different gene heavily implicated in energy metabolism, mutations in which can be inherited in an autosomal manner (dominant or recessive). Disruptions in POMC expression affect leptin signaling-due to leptin influencing POMC-expressing neurons- and MC4R neurons' activations via reduction of α-MSH production [76]. Newborns with POMC mutations will soon present cortisol and ACTH deficiency, mild central hypothyroidism and hyperphagia leading to the establishment of severe obesity [76,79]. Interestingly, reduction of α-MSH signaling has been associated with the presence of red hair in Caucasian populations [80], providing evidence for the potential effect of different ancestry in phenotype expressivity.

To boot, PCSK1 expression is involved in the production of the prohormone convertase 1/3 family who are, in turn, implicated in the cleavage of metabolism-related prohormones and the subsequent activation of multiple functioning hormones, such as insulin, glucagon, GLP-1, α-MSH, TSH and GnRH [36,76]. Mutations in the PCSK1 gene are subsequently associated with severe obesity and consequences following the disruption of the HPA axis, similar to the ones mentioned above (e.g. hypogonadotropic hypogonadism, adrenal insufficiency [79].

Apart from genes solely involved in energy homeostatic mechanisms, mutations in genes related to neurophysiological activity are also shown to affect the onset of severe obesity. Rare cases of mutations in the BDNF and the NTRK2 gene have been observed and associated with the presence of severe obesity and cognitive impairment in two cases of young children (an 8-year-old girl presenting insufficiency of BDNF and an 8-year-old boy presenting hyperphagia and obesity with a de novo mutation in NTRK2) [76,79]. In more detail, the NTRK2 gene is responsible for expressing both BDNF and its tropomyosin-related kinase B receptor, being, thus, involved in energy metabolism through MC4R-affected cascades [79]. More cases of less severe mutations in its entirety have been observed in patients with obesity or cognitive impairment, without, however, their direct effect on the obesity phenotype to have been fully elucidated [79]. A different characteristic example of gene mutations involved in neurological functions involved in energy homeostasis refer to mutations in the SIM1 gene. As the latter is involved in the development of PVN and subsequent cascade of POMC signaling, mutations in its entirety have been associated with obesity phenotypes, hyperphagia and other neurological dysfunctions. Those can, in turn, affect brain centers indirectly related to energy homeostasis, such as regions affecting cognitive abilities, memory and hypopituitarism [76,79].

Finally, also autosomal inherited, monogenic obesity-related mutations are further observed in the MC4R gene. Innately implicated in energy homeostasis, MC4R mutations are account for 0.2-5.8% of severe obesity cases [80] and are heavily involved in disrupted satiety signaling, leading to severe obesity onset due to hyperphagia; a tendency which appears to reduce over the aging years. Homozygote status appears to be of importance in phenotype severity, as heterozygotes for MC4R mutations can present great variability in weight-related phenotypes and allelic heterogeneity appears to be related with different pathogenic mechanisms [76]. As it will be further analyzed below, such mutations penetrance and expressivity greatly varies also based on the interactive effect of environment. MC4R mutations are, therefore, placed in the range between monogenic and polygenic obesity, with

Huvenne et al characterizing them as contributing to "oligogenic" obesity [79]. Oligogenic obesity, such as melanocortin 4 receptor(MC4R)-linked obesity, is characterized by a variable severity of obesity, partly dependent on environmental factors and the absence of a specific phenotype. This type of obesity is responsible for 2–3% of obesity in adults and children [79].

Table 3. Genes implicated in monogenic obesity [36, 76].

Gene	Chromosomic position	Inheritance Manner	Obesity-related phenotypes
LEP	7:128241278-128257629	Autosomal Recessive	Morbid Obesity, Hyperphagia, Hyperinsulinemia, Hypogonadotropic Hypogonadism, observed within days after birth
LEPR	1:65420652-65641559	Autosomal Recessive	Morbid Obesity, Extreme Hyperphagia, Hyperinsulinemia, Hypogonadotropic Hypogonadism, observed within days after birth
POMC	2:25160853-25168903	Autosomal Dominant or Recessive	Morbid Obesity, Hyperphagia, Hyperinsulinemia, observed within months after birth
PCSK1	5:96390333-96434143	Autosomal Recessive	Hyperphagia, Hyperinsulinemia, HPA axis disturbance, observed during early childhood
BDNF	11:27654893-27722058	-	Morbid Obesity, Hyperphagia, observed during childhood/adulthood
NTRK2	9:84668375-85095751	-	Hyperphagia, increased weight gain, observed within months after birth
SIM1	6:100385009-100464921	-	Hyperphagia, increased weight gain, observed during childhood
MC4R	18:60371062-60372775	Autosomal Dominant or Recessive	Hyperphagia, increased weight gain, observed during early childhood

Polygenic Obesity

The majority of cases of overweight or obesity are, however, attributed to the cumulative action of a multitude of genes, usually exerting an aggravated impact in the presence of favorable contextual factors such as unbalanced diet or lack of physical activity. Obesity is, thus, viewed as a multifactorial disorder with many an interesting approach attempting to lay the ground for the principles of its understanding. Perhaps two of the most well-known theories surrounding obesity etiology lie in the "Thrifty" and "Drifty" gene hypotheses. The former argues that the existence of genes implicated in the etiology of diabetes survived through the ages via the process of natural selection. In this way, sufficient weight gain and fat accumulation promoted survival in times when food intake or availability were scarce [36,81]. Due to the lack of backing evidence such as the fact that not all people who have survived presented the same "protective" genotype as the "Thrifty" hypothesis would dictate, the "Drifty" hypothesis rose to eminence. The latter argues that the observed increase in obesity prevalence derives from the genetic drift favoring appetite-regulating genes , which cannot be controlled under the process of natural selection [36, 82]. A milestone in the research surrounding the genetic effect on obesity incidence concerns the creation of the Genetic Investigation of Anthropometric Traits (GIANT) consortium [83]. To date, GIANT constitutes the cornerstone of BMI-related genetic research, by accumulating the majority of

genome-wide association studies (GWAS) in large populations from around the world. Evidently, it is found that approximately 40-70% of the variation in BMI levels can be attributed to genetic factors, while the same percentage reaches the 30-60% range when it comes to WHR [83,84].

It is, therefore, arguable that the action of genes participating in energy-regulating metabolic pathways lies in the center of the synergistic procedures taking place for the onset and the establishment of polygenic obesity. As shown in Tables 4 and 5 current literature has identified a multitude of observed SNPs located in key genes affecting the regulation of energy-related hormones. A key gene in the research surrounding obesity onset and gravity concerns the cornerstone fat mass and obesity-associated (FTO) gene. GWAS conducted during the past two decades has demonstrated constituent associations between SNPs located in this chromosome 16 gene and an increased risk for commo obesity, elevated BMI, body fat accumulation [85], cardiometabolic or T2D risk [86] and even cancer onset [87-89]. Despite exhaustive efforts in rodents and humans, the mechanisms by which FTO SNPs contribute to obesity establishment have yet to be fully elucidated [85]. Current evidence suggests that FTO presents significant enzymatic activity, in turn affecting pathways related to energy metabolism. One of its most significant roles discovered to date concerns its potential epitranscirptomic action as a N6-methyl-adenosine (m(6)A) RNA demethylase promoting m(6)A demethylation, which is essential for fat metabolism and adipogenesis [90,91]. Moreover, the FTO-produced protein has been implied to play a part in mRNA regulating factors, therefore centrally shaping RNA transcription and translation processes [90,91]. Recent systematic reviews and meta-analyses have underlined the effect of several FTO SNPs on obesity-related outcomes, namely the rs9939609, rs1421085, rs8050136, rs17817449, rs1121980 [90,91]. Out of those, the former constitutes perhaps the most well-known one, with Ali et al underlining the distinct association between presence of the A risk allele and increased chances for obesity, in their recent 2021 meta-analysis [92]. Table 6 summarizes the properties of the most well-identified, obesity-related FTO variants.

Table 4. List of SNPs in hormonal regulation-related genes and their associations with obesity-related traits.

Hormone	Function	Gene Name	Chromosomic position	Associated SNPs	Alleles	MAF	Associated Index	Effect Allele	Direction of Effect
Ghrelin	Orexigenic	Ghrelin and Obestatin Prepropeptide (GHRL)	3:10285666-10292947	rs143729751	G/T	0.002 (T)	Ghrelin levels	T	Negative (β=-0299)
GRP	-	Gastrin Releasing petide (GRP)	18:59220158-59230774	rs7243357	T/G	0.19 (G)	BMI, BMI-adjusted-for-smoking	T	Positive (β= 0.015 to 0.025)
CCK	Anorexigenic	Cholecystokinin (CCK)	3:42257825-42266185	rs1517037	C/T	0.24 (T)	Adult Body Size	C	Positive (β= 0.009)
				rs8192473	C/T	0.05 (T)	BMI	T	Positive (β= 0.035)
				rs754635	**C/A/G/T**	**0.23 (C)**	**BMI**	**G**	**Positive (β= 0.036)**
				rs10460960	G/A/T	G (0.24)	BMI	A	Positive (β= 0.02)
				rs9839267	**T/A/G**	**G (0.18)**	**BMI**	**T**	**Positive (β= 0.02)**
				rs4377469	G/A/C/T	G (0.27)	BMI	T	Positive (β= 0.04)
				rs75128851	**C/A/G**	**C (0.23)**	**Body Weight**	**G**	**Negative (β= -0.017)**
				rs111768603	G/A/T	T (0.14)	Adult Body Size	G	Positive (β= 0.016)
				rs111768603	**G/A/T**	**T (0.14)**	**Visceral Fat**	**T**	**Positive (β= 0.024)**
				rs8192472	C/G/T	T (0.40)	zBMI	A	Negative (β= -4.85)
PYY	Anorexigenic	Peptide YY (PYY)	17:43952733-44004469	rs116953263	C/T	0.01 (T)	Bone Mineral Density	T	Positive (β= 0.091)
PP	Anorexigenic	Pancreatic Polypeptide (PPY)	17:43940804-43942476						
Insulin	Indirectly anorexigenic	Insulin (INS)	11:2159779-2161221	rs3213225	G/A	0.45 (A)	Birth Weight	A	Positive (β= 0.009)
				rs11564722	C/T	0.33 (T)	HC-adjusted-for-BMI	T	Negative (β= -0.024)
				rs35506085	G/A	0.28 (A)	HC-adjusted-for-BMI	A	Negative (β= -0.027)
				rs4244808	T/A/G	0.35 (G)	HC-adjusted-for-BMI	G	Negative (β= -0.017)

				rs1003484	A/G/T	0.40 (A)	HC-adjusted-for-BMI	A	Negative (β= -0.021)
Leptin	Anorexigenic	Leptin (LEP)	7:128241278-128257629	rs28954105	G/T	0.03 (T)	Leptin Measurements	T	Negative (β= -0.218, 0.049-0.063)
				rs12537573	A/G/T	0.26 (G)	Leptin Measurements	A	Negative (β= -0.06)
				rs791600	G/A	0.44 (A)	Leptin Measurements	A	Negative (β= -0.043 – 0.088)
				rs104878505	G/C/T	0.50 (G)	Leptin Measurements	G	Positive (β= 0.033)
				rs104878505	G/C/T	0.50 (G)	Leptin Measurements-adjusted-for-BMI	G	Positive (β= 0.023 0.033)
				rs791600	G/A	0.44 (A)	Leptin Measurements-adjusted-for-BMI	A	Negative (β= -0.054, -0.118)
				rs17151919	G/A	0.03 (A)	Leptin Measurements-adjusted-for-BMI	A	Negative (β= -0.233 -0.333)
				rs104878505	G/C/T	0.50 (G)	BMI	C	Positive (β= 0.061 – 0.07)
				rs104878505	G/C/T	0.50 (G)	BMI	G	Negative (β= -0.046 -0.055)
Leptin	Anorexigenic	Leptin Receptor (LEPR)	1:65420652-65641559	rs12077336	G/T	0.14 (T)	Leptin Receptor Levels	T	Negative (β= -1.398)
				rs10789188	A/C/G/T	0.30 (A)	Leptin Receptor Levels	G	Negative (β= -0.36)
				rs191246201	G/A	0.001 (A)	Leptin Receptor Levels	A	Negative (β= -1.064)
				rs9436748	G/A/T	0.23 (T)	Leptin Receptor Levels	T	Negative (β= -0.337)
				rs3790438	T/A/G	0.14 (A)	Leptin Receptor Levels	A	Negative (β= -1.37)
				rs34291655	T sequence		Leptin Receptor Levels	CT	Negative (β= -0.34)

	rs183790625	G/A	0.10 (A)	Leptin Receptor Levels	A	Negative (β= -1.32)
	rs11805970	T/C/G	0.15 (G)	Leptin Receptor Levels	G	Negative (β= -1.44)
	rs114123539	G/A/T	0.004 (T)	Leptin Receptor Levels	T	Negative (β= -1.065, 1.20)
	rs6588147	G/A	0.37 (G)	Leptin Receptor Levels	G	Positive (β= 0.314)
	rs143288541	A/G	0.006 (G)	Leptin Receptor Levels	G	Negative (β= -1.094)
	rs17097193	T/C	0.05 (C)	Leptin Receptor Levels	C	Positive (β= 0.381)
	rs72683113	T/C	0.09 (C)	Leptin Receptor Levels	C	Positive (β= 0.341)
	rs145770123	A/G	0.008 (G)	Leptin Receptor Level	G	Negative (β= -0.74)
	rs10889560	C/A	0.16 (A)	Leptin Receptor Level	A	Positive (β= 0.341)
	rs7535099	A/G	0.22 (G)	Leptin Receptor Level	G	Positive (β= 0.345)
	rs174412403	T/C	0.21 (C)	Leptin Receptor Level	C	Negative (β= -0.328)
	rs17415296	C/A	0.10 (A)	Leptin Receptor Level	A	Negative (β= -1.4)
	rs112585178	G/T	0.01 (T)	Leptin Receptor Level	T	Negative (β= -0.97)
	rs12116840	G/A/C	0.19 (C)	Leptin Receptor Level	C	Positive (β= 0.236)
	rs76962533	C/T	0.01 (T)	Leptin Receptor Level	T	Negative (β= -0.03)
	rs11208660	C/T	0.13 (T)	BMI	T	Negative (β= -0.019)
	rs9436303	A/G	0.28 (G)	BMI	G	Positive (β= -0.07)
	rs2767486	A/G	0.31 (G)	BMI (age=3, 6m, 1, 1.5, 3y)	A	Negative (β= -0.12, 0.14)
	rs2767486	A/G	0.31 (G)	BMI (age=3,6,8m, 1y)	G	Positive (β= 0.128, 0.169)

	rs10889551	A/C/G	0.37 (A)	BMI (age=3, 6m, 1.5, 2y)	A	Negative (β= -0.063, 0.088)
	rs9436299	C/A/T	0.36 (C)	BMI-adjusted-for-WHR	A	Positive (β= 0.011)
	rs2767486	A/G	0.31 (G)	Body Size (age=10y)	A	Negative (β= -0.797)
	rs12409877	A/G	0.43 (G)	HC	A	Positive (β= 0.041)
	rs141707226	T sequence		WHR-adjusted-for-BMI	GT	Positive (β= 0.029)
	rs7511672	A/G	0.46 (G)	WHR-adjusted-for-BMI	A	Positive (β= 0.015)

Table 5. List of SNPs in key genes associated with obesity-related traits [36].

SNP	Gene	Chr	Chromosomic Position	Alleles	MAF	Effect Allele	Related Phenotypes
rs2815752	NEGR1	1	1:72346757	G/A/C	G:0.32	A	BMI
rs2568958	NEGR1	1	1:72299433	G/A/C	G:0.32	A	Obesity, BMI
rs6548238	TMEM18	2	2:634905	T/C/G	T:0.12	C	Obesity, BMI
rs7561317	TMEM18	2	2:644953	A/G/T	A:0.16	G	BMI, Body weight
rs6265	BDNF	11	11:27658369	C/T	T:0.20	C	BMI, WC, WHR
rs925946	BDNF	11	11:27645655	T/A/C/G	T:025	T	BMI
rs7498665	SH2B1	16	16:28871920	A/G/T	G:0.26	G	BMI
rs17782313	MC4R	18	18:60183864	T/A/C	C: 0.24	C	Obesity, BMI
rs12970134	MC4R	18	18:60217517	G/A	A: 0.21	A	BMI, WC, T2D
rs52820871	MC4R	18	18:60371599	T/G	G:<0.01	G	Obesity
rs2229616	MC4R	18	18:60372043	C/T	T:0.01	T	Obesity, WC
rs1805081	NPC1	18	18:23560468	T/C	C:0.22	A	Obesity
rs11084753	KCTD15	18	19:33831232	A/C/G/T	A:0.43	G	BMI
rs29941	KCTD15	19	19:33818627	A/G/T	A:0.39	G	BMI

Table 6. List of FTO SNPs associated with obesity-related traits [36].

SNP	Chromosomic Position	Alleles	MAF	Effect Allele	Related Phenotypes
rs9939609	16:53786615	T/A	A:0.34	A	Increased BMI and risk for T2D
rs1421085	16:53767042	T/C	C:0.23	C	Obesity, increased BMI, WC,HC and risk for T2D
rs9930506	16:53796553	A/G	G:0.29	G	Obesity
rs7202116	16:53787703	A/G	G:0.34	G	Increased BMI
rs3751812	16:53784548	G/T	T:0.22	T	Increased BMI
rs17817449	16:53779455	T/A/G	G:0.31	G	Obesity, increased BMI

Obesity Epigenetics

Another pillar in obesity establishment concerns the role of epigenetic modifications. The latter mainly refer to mechanisms surrounding DNA methylation in region rich in C/G bases (CpG sites), histone modifications and the activity of non-coding microRNAs. As it will be further analyzed below, the manifestation of the gravity of epigenetics in obesity prevalence derives from the impact of contextual determinants both in a direct and in an indirect way [93]. It is now established that environmental parameters, such as the quantity and quality of the consumed food, PA, smoking or other lifestyle habits can disrupt circadian regulation, increase DNA methylation rates and affect post-translational histone modifications. Therefore, the impact of nutritional compounds is located in the center of stimulation of epigenetic processes, with literature dictating the vital role of environmental characteristics during the first days of life after conception in the establishment of epigenetic procedures shaping disease predisposition. The Developmental Origins of Health and Disease (DOHaD) approach, highlights that the origins of various types of adult disease can be traced back to endometrial life, with nutrition throughout all stages of

endometrial and early life to have been found associated with different birth phenotypes, which are, in turn, related to environmental adaptations during infanthood and childhood, and, even, metabolic abnormalities or mental health in adulthood [94]. Furthermore, perinatal factors can also affect epigenetic changes. One of the most well-known nutrition-related factors concerns the period of breastfeeding [95]. The benefits of breastfeeding are well-documented with children who have been breastfed to have been consistently showing protective effects in later-on manifestation of inflammatory or cardiometabolic disorders [95]. Data obtained by animals show that human breast milk reduces chance for adult obesity due to its fat components activating the PPARγ receptor which de-methylases the Fgf21 factor; a procedure known to be associates with lower chances for adult obesity [36]. Generally, the epigenetic processes appear associated with the metabolic procedures of the fat tissue and subsequent levels of adipose tissue hormones, such as leptin.

Nutritional practices or macronutrient/micronutrient compounds have also specifically been connected to metabolic procedures via their impact on epigenetic mechanisms [36]. It is interesting that the different compounds can oppositely affect disease risk by increasing or decreasing the same epigenetic processes. Naturally, nutrients such as SFA, sugars or dietary patterns such as the WD have been associated with increased oxidative stress and subsequent inflammation rates, as well as an elevated risk for cardiometabolic disorders and obesity establishment. On the contrary, beneficial dietary compounds such as MUFA, PUFA, fiber, vitamins or dietary regimens like the Mediterranean Diet-MD present protective effects by reducing oxidative stress and preventing metabolic disorders such as insulin resistance, cardiovascular disease and obesity [36].

1.1.5. Metabolomics of Overweight and Obesity

The need to deepen the understanding of the multifactorial obesity etiology led the focus of research to multiple fields related to disease-associated biomarkers' discovery. Recently, studies in the field of metabolomics – the profiling of molecules associated to traits of interest- yielded significant findings regarding indices associated with weight gain. Traditionally, metabolite identification occurs via use of mass spectrometry or nuclear magnetic resonance targeted or non-target metabolomic analyses [96-98] but different methods such as machine learning have been employed for the discerning of novel biomarkers [99].

Presence of obesity or weight gain is imprinted on perturbed metabolic signatures of indices implicated in various protein, lipid and glucose metabolic pathways, such as branched-chain amino acids (BCAA) or short-chain fatty acids (SCFA) breakdown, the urea or Krebs cycles, as well as biological processes involved in inflammation, oxidate stress and immune system or mitochondrial function [100]. By and large, during the generalized disturbed metabolic state that is obesity, a continuous and reciprocal circumstance is observed where increase of one metabolite can cause a cascade of events leading to the disturbance of the production of multiple other substances, in

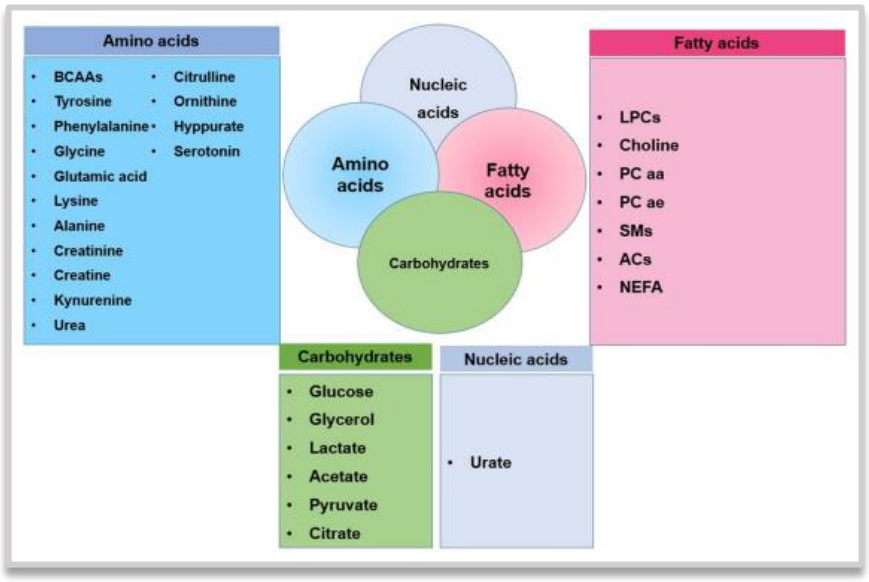


Figure 12. Obesity-related metabolomic biomarkers [101]

turn bilaterally affecting the secretion of the former and vice versa. In a systematic review of obesity metabolomics, Payab et al showed that presence of obesity or metabolic syndrome was associated with elevated levels of several amino acids, namely the leucine, isoleucine and valine branched-chain amino acids (BCAAs), phenylalanine, tyrosine, glycine and glycerol, among other amino acids. The study presented that anthropometric indices such as fat mass and waist circumference measurements were further associated with increase in BCAAs, tyrosine, phenylalanine, alanine and glutamic acid but decrease in glycine and choline (Figure 12) [101]. Furthermore, emphasis was also given on the altered levels of lipids such as lysophospatidylocholines [101].

Similar findings were presented by a different systematic review from 2019 which also highlighted the role of BCAAs and further supported the existence of increased levels of tryptophan, citrulline, acylcarnitines, ketoglutarate, serotonin and lipids like saturated or polyunsaturated fatty acids (SFA and PUFAs) and phospholipids such as sphingomyelins and lysophospholipids. The study additionally underlined the possibly dual role of adenosine, which increases in obesity presence but can ignite a compensatory effect in reducing further obesity establishment [102].

In like manner, Dias-Audivert et al used machine learning techniques to identify novel biomarkers related to weight gain. Their analyses indicated the role of five biomarkers associated with inflammatory and disturbed metabolic pathways, namely: Argininosuccinate, 3- carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), Dihydrobiopterin, a leukotriene metabolite and aprostaglandin (PGB2) [99]. Moreover, Vijay et Valdes underlined the possible effect of gut microbiome- affected

biomarkers, such as SFCA, on metabolic changes during weight gain via implicating key energy-related molecules, such as insulin [100].

The metabolomic profile of obesity has also been specifically studied in younger populations. Handakas et al distinctively presented a collective sample of increased BCAA, phospholipid and steroid levels in children with obesity [103]. The same study also underlined the findings of Lopez-Contreras et al who showed an inverse relation between presence of obesity and levels of the gut microbiome Bacteroides plebeius and Christensenellaceae strains, as well as the negative relations of the latter with the positively-related with obesity phenylalanine levels [103,104]. In like manner, levels of the weight-gain-related and microbial strain-influenced urine trimethylamine N-oxide (TMAO) index were also linked with childhood obesity, where increased amounted were related with higher BMI [102].

Altered obesity metabolomic profiling has also presented differences between men and women, again implicated in multiple pathways of amino acid, glucose, lipid and gut-microbiome-related metabolism [105]. In a sample of adult female participants of the TwinsUK study, Menni et al showed positive associations between 30 metabolites and weight indices, with four out of them maintaining associations in replication analyses in the KORA cohort. More specifically, increased urate, valine and butyrylcarnitine levels were positively associated with weight gain, whereas elevated 3-phenylproprionate presented negative relations [106]. Similarly, results from metabolomic analyses in children from the Childhood Overweight BioRepository of Australia (COBRA) cohort revealed differentiated associations between males and females, with the former showing relations between increased BMI z-score and very low density lipoprotein (VLDL) content [107].

In 2018, Cirruli et al used non-targeted metbaolomics approached to attempt to decipher the metabolomic perturbations observed in obesity indices of participants of the TwinsUK cohort. The study identified a 48-metabolite metabolome signature

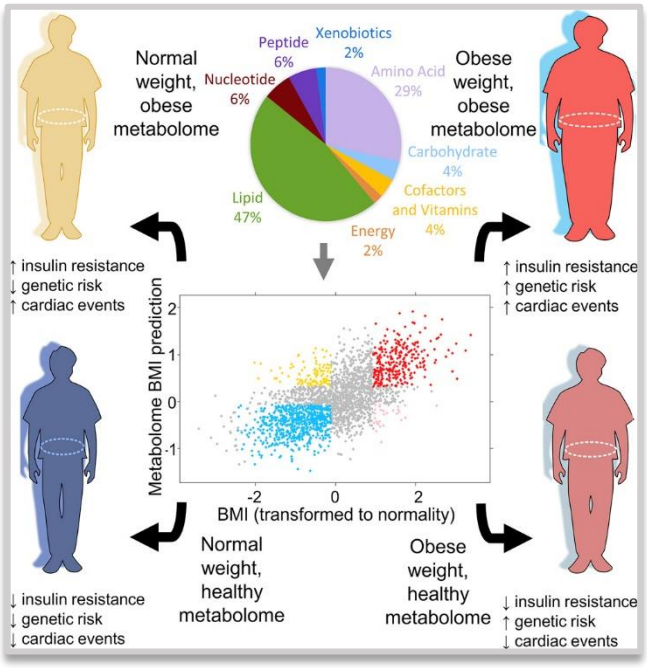


Figure 13. Metabolomic markers and obesity phenotypes [98].

consisting of BCAAs and other substances implicated in DNA and RNA processes to be strongly associated with BMI and BMI-related metabolic factors (Figure 13) [98]. It is worth noting that the percentage of BMI variation explained by a model containing age, sex and the 49-metbaolite signature lied in an impressive 43% [Figure 13) [98]. Overall, these findings could potentially be used as predictors and facilitators of

obesity prevention and treatment and be further combined in investigating the gravity of the effect of lifestyle choices in modifying the magnitude of the associations.

1.1.6. The Role of Lifestyle in Overweight and Obesity

Genetic predisposition, alone, is not always the main reason leading to the establishment of conditions associated with increased weight. Naturally, diet is one of the cornerstones of overweight and obesity development, where positive energy balance and excessive dietary intake of foodstuffs with high fat or sugar content can directly lead to weight gain.

Diet Quality

There are multiple measures used to assess diet quality, primarily based on the macronutrient content of the consumed food groups. Measures such as the Alternate Healthy Eating Index (AHEI); the Diet Quality Index (DQI); the Healthy Diet Indicator, and the Mediterranean Diet Score (MedDietScore) are considered the original, key-indicators for diet quality assessment, consisting of the aggravation of the consumption of food groups (i.e. protein sources, fruits and vegetables, among others) and even micronutrients (i.e. total cholesterol-TC, SFA, PUFAs or MUFAs, among others), to provide a summed score reflecting satisfying or poor levels of diet quality [108]. In their 2017 systematic review, Asghari G et al overall demonstrated that higher score values (denoting better diet quality due to higher consumption of healthier foods) were generally associated with better adult anthropometric measurements, lower weight and increased health status, while also presenting variations in the observed relations based on sex, origin and other individual and lifestyle characteristics [109]. A different systematic review focusing on children and adolescent populations identified 128 pediatric indices used to assess diet quality, with half of them including dietary intakes and increased levels of which were positively associated with better anthropometric, cardiometabolic and cognitive measurements [110].

In general, apart from the use of diet quality indicators, the role of dietary regimens consisting of increased consumptions of high-fat or sugar sources, shows a direct cause-and-effect relationship with increased levels of BMI and higher risk for BMI-related disorders. A characteristic example of increased BMI-related regimens lies in the adoption of the WD. The latter specifically refers to increased caloric intake via intake of “unhealthy”, processed foods with high caloric, fat, sugar and/or sodium content (i.e. red or processed meat, fast food, ready-to-make meals and sweets, among others), along with a simultaneous reduced intake of unprocessed, high-in-fiber and vitamin food groups, such as fruits and vegetables. Studies in both sexes and populations of multiple origins have shown that attrition to WD can be directly associated with higher BMI values, in a dose-responsive manner. In fact, recently, Eng JY et al showed that adults in the highest quartile for adherence to WD showed a 14-fold increased chance of presenting overweight or obesity [111]. In a similar context, analyses identifying dietary patterns have also highlighted associations between patterns including WD components -usually incorporating the term WD in the pattern’s name- and elevated BMI scores or direct existence of overweight or obesity [112].

On the contrary, adherence to diets or dietary patterns comprising healthy, low-fat content foods like fruit and vegetables, appear to be associated with lower risks for overweight or obesity presence. “Prudent” dietary patterns are regularly related with better quality of anthropometric and biochemical measurements in literature, with the most characteristic example referring to the holistic adoption of the MD, the health advantages of which have been extensively described in current literature [113]. MD is characterized by the timely and balanced consumption of all food groups by: i) minimizing the intake of potentially detrimental food stuffs like processed foods or red meat (recommended consumption: one portion per week); ii) centrally including the frequent consumption of vitamin and micronutrient-rich groups, such as fruits and vegetables (recommended consumption: three portions each per day), grains (recommended consumption: two portions per week) or low-fat dairy (recommended consumption two portions per day); iii) focusing on the consumption of natural ingredients with better quality of fat and micronutrient sources, such as olive oil and nuts (PUFA, MUFA and micronutrient source) instead of butter (source SFA and trans-FA), or chicken and fish (recommended consumption: a minimum of two portions per week) instead of meat; and iv) focusing on minimizing the consumption of high-sugar, processed or fast foods to few times per month. MD is, therefore, largely considered one of the most metabolically beneficial food regimens, not only due to its balanced macronutrient content, but also due to its sustainable nature which renders is easy and efficient to adopt. Accordingly, in their 2020 “umbrella” review, Seifu et al demonstrated that systematic reviews showed MD being inversely associated with obesity presence and better diet quality being associated with lower risk for increased weight or BMI and/or weight gain [114].

Furthermore, apart from the MD, other beneficial regimens have also been reported in literature, where it is natural that diets emphasizing on the consumption of high quality, unprocessed food groups and the restriction of high-calorie foodstuffs present positive associations with better weight status. Koutras et al emphasized that dietary patterns with increased content of unprocessed sources were related with better adult weight status, and the adoption of a “lacto-vegetarian” pattern was associated with reduced long-term weight gain and more steady weight maintenance [115]. Additionally, a 2022 systematic review by Jarvis SE et al underlined the protective association between plant-based diets and weight gain, with the effect more strongly shown for plants of better overall quality [116].

The Obesogenic Environment

Apart from the role of individual choice in the formation of dietary habits, the latter is further largely influenced by other environmental factors pertaining to the formation of diet and weight-related daily patterns. The term “obesogenic environment” is used to describe contextual/ environmental circumstances determining nutrition and other weight-related parameters in a way which promotes unbalanced dietary intake and reduced energy expenditure, thus aiding overweight and obesity establishment [117]. Nowadays, establishment of obesogenic environments is multifold and partly attributed to globalization trends which have led to the massive commercialization of WD in the majority of developed countries, allowing for the creation of new eating styles including both Western characteristics and country-specific patterns. By familiarizing more populations with the principles of

high-fat and sugar consumption observed in the context of WD, both local and global diet quality reduces, subsequently strengthening the further establishment of obesogenic contexts. Moreover, obesogenic circumstances are further promoted due to financial aspects shaping local food environments, for example via reduced accessibility to high-quality, expensive food, versus the high and easy accessibility to low-cost fast food or ready-to-make meals, in neighborhoods with low levels of socioeconomic status (SES) [118]. Especially in populations such as children and adolescents who are both in need of high-quality foodstuffs to ensure proper development, but also sensitive to the effect of external stimuli, the effect of food accessibility and promotion has been multiply demonstrated. Living in neighborhoods with easier access to super-markets, rather than fast-food restaurants was associated with lower z-BMI levels in children [119].To boot, Osei-Assibey et al showed that individuals of those age groups are most susceptible to exposures concerning portion sizes, sugar-sweetened beverages and food promotion [120], while Goncalves et al, showed the importance of food exposure in the school context on the formation of adolescent weight status [121].

Obesogenic circumstances can further affect weight status by pertaining to other health parameters like the natural environmental context via air pollution or sunlight exposure, with Wilding et al showing that local residence greenspace observed at the time of birth was negatively associated with the chance of presenting overweight or obesity at ages 10-11 [122]. Although lack of measure uniformity and built environment diversity across different countries hinder the creation of a conclusive body of evidence to support direct causal relationships between environment and obesity [123-125] contextual factors such as urban-sprawl and the built-in environment appear to be able to influence and aggravate the obesogenic effect [124,125]. The latter is, thus, considered to be able to affect lifestyle parameters such as PA; however, it appears that personal choice can reverse potentially negative effects, in cases where individuals make conscious attempts to overcome physical obstacles and adhere to healthier lifestyle routines.

Physical Activity

PA habits constitute another distinct pillar in the formation of weight levels, with a standard recommendation of 20-30 minutes of moderate physical activity provided to sustain good health [126]. As expected, due to the provoking increase in energy expenditure, recreational PA or PA not aiming at muscle mass increase, is directly inversely associated with body fat and obesity presence [127]. Either alone or when combined with increased caloric intake in the context of generalized lower level of life quality, reduced levels of physical activity or exercise (defined as a minimum of 10 continuous minutes of conscious physical activity) can lead to weight gain and aggravate overall individual health status. The beneficial effects of PA on overall quality of life via improvement of health status are even seen in individuals with increased weight, where PA is generally recommended or recreationally conducted, without being necessarily associated with weight loss purposes. A recent systematic review by Pojednic et al, showed that physical activity in people with obesity can present beneficial physiological, cardiorespiratory, cardiometabolic and immunological effects, irrespective of observed weight loss [128]. Similarly, Soares et

al showed that recreational PA is associated with amelioration in body composition of children and adolescents with overweight or obesity [129].

Smoking Habits

A different aspect pertains to individuals' smoking habits, the negative effects of which on overall health status, disease risk and mortality rates are already well-known [130]. As evidenced by current literature, smoking and lifestyle are involved in a bidirectional relationship, where unfavorable contextual determinants (eg stress or other psychological conditions) can promote or increase smoking practice, which can, in turn, influence the quantity and quality of food intake or PA. Smoking directly impacts metabolic regulation through nicotine's effect on homeostatic and hedonic pathways related to food intake [131]. Smoking affects the secretion of appetite hormones, in ways such as increasing leptin and down-regulating ghrelin production [132]. In their 2019 review, Chao et al showed that adult tobacco smokers tended to present higher levels of BMI compared to individuals who did not smoke, whereas smokers presented a significant weight gain after proceeding to smoking cessation, due to the observed metabolic dysregulations [132].

Sleeping Habits

Subsequently, the cumulative impact of such unfavorable habits can promote more unhealthy lifestyle routines, which can, in turn, further enhance the establishment of metabolic dysregulation related to obesity phenotypes. One example of this pertains to sleep quality and habits, where we observe a reciprocal interplay between sleep irregularities and body weight outcomes. As previously mentioned, sleep plays a major part in sustaining metabolic functions, as circadian rhythms are directly related to energy metabolic pathways [37]. Sleep disruptions such sleep deprivation or decreased quality of sleep can result in disruptions in neuroendocrine functions, hormonal and glucose metabolism, directly increasing inflammatory stress and cardiometabolic risk [133]. In their 2018 review, Cooper et al showed that adults sleeping for less than 7 hours per night presented higher chance of having increased BMI and obesity, compared to the ones who slept more [134]. On the other hand, increased weight can also affect the quality and/or quality of sleep. In the most characteristic manifestation of the observed bidirectional effect, individuals with increased weight are more likely to show symptoms of sleep disorders, the most noteworthy one being obstructive sleep apnea (OSP) [135]. Weight loss is subsequently the primary modifiable factor considered for amelioration of the disorder due to the profound impact of the observed fat accumulation and accompanying inflammatory response to the symptoms of the disease [136,137]. Proper sleep habits are, therefore, situated in the center of an overall good health status. Notably, regular sleep habits are central in maintaining well-regulated metabolic pathways even in the presence of increased body weight. This can, in turn, even be essential in predicting the outcome of success in efforts related to amelioration of cardiometabolic risk, eg dietary interventions for weight loss. Therefore, it appears that it is rather a holistic unhealthy lifestyle approach consisting of the interplay of multiple determinants which aggravates the establishment of increased weight, than the sole effect of separate risk factors. The impact of this

interaction subsequently extends to additional parameters of life quality, the worsening of each can reciprocally further aid the disruption of lifestyle habits pertaining to more weight-related metabolic alterations.

1.1.7. Gene-lifestyle Interactions in Overweight and Obesity

In light of the aforementioned, literature has focused on the combined effect of gene-environment interactions (GEI), with special focus on the modifying or mediating effect of the obesogenic environment on the penetrance and expressivity of genes associated with obesity susceptibility. A growing body of evidence has, therefore, focused on highlighting the role of GEI in obesity and cardiometabolic phenotypes. More specifically, studies have focused on the effect physical activity habits or gene-diet interactions in obesity-associated phenotypes. Although a positive energy balance and an excess in the dietary intake of foodstuffs with high fat or sugar content can directly lead to increased weight, it is further their subsequent interplay with a favorable genetic background that ultimately leads to establishment of weight disorders, as well as determining their respective gravity. The field of nutrigenetics investigates the impact of the genetic factor on the metabolic and other responses to food intake. In similar context but on the opposite side, the term nutrigenomics is used to describe the field specializing in unraveling the modifying role of nutritional components in gene expression.

In 2011, Kilpelainen et al used data from approximately 218.200 adults to conclusively demonstrate that presence of physical activity successfully attenuated the favorable effect of the rs9939609-A allele in increasing obesity risk [138]. Similar findings were reported by Xi et al for five obesity-related variants (i.e. rs7138803, rs1805081, rs6499640, rs17782313, rs6265), where the effect of which tended to be more evident in children with low versus children with increased PA [139]. An early 20212 review by van Vliet-Ostaptchouk et al highlighted a multitude of GEI interactions, with emphasis of the effect of macronutrient intake or physical activity habits [140]. Unhealthy lifestyle choices including a sedentary way of living or increased energy or fat intake were associated with increased risk for obesity in carriers or BMI- or fat raising alleles of variants in genes such as the ADRB2 and PPARγ ones [140]. Moreover, interactions between fat intake and obesity-favorable genotypes in the apolipoprotein genes were further associated with obesity phenotypes [140]. In 2009, Sonestedt et al used data from 4939 participants of the Malmo Diet and Cancer study to examine FTO gene-diet interactions in BMI levels. The study found that an obesity-favorable FTO genotype was not associated with BMI in participants who reported lower fat intakes versus the ones who reported higher ones. Additionally to that, it was further shown that the genetic impact was more evident to individuals with lower rather than the ones with higher PA levels [141].

Qi et al attempted to shed a light on the potential impact of sugar sweetened beverages on genetic predisposition for obesity. Data from almost 11.000 participants showed a stronger impact of a 32-SNP GRS for BMI in obesity presence when reporting higher consumption of the beverages [142,143]. In 2015, Kontinnen et al investigated the effect of appetite-dictated tendencies on the effect of genetic susceptibility for obesity using data for approximately 5900 individuals from the Dietary, lifestyle and genetic determinants of obesity and metabolic syndrome Study and the FinnTwin12

Study. The study showed that the effect of a 90-SNP GRS for BMI was partly mediated by emotional or excessive eating [144,145]. Similarly, Brunner et al showed that disinhibition mediated the effect of a 92-SNP GRS for BMI in the 5-year BMI trajectories of 2464 adults [145, 146]. In 2023, Nakamura et al accordingly showed a significant interaction between a 76-SNP GRS for BMI and SFA intake in increasing the risk for obesity presence [147]. In like manner, in their 2019 review, Ahmad et al conclusively demonstrated the modifying effect of the dietary component on the genetic susceptibility to obesity in populations of South Asians [148].

Gene-diet interactions can also have effects on various characteristics of quality of life. GWAS on characteristics of sleep quality and quantity have identified various loci presenting strong associations. A 2016 meta-analysis of GWAS on sleep duration and morningness, investigated previously shown associations with genetic variants and further identified novel loci for both traits [149]. Another 2016 GWAS identified various genetic loci associated with sleep duration, insomnia and sleepiness during daytime, as well as loci related to more than one sleep quality characteristics [150]. A different GWAS by Lane JM et al, in 2019, further identified 57 loci related to insomnia [151]. Other studies have highlighted that gene-sleep interactions may attenuate genetic susceptibility to obesity when favorable sleep habits are present [152]. In the same context, depression symptoms have also shown a positive association with overweight/obesity existence [153] and a reciprocal interplay between the two has also been suggested [154]. GWAS and meta-analyses of GWAS on depression symptoms, have shown associations with more than 80 replicated genetic loci [155] and associations with 102 independent variants, respectively [156]. GWAS have investigated relations of genetic loci, namely, with stressful life events [157] and bipolar major depressive disorder [158].

Figure 14 summarizes the cascade of events followed by macro- and micronutrient intake, placing diet in the center of the interactions with other factors affecting metabolic pathways. Gene-diet interactions are simultaneously implicated in inflammatory, metabolic, epigenetic and gut microbiome-related procedures, ultimately modifying the risk, onset and gravity of cardiometabolic and inflammation-related disorders. The role of nutrigenetics and nutrigenomics is, therefore, deemed increasingly essential in both understanding disease etiology but also further proceeding to the creation of prevention and treatment strategies [159]. When discussing the role of gene-diet interactions in complex disease, Qi et al concluded that the collection of nutrigenetic data would enhance healthcare via means of identifying high risk individuals and/or individuals who would differently benefit from the adoption of various dietetic approaches. Qi's study concludes by emphasizing the encouragement of the National Human Genome Research Institute in putting the collection of genomic information in the center of strategies concerning behavioral interventions [160].

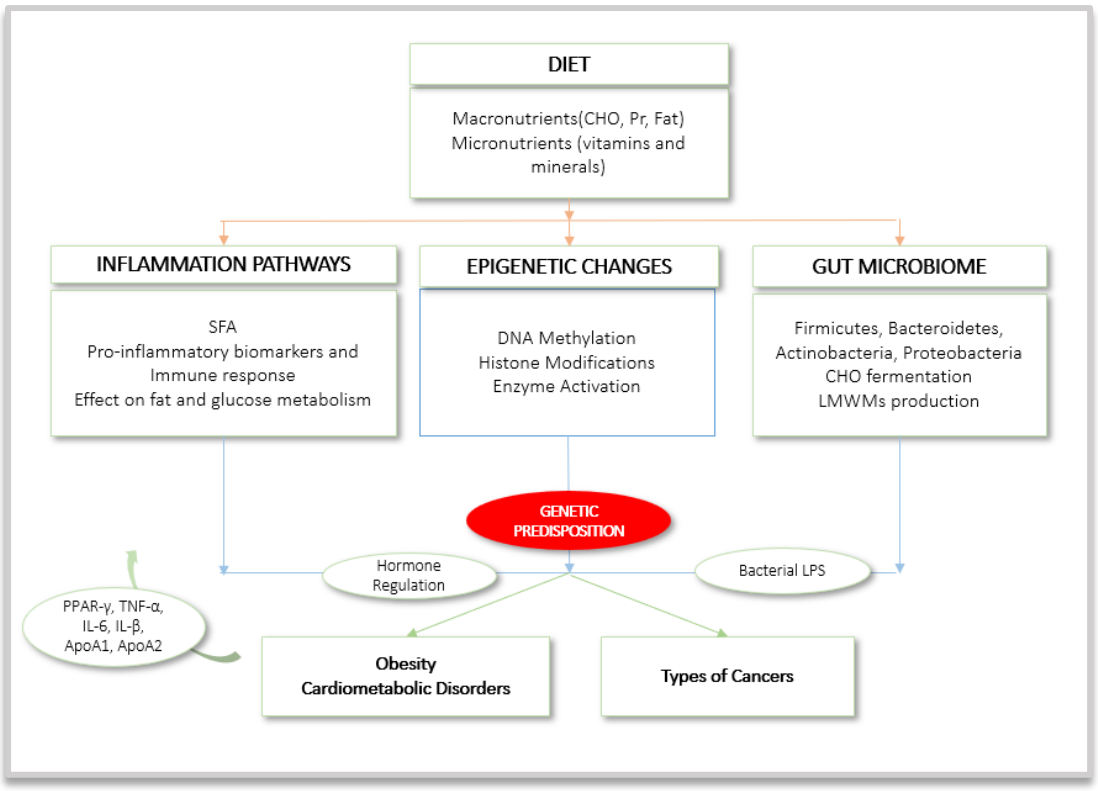


Figure 14. Basis of gene-diet interactions based on current literature [36].

1.1.8. Genetic Risk Scores in the field of Weight Management

The following information in 1.1.8. constitute information published under the publication. J Atherosclerosis Prev Treat. 2023; Jan-Apr;14(1):35-43. doi.: 10.53590/japt.02.1045 and can be found in Appendix A.

Deciphering disease etiology by quantifying the impact of genetic predisposition constitutes the focal point in the conduct of research surrounding genetics during the last years. Identifying and investigating the effect of disease-associated single nucleotide polymorphisms (SNPs), as well as using them to create aggravated genetic scores, provided encouraging results in the field of cardiovascular (CVD) and cardiometabolic disease [161,162]. Those findings shed a quantifiable light on the role of genetic makeup while expanding the horizons for the potential creation of new and personalized treatment approaches. The construction of polygenic risk scores (PRSs) thus quickly expanded to the notion of potentially contributing to determining disease risk and subsequently contributing to effective disease prevention, diagnosis and even treatment [161-163]. The need for more extensive research resulted in the gradual evolution of continuously enhanced methodological approaches for PRS extraction [164]. As the latter examine the effect of multiple variants on the outcome of interest based on a large SNP pool in populations of increased size, their creation and use were extensively investigated through genome-wide association studies (GWAS) in large consortia. The increasing presence of PRSs for multiple phenotypes in the current literature ultimately led to the creation of PGS catalog; an inclusive database comprising of all PRS entries created to date [165].

Discussion and research around PRS use as a prediction and treatment tool has recently yielded encouraging results, with studies reporting beneficial effects in

cardiovascular and cardiometabolic disease [161,1622]. Provision of lifestyle recommendations appeared to significantly contribute to obesity treatment [2] and coronary artery disease (CAD) prediction and greatly benefit individuals with high PRS across the spectrum of CVD, with PRSs even constructed for stroke and hypertension [161,163]. In like manner, the American Heart Association recently focused on the potential utility of PRS in CVD and other cardiometabolic disorders such as type 2 diabetes (T2D), underlining the need for the conduct of additional research to strengthen PRS inclusion in current practice [162]. Subsequently, discussion around the integration capacity of PRSs as a way to promote precision medicine and personalized nutrition is ongoing, with special attention to ameliorating relevant challenges, namely the differentiational influencing capacity following interaction with environmental stimuli, the diverse methodological approaches in PRS extraction and the understanding of the true meaning of genetic information both from professionals and patients alike.

PRS and weight-related parameters

Evaluation of genetic risk in the form of summed risk scores primarily treated CVD danger but quickly expanded to other disorders of cardiometabolic profile [162]. The conduct of extensive GWAS was accompanied by the development and expansion of the GIANT consortium [83]. This led to the identification of multiple Body Mass Index (BMI)-associated loci with the milestone discovery of the first 97 loci accounting for about 2.8% of the marker's variation [166]. Nowadays, approximately 6% of BMI variance is explained by 785 near-independent genome-wide significant SNPs [8,9]. Thus, the beginning approaches of quantifying genetic predisposition mainly involved the literature-based, a priori selection of disease-related variants and the subsequent investigation of the impact of their added effects. Therefore, various genetic risk scores of tens of SNPs were created and used in the examination of associations between increased genetic risk and disease manifestation or severity. In like manner, research on personalized approaches for combatting cardiometabolic and weight-related disorders primarily focused on examining the combined effect of target SNPs with different dietary regimens. In this context, the first large initiatives such as the FOOD4ME project and the POUNDS lost clinical trial [167,168], attempted to unveil the interactive role of genetic makeup and nutritional habits in overweight and obesity. Focusing on target SNPs and macronutrient content, the projects provided limited, but encouraging, evidence on the effect of gene-diet interactions on anthropometric traits.

Based on GIANT-derived information or the conduct of independent GWAS, different teams proceeded to the development of PRSs for BMI in populations of various sizes. To date, PRSs associated to anthropometric traits and body measurements account for 154 of the database entries [165]. Indeed, nowadays, attempting to decipher the multifactorial obesity etiology using genetic information has become central in research surrounding BMI, with efforts made to explain the polygenic prediction of weight formation throughout the life course [169,170] Khera et al. highlighted the role of including a multi-variant PRS in explaining weight variance in populations ranging from birth cohorts to middle-aged individuals [169]. Correspondingly, Shi et al recently constructed a different BMI PRS to investigate potential associations with overall cardiometabolic health from early age to

adulthood. The study revealed significant associations between the score and other indices of cardiometabolic profile, namely fasting glucose and systolic blood pressure [170]. Building on the data and the role of genetic makeup in overweight or obesity presence, current research also focuses on the potential influence of genetic markers on weight loss. A study by de Toro-Martín investigating the extent of the genetic effect on the success of bariatric surgery, showed an increase in the prediction model accuracy when including PRSs, as well as significant interactions between the scores and the reduction in post-surgery recovery and surgery type [171]. In the same context, Katsareli et al showed that adults with increased genetic risk score for obesity noted a decrease in post-bariatric surgery loss of excess weight, with each unit of the score being associated with a 4.618% decrease in the 12-month observed weight loss [172].

In the same spectrum and building on the findings of previous key projects, emphasis should also be given on studies looking into the potential interactions between genetic scores and macronutrient content [112]. Moreover, studies focusing on the genetic influence on the observed weight loss after lifestyle interventions to combat overweight and obesity even outside of a clinical environment are also needed. Research on this field could unravel the gene-diet interactions surrounding weight management and loss and ultimately maximize the impact of individualized recommendations using genetic data to determine optimal treatment strategies. As a result, effectively unravelling the genetic proportion of body weight variance could progressively allow for the formation of more inclusive strategies to its management.

PRS Interactions with Lifestyle Determinants

In addition to accounting for the risk attributed to genetic makeup, the impact of PRS interactions with lifestyle factors such as diet, ultimately influencing weight management have also been studied. In a 2021 study by Wang et al, a 60-SNP PRS was constructed using variants found to be associated with birth weight and later-life disease. The interactions between the genetic score and dietary parameters showed that healthy habits during early life, such as breastfeeding, were beneficial in reducing the risk for worse lipidemic profile in adult life in participants with higher genetic risk [173]. The significant modifying effect of diet was also demonstrated by Tan et al, who showed that individuals with higher PRS for obesity indeed presented higher levels of C-reactive protein but those levels appeared reduced in the presence of high dietary protein intake [174]. Similarly, middle-aged individuals with a higher genetic risk score for thinness presented lower body weight; an association aggravated with high protein and low carbohydrate intake, among others [175]. The multidisciplinary character of genetic risk-associated interactions is evident throughout the reciprocal interplay between the formation of anthropometric characteristics' levels and the formation of the lifestyle choices surrounding them. In adult populations, Dashti et al. showed that adults with higher genetic risk for obesity were less likely to make healthier food choices at workplace and more likely to purchase more food and adhere to unhealthy dietary habits such as delaying or skipping breakfast and homemade meals [176]. However, Lee et al showed that BMI PRSs were related to body weight in Korean adults, but not to their respective caloric or macronutrient intake [177]. Similarly, Konttinen et al highlighted that elevated genetic risk was more

correlated with increased weight gain during a 7-year period in individuals not demonstrating restrained eating than those who adhered to it. However, the study attributed the effect to the role of previous processes entailing weight gain and nutritional habits, rather than a separate factor which would influence future weight gain [178]. Extended associations have also been explored, with Park et al showing that individuals with a high genetic risk for BMI, early menarche and attrition to an unhealthy diet (i.e. high consumption of fried foods and low consumption of fruits and vegetables) presented an increased obesity risk compared to those with late menarche and attrition to a healthier diet [179]. A different study focusing on European children and adolescents, underlined the modifying effect of diet, where genetic influence was attenuated by fiber intake in participants presenting higher genetic risk for obesity [180].

To boot, PRS-lifestyle interactions constitute a focal point across the spectrum of understanding more weight-related diseases. The emphatic effect of nutrition is underlined in studies of approximately 70000 participants of the UK Biobank, where adherence to a healthier diet was associated with reduced risk for cardiovascular disease, even in individuals with a high genetic risk score. Similarly, adoption of a healthier lifestyle was linked to lower CVD risk and overall mortality, again irrespective of genetic danger. [181,182]. Moreover a different large study with data for almost 340000 UK Biobank participants showed that increased genetic risk for type 2 diabetes (T2D) was associated with higher chances for CVD manifestation; an effect reduced in individuals with better quality of lifestyle [183]. With regards to T2D alone, increased values of a PRS for the disease and attrition to the Western dietary pattern were associated with higher levels of fasting glucose [184]. Likewise, López-Portillo et al demonstrated that fasting glucose levels were higher in non-diabetic individuals with increased genetic risk for T2D and higher consumption of sugary beverages, compared to those with lower genetic risk scores and reduced intakes of the latter [185]. Biochemical interactions have also been studied, where PRS for T2D have been found to significantly interact with triglyceride and cholesterol levels in the subsequent formation of fasting glucose levels [186]. Merino et al showed the dominating effect of unhealthy diet in increasing T2D risk even by 30%, again irrespective of genetic risk [187]. Additionally, although Zhang et al did not show significant interactions between genetic risk and adherence to the plant-forward EAT-Lancet diet for T2D onset, their study did note that individuals with increased genetic risk and lower attrition to the dietary pattern did present the highest risk for T2D presence during a 24-year follow-up period [188]. Correspondingly, PRS-diet interactions have been evident in more disorders, such as cancer and dementia, where an increased diet quality lower the chances for disease onset, even in individuals of high genetic risk [189-191]. In a similar context, lifestyle can also indirectly affect the gravity of genetic risk on actual disease manifestation via increase in weight-related anthropometric measurements alone. Esteve-Luque et al showed that higher values of BMI significantly interacted with genetic risk in increasing triglyceride levels and the subsequent risk for hypertriglyceridemia [192]. A different study underlined that obesity presence led to higher risk for T2D, even in individuals with lower genetic risk and better lifestyle quality [193].

PRS Utility in Personalized Recommendations

Research around the potential role of PRS use in clinical practice has shown that inclusion of PRSs in models for cardiometabolic disorders such as cardiovascular disease (CVD) can account for risk prediction in a manner similar to established contributing factors such as cholesterol levels [194-196]. The Task Force of the International Common Disease Alliance has further underlined the importance of PRS inclusion in increasing the accuracy of predicting CVD disease risk and severity, throughout one's lifetime [197], and the weighted contribution of PRS to maximizing patient outcomes [197]. Given the potential increase in accuracy observed in prediction models after the addition of PRS, testing their potential utility has also expanded to the field of anthropometrics. Choe et al showed that a BMI PRS was associated not only with longitudinal BMI change, but also with other cardiometabolic phenotypes, such as fatty liver [198]. A similar attempt was made by Padilla-Martinez et al., who displayed significant associations between PRSs for T2D and obesity and manifestations of prediabetes and other disrupted cardiometabolic parameters [199].

In this context, PRS use could be seen as a useful tool to increase disease prevention through successful prediction and/or early detection. This notion carries both favorable effects for public health and financial parameters of healthcare systems, as well as optimizing individual understanding and ability to choose and decide optimal combatting strategies [200]. Although the inclusion of PRSs and relevant interactions can explain cardiometabolic disease risk [45], the conversation around its clinical validity underlines the importance of real-time context on PRS information evaluation and decision-making in order to avoid confusion with genetic determinism [200,201]. This sheds a light on the vital role of both development of valid methodologies to increase PRS reliability, transferability and accuracy, as well as the professionals' familiarization with the interpretation of its information. This is also why the education of healthcare professionals is put in the center of integrating genetic information into daily practice.

Furthermore, taking PRS information into account can prove beneficial on its own accord in patients with extremely high genetic risk [201] and, thus, PRS utility is also discussed at personal level [38]. PRS information can be differentially valuable to each individual, according to both their personal interest and understanding of the information, as well as relevant genetic risk in outcomes of interest. The latter might not always correlate to matters of clinical importance, but do account for increasing awareness on genetic predisposition for various matters significant to the individual. It is therefore why, a reliable approach to PRS calculation for various traits, with easily understandable and interpretable results is central in future research surrounding PRS use [195]. Especially in cases regarding cardiometabolic disorders such as overweight, obesity and type 2 diabetes, finding ways to efficiently include PRS prediction in easily applicable risk tools is considered a priority for the maximization of PRS efficacy.

Challenges in PRS Construction and Interpretation

Although inclusion of PRSs in disease prognosis can be beneficial, several considerations arise when discussing the methodological aspect of PRS construction, the efficacy of the various PRS development methodologies presented in current literature and the real-time interpretation capacity in clinical and non-clinical settings. Firstly, the fundamental limitation of PRS' universal application concerns the underrepresentation of data used from populations of different genetic ancestry

[200]. To date, although several attempts for PRS construction using data from various populations have been made, PRSs presented in literature mainly focus on European ancestry. The lack of existent PRSs deriving from large cohorts of global populations affects their translational capacity in less frequently examined populations where contextually phenotype-associated variants, SNP linkage disequilibrium (LD) or allele frequency may vary. Therefore, a preceding necessity for developing more PRSs using data from populations around the globe is formed before discussing their maximum use, in order to ensure universal application capacity.

Another pillar of PRS development refers to the biases of the different methodological approaches undertaken in calculating the scores [200]. Diverse current practices consist of: i) the replication of simple aggravations of the risk-alleles for phenotype-associated variants using their respective effect sizes from current literature (i.e. consortia such as the GIANT one or data from large studies such as the UKBiobank [46] or the Twins Early Development Study -TEDS [203]) ; and ii) the conduct of novel GWAS in populations of sufficiently large sample sizes, extraction of summary statistics, subsequent identification of phenotype-associated variants and their risk alleles' aggravation in a holistic score. As PRS development and phenotype examinations are ongoing, research may simultaneously focus on the identification of novel phenotype-associated variants and the replication of previously identified ones. As a result, the statistical design and assessment may significantly differ across studies and the final choice for the optimal model to be used may lie in the discretion of the researcher according to the needs of the research question at hand. Additionally, differences in samples sizes significantly matter in effective PRS validation. Although the effect of using target-SNPs outside of reference populations can be limited, current discussion around the role of population size has shown that cohorts with a few thousands of participants can be of use in replicating results and using SNPs from PRSs deriving from even larger populations [204]. Moreover, the additional variety in statistical methods (i.e. p-value thresholds, clumping, Bayesian or lasso-based penalization), packages (eg PRScs, LDpred2) and assessment applied can largely affect the end product which may be ultimately differentiated across studies. It is therefore highlighted that standardization of the PRS extraction process [205] is central to facilitating their validation and sequentially increasing their predictive ability. Additionally, in this context, attempts to practically compare PRS results and methodology [164, 203-205] can provide useful data for the next steps in the need for a unified, applicable approach to allow for PRSs capable of yielding rapid but reliable results and effective comparisons of findings between populations of different characteristics.

Moreover, familiarization with the true meaning deriving from the information of the PRS is vital in its correct interpretation. Understanding the potentially indirect effects of SNPs included in the models and weighing the environmental factor in are key considerations in constructing future PRSs as reliable disease prediction risk tools. Apart from the technical aspects, a different cornerstone of practical PRS use appertains to the familiarization of healthcare professionals with the field. Proper assuefaction with the practical meaning of PRS information is critical for professionals to address disease risk and convey the appropriate message to patients. The delicate understanding of individual risk and its practical meaning in ultimate disease manifestation can be challenging in cases where the risk is small or the patient is not

properly acquainted with the details of their genetic profile. As a result, both professional and patient education and perceptions around PRS utility are integral in its successful use as a disease screening and treatment tool [196,200].

PRS and Nutrigenetics/Nutrigenomics in Future Healthcare Practice

Although there is a limited number of studies investigating and discussing the extent of PRS effective translation to date, future directions can be encouraging on the incorporation of PRS methodologies in the daily practice [161-163]. PRS inclusion in disease screening and the formation of personalized recommendations could potentially offer a solution to the growing pressure applied to healthcare systems for more inclusive strategies and efficient use of financial resources [49]. In the field of nutrigenetics (i.e. the impact of SNPs on certain nutrient interaction or role in metabolic pathways) and nutrigenomics (i.e. the impact of nutrients on gene expression), PRS use can be considered as a promising tool in the advancement of personalized nutrition.

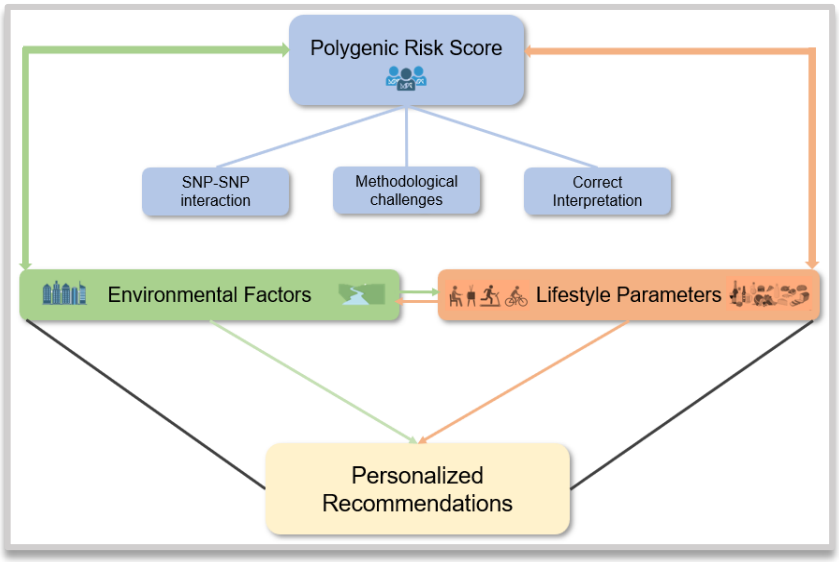


Figure 15. Polygenic Risk Score (PRS) in Personalized Recommendations (created with BioRender.com).

Understanding the connective links between research conduct and translation is substantial in order to be able to reinforce PRS practical use. An integral part to such an effort would be the effective translational communication between bioinformatics and healthcare sectors in order to enhance proper PRS use and interpretation [205]. Especially when referring to the use of PRSs in cardiometabolic and weight-related disorders, understanding, quantifying and translating the contribution of genetic predisposition is vital in interpreting genetic impact. Incorporating genetic information in medical and nutritional advice can maximize the success of the proposed strategies, while informing the individuals in main aspects of their genetic profile. In this spectrum, PRS interpretation in weight-related disorders can only be effective when conducted and evaluated alongside the effect of other lifestyle determinants (Figure 15). This can allow for increased motivation on behavioral change and lifestyle adaptations [197] to the proposed measures, which can subsequently strengthen the disorders' effective management.

In an attempt to dissect the steps of including genetic details in current practice and promote personalized nutrition, in 2022 the Academy of Nutrition and

Dietetics published the creation of a *Nutrigenomics Care Map* specifying the timeline of nutrigenetic information integration in nutritional assessment [209]. The map puts professional formation on the forefront of the practice, by inserting the sufficient nutrigenomics training prerequisite as the first out of the four steps of the process. Patient screening, genetic testing and communication of genetic profiling results as part of the nutritional assessment and the setting of SMART (specific, measurable, attainable, relevant and time-based) goals complete the suggested procedure [209]. Such an approach aims to maximize nutritional consulting by actively involving the patient in the formation of goals and dietary regimens optimally corresponding to their genetic profile. Integration of PRSs in this effort could allow the practice to move forward from personalized advice provided only based on specific genotypes of key genes associated to body weight or obesity [210,211]. As a result, more research in the form of Randomized Clinical Trials (RCTs) is needed, regarding the interactions between BMI PRSs and dietary regimens in order to establish the evidence-based approaches required for the nodes of individualized advice. Such efforts would subsequently enhance our understanding and forming of optimal recommendations, each-time targeting the outcome of interest and adopting the literature-based, corresponding strategy (eg advice on adherence to a dietary regimen of specific macronutrient content for the achievement of weight loss in individuals with specific PRS for obesity). Due to the current increase observed in the offer of nutrigenetic services, establishment of scientific, quality guidelines for directing healthcare professionals is vital [212].

Furthermore, on principle, the meaning of PRS information differentiates itself according to the nature of the disorder in reference. For example, a PRS will be differently interpreted in cases of monogenic rather than polygenic diseases, such as the cardiometabolic and weight-related ones. The multidisciplinary character of those disorders therefore reciprocally affects the creation of the appropriate framework in which it will be communicated. This interplay between genetic information communication and healthcare setting factors centrally affects both the formation and the influencing capacity of public health policies in precision medicine and nutrition [194-196]. The latter, thus, re-enforces the need for sectors simultaneously operating on unravelling the relations between the creation, interpretation and communication of genetic information across healthcare professionals. These could, in turn, be incorporated into screening tools for multiple traits and contribute to the creation of individualized disease prevention or treatment strategies.

Future incorporation of PRS information in the daily healthcare practice could present considerable advantages to advancing precision medicine and personalized nutrition. Creation of sound methodologies, accounting for the extent of the impact for environmental stimuli and simultaneously able to allow for the effective inclusion of PRS results in disease prediction, diagnosis and prognosis is deemed vital in bringing PRS research and application forward. PRS information on cardiometabolic and weight-related disorders can increase the prognostic validity of already existent tools and the fruitful formation and implementation of individualized recommendations. However, sufficient familiarization of healthcare professionals with the meaning and contextual translation of PRS results plays a major part in its proper communication where attention must be given in the role of the interactions between SNPs, environment and lifestyle determinants in ultimate disease manifestation. Future

initiatives should aim at uniformly enhancing both methodology development and educational formation in attempting to firmly establish, integrate and distribute PRS use as a daily practicum.

1.2. Connective dots between body weight and cardiometabolic risk factors

1.2.1 Glycemic and Lipidemic Profile

Presence of overweight or obesity and the accompanying increase in fat accumulation exerts significant effects in metabolic parameters [213] (Figure 16). Essentially, presence of increased body weight presents deleterious effect in most metabolic pathways, practically affecting the function of most organs to some extent. Either directly or indirectly, obesity affects peripheral health elevating the risk for most types of NCDs (i.e. T2D, CVD, NAFLD, respiratory, neurodegenerative, digestive and other).

As shown in Figure 17, (24), the previously discussed unfavourable combination of genetic makeup, presence of low PA and a chronic positive energy balance led to increase in fat deposition and elevated ectopic fat accumulation. The establishment of systemic,

Figure 16. Major obesity-related diseases

low-grade inflammation follows, promoting disrupted hormonal and metabolic signals which ultimately result in the manifestation of additional metabolic disorders. As previously mentioned, the adipose tissue is currently viewed as an active endocrine gland, with the majority of its secreted hormones playing significant roles in energy regulation. Expansion of the adipose tissue in the presence of increased weight is associated with: i) disrupted production of adiponectin (known as "metainflammation" [214] ; ii) increase in the adipocyte determination and differentiation factor-1/sterol regulatory element-binding protein-1c transcription factor which increases lipogenesis and reduces fat oxidation; iii) reduction in the expression of IRS-1 in adipocytes and subsequent reduction of the PI3K activity and reduction of PI3K activity in the skeletal muscle; and iv) increased production of inflammatory cytokines like TNF-α [215].

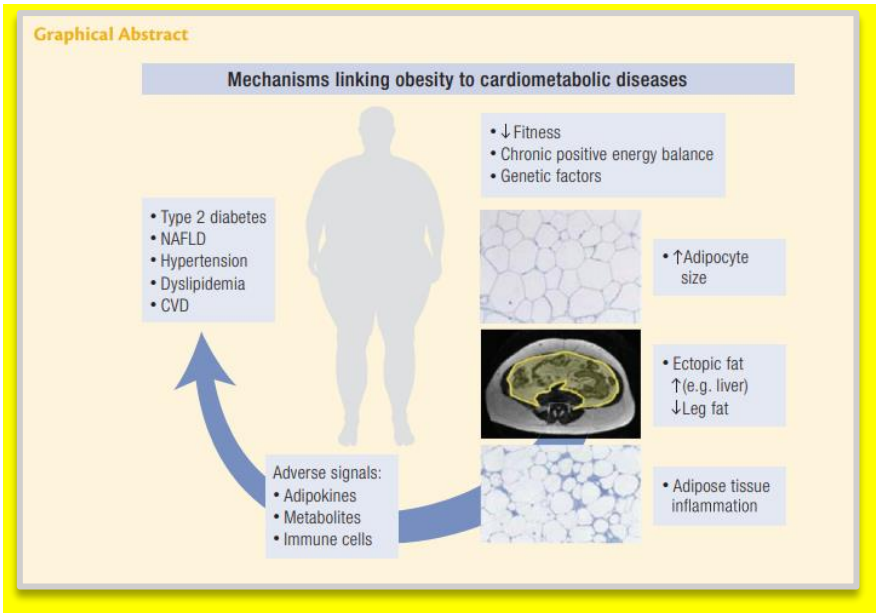


Figure 17. Linking mechanisms of obesity and cardiometabolic diseases [24].

Those effects directly impact glucose metabolism by decreasing insulin sensitivity and progressively strengthening the presence of insulin resistance as the establishment of obesity and the expansion of fat tissue continue uninterrupted. In like manner, fat metabolism also appears dysregulated in the obese state. Obesity is accompanied by elevated levels of circulating FFAs and increased production of TGs as a means to metabolize the circulating glucose not properly uptaken due to the insulin resistant state [215].

In this context, we can argue that obesity can be viewed as sine que non for other cardiometabolic disease. A combined cluster of overweight/obesity presence and their interplay with obesity-related traits related to the observed fat accumulation and subsequent insulin resistance, can further increase the risk for cardiovascular disease, through establishment of the metabolic syndrome (MetS) [216]. Based on the ATP III criteria for the Clinical Identification of the Metabolic Syndrome [217], the latter is defined as having 2 or more of the following traits: i) WC higher than 102 cm for men and 88 cm for women; ii) TG levels ≥ 150mg/dL; iii) HDL-C below 40mg/dL for men and 50mg/dL for women; iv) SBP ≥ 130mmHg and diastolic pressure ≥ 85mmHg; v) fasting glucose levels ≥ 110 mg/dL. As globesity further establishes, MetS presence steadily increases with its global prevalence ranging from 10 to 84 %, as of 2014 [218]. In 2021, Bagheri et al examined progression pathways for the MetS and concluded that hyperglycemia was the primary factor if not accounting for any prior effect or activity (Markovian approach), whereas overweight/obesity led the way for MetS establishment according to the non-Markovian approach [219]. Presence of the syndrome is accompanied by metabolic or metabolomic dysfunctions that may not be observed in the presence of obesity alone. For example, presence of MetS has been associated with systemic mitochondrial dysfunction. Further analysis has also shown that modifying the availability or metabolism of compounds such as SFA may even limit the inflammation associated with obesity leading to metabolic syndrome [105]. Interestingly, apart from the obvious associations with hyperlipidemia, insulin resistance and hypertension, a 2016 systematic review in almost 62.000 patients with

MetS showed that the disorder is also associated with a lower quality of life, either directly or indirectly through effects on BMI or even depression [220].

This cluster of abnormal pathogenetic mechanisms directly elevates the risk for more obesity-related disorders. In addition to the finding that suggests that metabolomic risk observed in the obese state can also cause other cardiometabolic disruptions like T2D [221], several compounds of the MetS have been associated with increased risk for T2D. Data from 95756 individuals of the Copenhagen General Population Study showed that a 1-unit increase in each of the MetS parameter was associated with vastly increased observational risk for T2D. Interestingly, only the increase in WAC and glucose levels also denoted an elevated genetic risk, whereas similar elevation for the other indices was not associated with corresponding increase in genetic risk [222]. Nowadays, MetS is considered to be a modifiable risk factor for T2D, capable of even being used as a prediction or management tool [223].

In 2015, the International Diabetes Federation (IDF) showed a 8.8% global prevalence of T2D among adults in the age range of 20-79 years old [218]. In their 2020 systematic review, Regufe et al showed that presence of MetS in a favorable environment (eg with increased genetic predisposition or other comorbidities present) constitutes an important factor to T2D development [218]. As described above, accumulation of excess ectopic fat burdens the proper function of multiple organs and obesity-associated presence of T2D is promoted by a cascade of events. Excess fat in liver or pancreas can lead to metabolic shift, where disrupted blood flow and lipotoxicity result in hepatic steatosis and disruptions in the production of insulin from the pancreatic β-cells. This can lead to a compensatory increase in insulin secretion to maintain the rate of glucose breakdown. Progressively and as insulin levels rise, the observed resistance to its function is aggravated up to the point where the metabolic demands can no longer be met with its innate production [214]. At the same time, the low-grade inflammatory response inducted by excessive fat leads to increased levels of adipokines, which, in turn, promote the deterioration of β-cell function via increasing cell death and inducing hypoxia. At this point, untreated hyperglycemia in the obese state gradually leads to the establishment of T2D [214].

1.2.2. Inflammatory Status

As previously described, obesity is considered a state of low-grade inflammation. Presence of excessive weight and fat increases inflammatory cytokine production, resulting in cell death and disruptions in glucose homeostasis [224]. The enlargement of fat tissue results in elevated production of chemoattractants which further activate the migration of macrophage cells in the adipose sites (Adipose Tissue Macrophages). Subsequently, the latter proceed to production of a variety of pro-inflammatory cytokines, such as CRP, TNF-α and IL-6, which further induce inflammation [225]. Such substances appear to up-regulate the expression of genes implicated in lipid and glucose metabolism, in a way that enhances lipid synthesis by suppressing insulin-mediated glucose metabolism and causing a state of glucotoxicity [226]. As a result, obesity progressively brings about insulin resistance which can promote degradation in the functions of multiple organs, such as hepatic steatosis and pancreatic dysfunction [226]. Simultaneously, the expansion of adipose tissue is accompanied by reduced blood supply causing a state of observed hypoxia [224]. This effect can cause distinct oxidative stress (OS) and further disrupt the production of

other adipose tissue-secreted hormones, such as adiponectin. It is, therefore, logical to argue that once entering this vicious circle, the cascade of inflammation-promoting events only aggravates the cardiometabolic dysregulations and risk to multimorbidity.

The following information constitute information published under the publication Nutrients 2023, 15, 1884. https://doi.org/10.3390/nu15081884 and can be further found in Appendix D.

Vascular endothelial growth factor A (VEGF-A) is involved in various biological functions, primarily as a major contributor to angiogenesis induction which extends its activities to cell proliferation, migration and even differentiation [227-229]. Due to its versatile roles in endothelial function [230], its involvement in activating the cortisol–adrenocorticotrophic hormone (ACTH) stress axis, its promotion of aldosterone [231] production as well as its multifactorial influence on energy homeostasis [228,232, 233], insulin resistance [228,234] and cardiac function [235], VEGF-A is involved in various reciprocal relationships influencing cardiovascular and cardiometabolic risk factors such as glucose sensitivity, lipidemic profile, obesity and blood pressure. Altered VEGF-A expression is observed in the presence of disturbed cardiometabolic states, denoting a requited relationship between the biomarker's levels and disrupted cardiometabolic profile. For example, VEGF-A is known to be involved in glucose homeostasis, where both its over- and under-expression can affect glucose tolerance [234], as well as lipid metabolism, through its regulation of lipases and the creation of chylomicrons [233]. In a similar manner, VEGF-A is highly expressed in the adipose tissue, where an increase in the number of adipocytes signifies increased VEGF-A and subsequent angiogenesis and further cell proliferation and differentiation [227]. Circulating VEGF-A levels have been conclusively demonstrated as greatly heritable [10]. The past decades have marked the conduct of large meta-analyses of multiple genome-wide association studies (GWAS), revealing key variants significantly associated with the marker's levels. More specifically, Debette and Visvikis-Siest et al. brought four key single-nucleotide polymorphisms (SNPs) to light, collectively explaining 48.7% of VEGF-A variation [236]. Subsequent studies have unveiled additional VEGF-A-related SNPs, which have, in turn, been further associated with adult cardiometabolic indices [237,238] and even the presence of neurodegenerative disorders such as Alzheimer's disease [239]. Selected VEGF-A-associated SNPs have even been directly linked to the presence of hypercholesterolemia and metabolic syndrome in adults [240,241]. In addition, the interplay between VEGF-A SNPs and dietary components has also been associated with multiple metabolic syndrome determinants [242,243]. An example of the importance of the interplay between VEGF-A, anthropometric indices and dietary compounds was recently highlighted in the finding that the effect of VEGF- A variants on circulating iron levels might depend on anthropometric indices (i.e., BMI [244].

1.2.3. Genetics of Cardiometabolic Risk Factors

As in the case of obesity, genetics also play a major part in the onset and gravity of other cardiometabolic disorders. In the case of T2D, literature highlights that despite its heterogeneity, the heritability of the disease lies in the impressive range of 30-70% [245]; a fact potentially denoting the large window or opportunity for the manifestation of contextual modifying effects, such as nutritional or exercise habits.

The effect of genetic makeup on glucose levels and even type 2 diabetes onset have been extensively researched through various GWAS. Indeed, about 143 loci have been identified having associations with type 2 diabetes onset [246], as well as loci directly associated to glycemic traits in general or β-cell function [247]. SNPs of indicative genes associated with type 2 diabetes of glycemic traits are presented in Table 7. GWAS have now located over 700 T2D-associated loci across populations with a range of diverse ancestries [245, 248].

Naturally, as previously explained, genetic makeup can affect the interplay between the fat accumulation accompanying overweight/obesity development and insulin resistance, among other glycemic traits. A shared genetic background is implicated in the pathogenesis of T2D and obesity presence, as the latter is based on pillars directly associated to glucose and fat metabolism (Figure 18). Currently, an increasing number of GWAS reveals loci that contribute to T2D onset and BMI variation [249]. Indicatively, the Stratification of Obese Phenotypes to Optimize Future Obesity therapy (SOPHIA) project sought out to unravel the genetic architecture connecting obesity to T2D onset [250]. The ongoing project sheds a light on the combined effect of genetic, transcriptomic and metabolomic factors in T2D pathogenesis highlighting the favorable effect of distinct, obesity-related geno- and phenotypes [250].

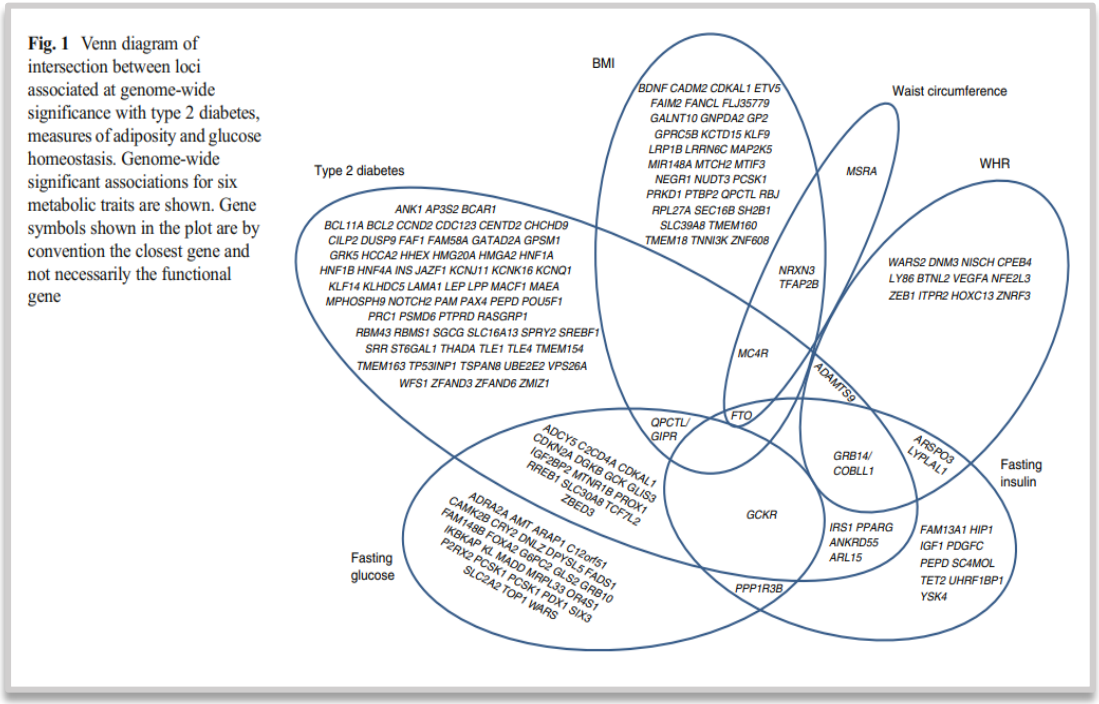


Figure 18. Overlap between obesity-related genes and genes implicated in T2D pathogenesis [251].

Table 7. List of indicative genes and single nucleotide polymorphisms (SNPs) associated with T2D and glycemic traits

Hormone	Function	Gene Name	Chromosomic position	Associated SNPs	Alleles	MAF	Associated Index	Effect Allele	Direction of Effect
PP	Anorexigenic	Pancreatic Polypeptide (PPY)	17:43940804-43942476	rs9957145	G/A	0.18 (A)	Type 2 Diabetes	G	-
				rs9319943	T/C	0.25 (C)	Type 2 Diabetes	C	Negative (β= -0.03)
				rs1517037	C/T	0.24 (T)	Type 2 Diabetes	C	Positive (β= 0.037)
				rs9957320	G/T	0.18 (T)	Type 2 Diabetes	G	Positive (β= 0.055)
Insulin	Indirectly anorexigenic	Insulin (INS)	11:2159779-2161221	rs689	A/G/T	0.35 (A)	Type 1 Diabetes	T	-
				rs3842753	T/A/G	0.35 (T)	Type 1 Diabetes	G	Positive (β= 0.308)
				rs1004446	G/A	0.33 (A)	Type 1 Diabetes	C/G	-
				rs3741208	A/G/T	0.33 (A)	Type 1 Diabetes	T	-
				rs4244808	T/A/G	0.35 (G)	Type 1 Diabetes	G	-
				rs4366464	G/A/C	0.10 (G)	Type 1 Diabetes	C	-
				rs3842727	G/T	0.36 (G)	Type 1 Diabetes in HLA individuals	A/T	-
				rs3842752	G/A	0.13 (A)	Type 2 Diabetes		Negative (β= -0.016)
				rs3842753	T/A/G	0.35 (T)	Type 2 Diabetes	G	-
				rs149483638	C/T	0.02 (T)	Type 2 Diabetes	C	-
Leptin	Anorexigenic	Leptin (LEP)	7:128241278-128257629	rs4731420	G/C	0.17 (C)	Type 2 Diabetes	C	-
				rs791595	A/C/G/T	0.23 (A)	Type 2 Diabetes	A	-
Leptin	Anorexigenic	Leptin Receptor (LEPR)	1:65420652-65641559	rs10889560	C/A	0.16 (A)	Type 2 Diabetes	C	Negative (β= -0.047, 0.088)

1.3. Weight Loss

1.3.1. *Influencing Parameters of Weight Loss*

Determinants of Weight Loss and Weight Loss Maintenance

Over the years, attempts to combat obesity by achieving weight loss mainly refer to the adoption of hypocaloric dietary regimens, the impact of which appears enhanced when combined with other healthy choices, such as increased PA. A different approach to more severe obesity cases such as the presence of morbid obesity and BMI levels of above 40kg/m^2, call for more drastic approaches like bariatric surgery [214].

Naturally, the reduction of fat mass accompanying weight loss is greatly beneficial to obesity-related comorbidities, as well as all-cause mortality in individuals with obesity [214]. Reductions in fat mass benefit the individuals' glycemic control and lipidemic profile by improving levels of TG, TC, LDL-C and HDL-C [214]. In reporting findings from Ebbert et al, Uranga et Keller underline that losing approximately 3kg of weight can results in reductions in TG and LDL levels and increase in HDL levels; such measurements present even enhanced improvement when a weight loss of 8kg is observed [214]. Although one would expect that lipidemic profile improves as weight loss increases, such a notion is not backed by current literature which hints to the potential existence of a weight loss threshold, higher values of which present smaller alterations in the improved indices observed [214].

The approaches adopted are not the sole determinant of a successful weight loss attempt. Metabolic risk factors also dictate the success of weight loss initiatives to some extent, with Stroeve et al attributing them 57% of successful weight loss variation [102]. The genetic factor also plays a major part in such efforts, with multiple obesity-related loci to have also displayed associations with rates of achieved loss in body weight. In their 2015 review, Martinez et Milagro investigated the effect of variants located in popular obesity-related genes, such as FTP, MC4R POMC, LEP, ADIPOQ and PPARγ, among others [252]. As it will be further analyzed below, their study showed multiple nutrigenetic effects, with obesity predisposing alleles being attenuating the effect of hypocaloric diets or significantly interacting with specific macronutrients in increasing or decreasing the rates of the observed weight loss [252]. In 2019, Lamiquiz-Moneo et al examined the effect of a 25-SNP GRS in the observed weight loss of 788 patients with overweight and obesity following a weight loss intervention program. The study found individuals with lower levels of the genetic score presented greater weight loss at a mean follow-up of approximately 6 years [253]. A similar attempt in a population of 1429 children of the Long-term Effects of Lifestyle Intervention in Obesity and Genetic Influence in Children (LOGIC) study showed significant associations between 2 (out of 56) examined SNPs and lower reduction in body weight for the risk alleles in the LOC100287559-rs7164727 and the RPTOR-rs12940622 variants [254].

Another pillar of weight change is located in the role of gut microbiome and their interaction with metabolic and metabolomic changes. Data from the TwinsUK cohort showed no associations between weight change and energy intake, while noting that only 41% of weight change was attributed to genetic factors. This, thus, led to the notion that the remaining proportion of weight change might be deriving

from other factors such as the role of gut microbiota [255]. Based on these principles, Cuevas-Sierra et al even proceeded to the creation of a predictive model including individual genetic and microbiome characteristics to determine optimal weight loss approaches in patients with overweight or obesity [256]. The study investigated relations between responses to either a high-protein or a low-fat diet and microbiota strains, ultimately creating two different predictive models. Use of those models combined with genetic information increased the selection of the diet most likely to succeed in both men and women [256].

Maintaining weight loss after the adoption of a behavioral intervention or the conduct of bariatric surgery has been a subject of discussion during the past decades. Weight loss is followed by body adaptations attempting to restore balance and prevent starvation and maintaining energy stores (eg increase in ghrelin and decrease in leptin). Therefore, periods of weight loss can induce compensatory mechanisms which, in turn, attempt to prevent further weight loss and make weight loss maintenance even more difficult [257]. In the case of lifestyle interventions, it is observed that only a quarter of individuals achieving weight loss after adhering to a hypocaloric diet manage to preserve that loss in the long run [258]. In a meta-analysis of almost 22.000 patients, Ge et al argued that most weight loss effects observed by behavioral interventions disappear at an approximate time of 12 months after program completion [259]. Similarly, a 2022 meta-analysis by Flore et al showed the importance of the intensity of the intervention followed, highlighting that more intense approaches resulting in increased weight loss early or during the intervention might benefit the maintenance of the weight loss observed during the acute intervention phase [258]. In an attempt to pinpoint the determinants that affect a successful maintenance of weight loss, Varkevisser et al highlighted the importance of cognitive and behavioral parameters in promoting practices of control of energy intake and increase of energy expenditure can benefit weight loss maintenance [260]. This finding is also backed by the systematic review by Ramage et al which underlines that successful interventions were mainly characterized not only by dietary components (i.e. energy deficit and increased intake of specific macronutrients like fiber), but also by increase in behavioral training and self-monitoring techniques [261]. Consequently, the adoption of healthy lifestyle strategies such as increase in PA or adherence to a balanced diet can only positively affect long-term weight loss maintenance.

Another important factor in the discussion of successful behavioral interventions lies in the delivery style of each approach. In 2016, Kelly et al assessed the effectiveness of electronically delivered dietary interventions for combatting chronic diseases in adults. The review showed that diet quality and several clinical indices (eg body weight, WC, TG, TC, SBP) were improved after following a telehealth intervention [262]. Similar findings of low-to-moderate quality were reported in a different 2022 meta-analysis by Barnett et al [263]. Apart from the obvious advantages of eliminating space-induced barriers, long-distance delivery can even present financial advantages; Moin et Mangione discussed the delivery of in-person compared to electronically delivered intervention for reduction in CHD risk, showing that the latter proved to be just as beneficial but, obviously, more cost-effective than the other [264].

In the question of whether macronutrient composition plays a part in promoting the latter, findings from the Diogenes study showed that individuals who had adherence to a 6-month hypocaloric diet of increased protein content presented lower weight regain at 12 months, when compared to participants of the low-protein diet group [265]. However, consistent results have yet to be yielded on specific macronutrient intake, allowing us to currently focus on the benefits of an overall balanced dietary regimen, rather than a specific macronutrient ratio. Regarding potential sex-differences, although women tend to present greater organization and motivation skills during weight loss interventions [266], men tend to present better results in weight loss leading to greater weight loss that men [267]. However, weight loss maintenance rates do not present significant differences between the two [266].

It is worth mentioning that in the case of bariatric surgery, better weight loss maintenance and metabolic profile are observed in the years following the surgery, with special attention to the Roux-en-Y bypass type [268]. This finding is in line with the fact that this type of surgery leads to a significant reduction in weight, usually larger than the one observed after adhering to behavioral interventions. We could, therefore, argue that although weight loss maintenance in this case can present better results, we must always keep in mind the weight loss and regain-ed at the end of the follow-up periods. Current evidence is limited in the 6-year follow-up period and do not allow for generalized conclusions on the success of long-term maintenance to be drawn. Jones et al also highlighted that a passive stance in the period after the bariatric surgery also negatively affects the maintenance and further pinpoint the role of active self-management skills in enhancing the observed maintenance [269]. We could, therefore, argue that approaches aiming at substantially shaping the individuals' conscientious understanding and practicing of healthy lifestyle approaches (i.e. healthy eating, exercising, reducing stress and having good sleep habits, among others), irrespective of undergoing a bariatric surgery or not, might present better chances of achieving both weight loss and long-term maintenance.

Dietary Interventions for Weight Loss

Naturally, energy deficit is essential in achieving weight loss. Meta-analyses have shown the superiority of following very low energy diets with less than 800 kcal per day (VLEDs) compared to low energy diets (LEDs) containing 800-1200 kcal per day, in achieving greater weight loss [270]. However, the differences between the two strategies appear to decline over time, seeing as individuals having followed LED present comparably similar rates in sustaining long-term weight loss to the ones having adhered to VLEDs [270]. However, the impact of VLEDs on body composition constitutes a matter of discussion, with Steur giving special focus on the potentially detrimental effects of such diets on skeletal muscle [271].

Another approach concerns the adoption of intermittent fasting techniques, where energy intake is limited within a specific time frame, usually concerning a 6- or 8-hour permissible window per day, or a 24-hour fasts on alternate days or even a 2-non-consecutive-day fast during the week [272]. In 2021 umbrella review, Patikorn et al showed that the practice was indeed associated with ameliorations in the modifications of multiple obesity-related traits [273]. In their 2020 review, Welton et al showed that IF techniques resulted in significant weight loss, but not substantially differentiated than the one compared to caloric restriction strategies. In this case, IF

was found to relate to better glycemic control in individuals following the practice [272]. Similar results were reported by Gu et al who showed no differences in weight loss between IF and caloric restriction, but an amelioration in glycemic indices such as HOMA-IR and insulin [274]. Interestingly, the study showed that IF provided significantly different results when compared to no intervention diets, meaning that practicing IF can be more beneficial than following other types of isocaloric regimens [274]. In this spectrum, Varady et al highlight the beneficial effect of IF not only on short-term weight loss but overall improvement of clinically-related, cardiometabolic factors [275].

Apart from caloric restriction in whichever form, several specific dietary regimens have been examined, with special focus on investigating the effects of low carbohydrate (<45% of total energy intake) or low fat content (<30% of total energy intake), or even comparing the two approaches. Table 8 summarized key dietary interventions in the field.

Table 8. Summary of key dietary interventions investigating the effect of different macronutrient content on weight loss.

Name	Proposed Intervention	Duration	Participants	Outcome
DPP	Placebo vs Metformin vs Intensive Lifestyle Intervention (Hypocaloric, low-fat diet)	9 months	3234 individuals at high risk for T2D	High-carbohydrate, low-fat diet associated with increased weight loss
Look AHEAD	Lifestyle intervention (Hypocaloric, low-fat diet and increased PA)	6 months with follow-ups up to 4 years	5145 participants with overweight/obesity	Low-fat diet associated with increased weight loss
POUNDS lost	Dietary Intervention [Hypocaloric moderate-fat, moderate-protein vs moderate-fat, high-protein vs high-fat, moderate-protein vs high-fat, high-protein diets)	6 months up to 2 years	811 participants with overweight/obesity	No difference in weight loss between the four diet groups
DIETFITS	Dietary Intervention (hypocaloric, healthy low-fat vs healthy low-carbohydrate)	12-months	609 individuals with overweight	No difference in weight loss between the two diet groups
DiOGenes	Dietary Intervention (Hypocaloric high-protein, high-glycemic index vs high-protein, low-glycemic index vs low-protein, high glycemic index vs low-protein, low-glycemic index	8-week low-calorie diet and 26 weeks ad libidum	932 individuals with overweight	No difference in weight loss between the four diet groups
DIRECT	Dietary Intervention (Hypocaloric low-fat vs hypocaloric, MD nuts vs isocaloric, low-carbohydrate)	2 years	332 individuals with obesity	MD and low-carbohydrate diets associated with greater weight loss

PREDIMED	Dietary Intervention (Hypocaloric MD+EVOO vs MD+ mixed nuts vs control)	One-year follow-ups up to 5 years	7447 at high CVD risk	Lower risk of CVD incidence in MD +EVOO vs MD + mixed nuts, vs control

Intake of low-carbohydrate regimens has gathered lots of attention, especially due to the increasing popularity of adopting schemes such as the Atkins or the ketogenic diet to achieve weight loss. Findings regarding long-term weight loss maintenance after adherence to low-carbohydrate regimens, such as the Atkins one, present contradictory results. In 2021, the Obesity Management Task Force proceeded to the publication of guidelines for very low energy ketogenic diets (carbohydrate intake usually accounts for 10% of total energy intake), in an effort to minimize potential complications and enhance the advantages observed in obesity-related traits after adherence to such diets. The guidelines suggested that these diets results in greater weight loss and improvement of glycemic and lipidemic profile compared with other interventions. Overall, the study concluded that the diets can be an effective way to achieve weight loss, always under the prism of personalization to avoid potential aggravating results in other disorders [276]. Regarding the adoption of Atkins diets (macronutrient content may range from 10% to 35% of total energy intake deriving from carbohydrates), several studies report better loss maintenance rates in the 2-year post-intervention period, while others find no significant differences with low-fat regimens [277]. Finally, low glycemic index (GI) diets also appear popular for weight loss, with current literature reposting inconsistent results as to their leverage over other dietary interventions for weight loss [277].

Regarding dietary strategies promoting the adherence to low-fat diets, Initiatives such as the Diabetes Prevention Program (DPP) [278] and the Look AHEAD study [279] have looked into the effect of the latter compared to standard care recommendations. Both studies reported an overall advantage of the low-fat diets in achieving great weight loss compared to the control groups [270]. Recent meta-analyses have showed that adhering to low-carbohydrate diets results in greater short-term weight loss compared to the one observed by following low-fat diets [280,281]. Multiple intervention trials in adults with obesity have shown favorable effects on weight loss after following 6-month hypocaloric diets with a low-carbohydrate compared to a low-fat content. However, data from 1-year follow ups report that weight loss maintenance rates remain comparably similar for the two groups [227]. According to Chao et al, low-carbohydrate diets result in higher 6-month weight loss by 3 or 4kg [270]. Intervention trials have also been designed to specifically test for differences between the two regimens in achieving weight loss in adults with overweight or obesity. The Preventing Obesity Using Novel Dietary Strategies (POUNDs lost) trial [168] examined the effects of four different hypocaloric dietary regimens (2x2 design) in 6-month and 2-year weight loss of adults with obesity. The four groups comprised of: i) a low-fat, moderate-protein diet (<20% fat, 15% protein, 65% carbohydrate); or ii) a low-fat, high-protein diet (20% fat, 25% protein, 55% carbohydrate); or iii) an MD-alike, moderate-fat, moderate-protein diet (40% fat, 15%

protein, 45% carbohydrate); or iv) an MD-alike, moderate-fat, high-protein diet (40% fat, 25% protein, 35% carbohydrate) [168]. The study showed a significant mean weight loss of 6kg at the end of the 6-month intervention period, but yielded no significant differences between the four dietary groups [168]. In a similar context, the Diet Intervention Examining The Factors Interacting with Treatment Success (DIETFITS) study sought to investigate the effect of matching different individuals to either a healthy low-carbohydrate diet or a healthy low-fat one, in the observed weight loss after a 12-month intervention period in adults with overweight or obesity [282]. In line with the findings presented by POUNDS lost, DIETFITS showed a mean weight loss of 6kg at the end of the 12 months, but without significant differences within the two diet groups [283]. With regards to the quality of the pooled data in current meta-analyses, Churuangsuk et al highlighted that meta-analyses of good quality tended to show no differences in weight loss achieved by the two regimens [284]. Overall, a 2020 systematic review by Smith et al highlighted that there are no sufficient evidence to argue over the superiority of low-carbohydrate to low-fat diets in body mass variation [285].

Diets with a high-protein content targeting weight loss are considered a potentially resourceful approach due to the proteins' promoting satiety, having markedly higher DIT-inducing capacity and preventing loss of FFM [286]. However, meta-analyses in the field have demonstrated little to no significant advantages in the weight loss achieved after a high-protein and a high-fat diet [270], with a systematic review even demonstrating that low-fat diets can even present a modest advantage for short -term weight loss [277]. One of the most well-known studies in the field refers to the Diet, Obesity and Genes (DiOGenes) study, which investigated the effect of four dietary regimens (high-protein, high-glycemic index vs high-protein, low-glycemic index vs low-protein, high glycemic index vs low-protein, low-glycemic index) in the observed weight loss of 932 adults with overweight after an 8-week intervention and a 26-week ad-libidum period. The study did not show significant differences in weight loss between the diet groups but showed amelioration of low-grade inflammation indices after following the low-protein diets [287]. A different study assessed data from the Measuring Eating, Activity and Strength: Understanding the Response-Using Protein (MEASUR-UP) and Protein Optimization in Women Enables Results-Using Protein (POWR-U) studies. The study examined the effects of following a hypocaloric, high-protein versus a hypocaloric, control diet for a 6-month period, on the observed weight loss 80 adults with obesity, finding no significant different in the weight loss observed between the two groups [288].

In terms of specific dietary patterns, the Dietary Approach to Stop Hypertension (DASH) diet has also gained ground . Initially well-known as the go-to dietary strategy for combatting hypertension, DASH has also expanded as an effective approach to combating hypertension-related disorders, such as obesity due to its rich content in foods inversely associated with increased weight and its low content Two systematic reviews in 2016 and 2021 showed that DASH resulted in significant weight loss compared to control groups [289]. However, literature has not yielded significant superiority over other dietary interventions for weight loss.

Lastly, as previously described, the advantages of MD on cardiometabolic risk factors are largely known [290]. Additionally, research suggests that adoption of MD is even beneficial for the modification of obesity-related traits and achieving optimal weight loss. The Dietary Intervention Randomized Controlled Trial (DIRECT) study attempted to examined the effects of MD in weight loss of 332 individuals with obesity after a 2-year intervention of either a hypocaloric, low-fat diet; a hypocaloric, MD diet or a low-carbohydrate, isocaloric diet [291] showed that participants in the last two groups achieved greater weight loss and noted better levels of glycemic or lipidemic indices, than the ones in the low-fat diet [291]. To boot, the Prevención con Dieta Mediterránea (PREDIMED) Study, constituted another a large, randomized clinical trial (RCT) targeting prevention of CVD in individuals at high risk for the disease. The study examined the intake of MD with olive oil versus MD with mixed nuts, comparing to a control group. Although MD was proven beneficial for reducing CVD incidence, no significant effects were observed for short- or long-term weight loss [292]. In their 2011 review, Nordamann et al showed that individuals having following MD interventions presenting better weight loss, glycemic, lipidemic and inflammatory profile than the ones in low-fat diet regimens at 2-years of follow-up [270]. Finally, Ge et al concluded that MD superseded all other dietary regimens, seeing as weight loss appeared to diminish for all other types of diets at a time of 12 months after intervention completion [293].

1.3.2. Metabolomics of Weight Loss

Continuing the discussion on the existence of specific metabolomic signatures in the presence of obesity, the role of metabolomic profile has also been examined in weight loss. In line with previous relations discussed, this effect also presents bidirectional influences, with findings showing that baseline metabolic profile can affect weight loss and, in turn, weight loss can cause differences in the levels of several metabolites. Overall, weight change is associated with distinct alterations in metabolomic profile either by inducing them or vice-versa. Disruptions in oxidative stress, mitochondrial dysfunction and the tricarboxylic acid cycle (TCA) are in the forefront of weight change-related metabolomic parameters. Numerous factors appear to be at play, with significant interactions observed between proposed behavioral interventions aiming at weight loss and changes in metabolites implicated in glucose and fat metabolism or even gut microbiota strains [100]

In patients with morbid obesity, predictive models performed best, including metabolites such as ketone bodies, TGs and AAs. In a study by Geidenstam et al, lower levels of BCAAs and other AAs, among other metabolites, were associated with greater weight loss. Insight on the weight-loss induced metabolomic effects have provided implications for several metabolic pathways. For example, administration of metformin in combatting obesity was shown to be associated with AMPK activation and subsequent increase in FA oxidation, thus promoting weight loss [105]. In a study of 3.176 women from the TwinsUK cohort, Menni et al found that urate, γ-glutamyl valine and 3-phenylpropionate were independently associated with changes in BMI. Specifically, adding the four metabolites in prediction models significantly increased

the AUC for BMI change, compared to accounting only for demographic or lifestyle variables [106].

Observed differences may vary according to the proposed interventions, with Rangel-Huerta et al summarizing the findings of current literature in their 2019 review [102]. Different studies have demonstrated the effect of energy restriction on changes in several metabolite concentrations urine trimethylamine N-oxide (TMAO), SFA, MUFA, OUFA and phospholipids, among others [102]. In the case of examining different dietary schemes, a hypocaloric diet with increased intake of dairy was associated with elevated levels of citrate, urea and creatinine but lower levels of TMAO. Results from the POUNDs lost study showed differences in BCAA and several AAs following weight loss; findings successfully replicated in the DIRECT study. In the case of adopting PA recommendations.

Metabolomic signatures also appeared altered after weight loss was achieved in populations of children and adolescents. Sohn et al showed modifications in glutamate, arginine and BCAA metabolic pathways after 6-months of a weight loss intervention in 40 children, with significant metabolomic changes also noted in the 6 to 18-month period following the end of the intervention [294]. Similarly, Rigamonti et al showed significant alterations in 64 metabolites of 42 teenagers with obesity after adhering to a hypocaloric diet for a total duration of 3 weeks [295]. Interestingly both the aforementioned studies showed increase in carnitine and carnitine-associated metabolites after the end of the proposed interventions, hinting at beneficial changes in fat oxidation post weight loss.

1.3.3. Gene-lifestyle Interactions in Weight Loss

In line with the insightful findings on the role of gene-lifestyle interactions in weight change, current literature presents a vast variety of proposed interventions examining different approaches for achieving optimal weight loss. Figure 19 depicts a representative network of published research surrounding the field of gene-diet interactions in weight loss throughout the past 14-year period.

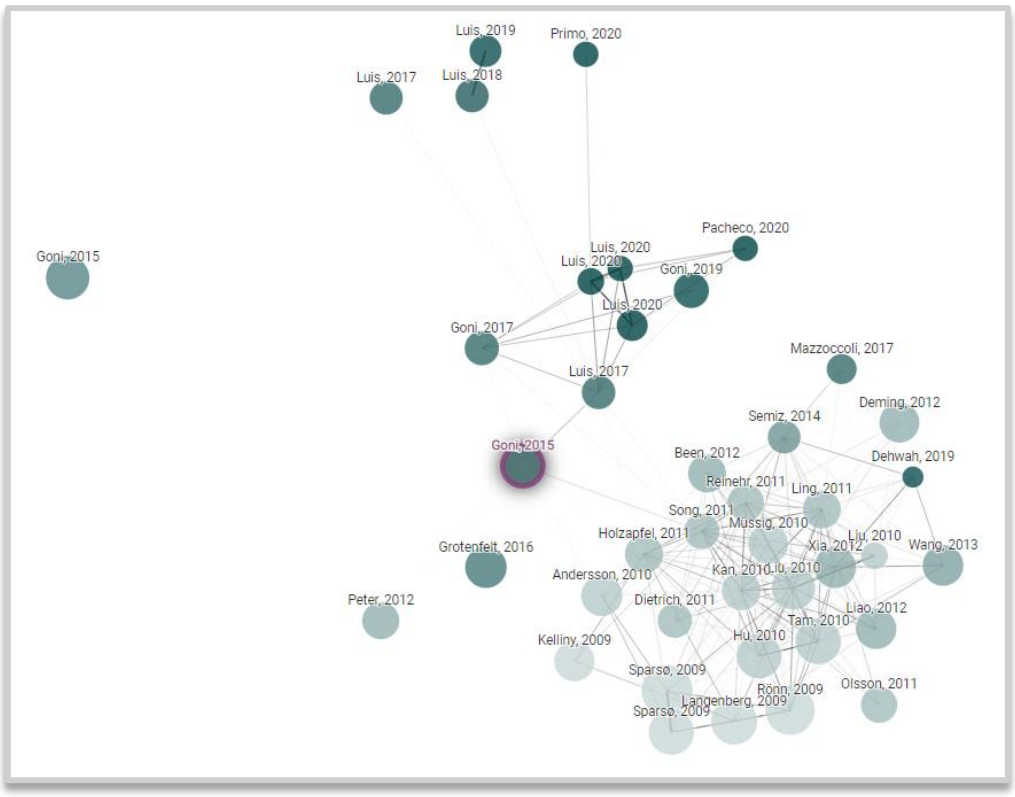


Figure 19. Papers concerning gene-diet interactions in weight loss [created with connected papers.com].

When proceeding to accounting for the additive effect of genetic makeup, findings display great interest in its implications on diet's mediating or modifying effects. Nutrigenetic interactions are observed in the manifestations of various obesity-related traits, whether they be of anthropometric or even behavioral nature. Rivera-Iniqguez et al investigated nutrigenetic associations in rewarding behaviors, among others, highlighting the influence of dietary acceptability and genetic compatibility in modifying eating behavior [296]. This constitutes a promising field of interest for future research surrounding the role of gene-diet interactions in intermediate or psychological phenotypes affecting obesity traits via impact on dietary habits.

In large, nutrigenetic effects have been studied in the context of either cross-sectional or retrospective analyses, or dietary interventions investigating the effect of hypocaloric diets or diets of different macronutrient content. A meta-analysis on data from 33.187 participants of the National Heart, Lung, and Blood Institute Trans-Omics for Precision Medicine cohorts showed a significant, UKBB-replicated interaction between the rs79762542 variant and carbohydrate intake in influencing HbA1c levels [297]. Dietary interventions have examined the combined impact of genetic factors and diets of various macronutrient composition on the modifications of body weight or other cardiometabolic risk factors, by providing advice based on genetic characteristics (nutrigenetic advice) (Table 9). Frankwich et al examined the impact of administering diets of different macronutrient contents but combined with nutrigenetic advice on weight loss, without observing vast differences in post-intervention results [297]. A different well-known study concerns the PREVENTOMICS

initiative. This study sought to examine the effect of providing personalized vs standard advice for 10-week period, on the observed weight loss of 100 individuals with overweight or obesity [298]]. Similarly to above, PREVENTOMICS did not show significant differences in the observed weight loss of the two groups [298]. Furthermore, the Nutrigenomics, Overweight/Obesity and Weight Management (NOW) RCT, built on the advice given in the aforementioned DPP trial (the Group Lifestyle Balance-GLB) and additionally provided recommendations based on genetic information. The trial managed to show increased fat reduction in the group receiving GLB and nutrigenetic advice, compared to GLB alone [299].

Moreover, the NUGENOB and Obekit initiatives attempted to investigate the effect of genetic variants in the observed weight loss of participants following a hypocaloric low-fat diet vs a moderate-fat (NUGENOB) [300] or a high-protein diet (Obekit) [301]. The former yielded no differences in weight loss between the two diets, but showed nominal associations between BMI-risk alleles and reduced weight loss in participants in the low-fat group [302]. Interestingly, findings from the study showed that participants with the AA genotype for the TFAP2B variant presented greater weight loss in the low-fat group, whereas GG homozygotes showed increased weight loss in the high fat group [303]. In a similar context, the Obekit trial showed that homozygotes for the rs1042713 variant showed lower reductions in TC and LCL-C in the low-fat compared to the moderately high-protein group [301].

Table 9. Summary of key personalized or nutrigenetic interventions investigating the effect of different macronutrient content on weight loss.

Name	Proposed Intervention	Duration	Participants	Outcome
Frankwich et al	Lifestyle Intervention (Nutrigenetic diet -i.e. balanced vs low-fat vs low carbohydrate vs MD vs standard recommendations)	8 weeks (primary) and 24 weeks	51 individuals with overweight or obesity	No difference in weight loss between the five diet groups
PREVENTOMICS	Lifestyle Intervention (Personalized diet vs standard recommendations)	10 weeks	100 individuals with overweight or obesity	No difference in weight loss between the two diet groups
NOW	Lifestyle Intervention (GLB vs GLB + nutrigenomics recommendations)	12 months	140 individuals	GLB + nutrigenomics group reduced fat intake at 12 months
NUGENOB	Lifestyle Intervention (Hypocaloric low-fat vs moderate-fat diet)	10 weeks	771 individuals with obesity	No difference in weight loss between the two diet groups
Obekit	Dietary Intervention (Hypocaloric moderately high-protein vs low-fat diet)	4-month intervention period and 6-month follow-up	260 participants with obesity	Various changes

Generally, regarding the role or genetic variation on weight changes induced by lifestyle interventions, a 2018 systematic review by Tan et al highlighted the versatility of current results and the fact that most studies up to that point, mainly reported findings on the interaction between specific candidate variants and dietary parameters in the modification of anthropometric measurements [304]. The most representative example concerns the case of SNPs in the FTO gene. Interestingly, carriers of the BMI-related, rs9939609-A allele have shown greater reductions in body weight after following hypocaloric dietary interventions in a limited number of RCTs [277, 305, 306]. To boot, several RCTs have shown no significant interaction between the variant and macronutrient composition in differentiating responses to weight loss attempts [277, 307, 308]. However, the POUNDS lost study demonstrated a significant increase in weight loss among carriers of the risk allele of the rs1558902 variant who adhered to the high-protein diet for the 2-year period [DOI 10.1007/s13668-013-0061-3]. Interestingly, the same study also yielded important results for other obesity-related loci. Presence of the obesity-related rs2943641-C and rs2287019- T alleles was associated with elevated weight loss and glycemic profile improvement in participants adhering to the low-fat diet, with findings pertaining mostly to the short-term (6-month) rather the long-term (2-year) intervention period [277]. Regarding FTO and glycemic profile, a recent meta-analysis by Parastouei et al reported no statistically significant differences between the risk allele carriers and non-carriers in changes observed for fasting glucose or HOMA-IR after dietary interventions with hypocaloric regimens [309]. In 2015, Martinez et Milagro explicitly summarized nutrigenetic

interactions observed in dietary interventions targeting weight loss as a way to set bases for personalized management strategies [252] Table 10 summarizes the findings presented in the study denoting significant interactions between genetic variants and dietary components in modifying adult weight loss. Overall, the gene- or GRS-diet interactions reported in the literature present contradictory results with many efforts showing significant interactions between the two factors and other yielding no statistically important findings. The thorough review concludes by highlighting the need for more research in populations of large sample size to validate current findings and provide more robust results [252].

Table 10. Table adapted from Martinez et Milagro, summarizing gene-diet interactions in the weight loss observed in adults following lifestyle interventions [252].

Gene	SNP	Proposed Intervention	Participants	Outcome
GIPR	rs228719	Four diets with different macronutrient content	757 individuals with overweight	rs228719-T allele x low-fat diet resulted in increased weight loss
GNAS	G(-1211)A	7-day fasting	87 individuals	GG genotype associated with increased weight loss
MC4R	rs1943218, rs17066866, rs17066856, rs9966412, rs17066859, rs8091237, rs7290064, rs12970134	6-14 month DPP	3234 individuals with T2D	Interactions between diet and variants in increasing weight loss
PLIN1	11482G->A	1-year hypocaloric diet	150 individuals with obesity	11482-A allele associated with weight loss resistance
PPARG	Pro12Ala	Diet and exercise	1456 inidivduals	Ala12 x high-fat intake resulted in lower weight loss
TCF7L2	rs7903146	10-week hypocaloric low- vs high-fat diet	771 individuals with obesity	TT genotype x low-fat resulted in increased weight loss
TCF7L2	rs7903146	9-month fat reduction and fiber increase	304 individuals	TCF72L x fiber affected weight loss rates
TFAP2B	rs987237	8-week hypocaloric with low- vs high-fat diet	771 individuals	rs987237 x energy deficit affected the impact of fat intake on weight loss
UCP1	-3826A/G	2-month diet	17 women with normal weight	-3826-G resulted in lower weight loss
UCP3	CGTACC haplotype	1-month VLED	214 women	CGTACC resulted in increased weight loss
FABP2	Ala54Thr	3-month hypocaloric diet	111 individuals with obesity	Thr54 resulted in increased weight loss

In a nutshell, the conduct of nutrigenetic studies has, so far, provided limited but encouraging findings on the effects of the interactions on anthropometric

parameters. Exploring future directions, with emphasis on dietary regimens with evidence-based beneficial characteristics, such as MD [310], poses a promising direction with extensions in effective obesity-combatting strategies.

1.4. Current Gaps in Literature and Thesis Research Questions

The purpose of the study is to assess the interaction of genetic makeup and lifestyle determinants, including gene-diet interactions, on obesity-related traits of healthy adolescents and adults with overweight or obesity. Following the literature review, the research questions constituting the cornerstone of the present thesis will attempt to decipher the following: i) the examination of associations between dietary and lifestyle habits and anthropometric and lifestyle indices in Greek adults with overweight or obesity; ii) the investigation of the impact of hypocaloric diets of different macronutrient content in the anthropometric, body composition and lifestyle parameters of Greek adults with overweight or obesity; iii) the effect of genetic makeup on the response to the hypocaloric diets and the observed modifications of the weight-related indices; iv) the identification of dietary patterns in adolescents and their associations with cardiometabolic indices and interactions with VEGF-A-related genetic variants; and v) the effect of genetic makeup on the BMI levels of Greek adults.

The innovation of the present thesis rests in: i) the first dietary intervention for weight loss examining two different dietary regimens in Greek adults with overweight or obesity; ii) the use of an online assessment tool to deliver the intervention and assess intervention outcomes; iii) the first analyses for gene-diet interactions following the hypocaloric diets in Greek adults with overweight or obesity; iv) the comparative evaluations of the dietary habits of adolescent populations of different origin; v) the never-before-conducted attempted evaluation of the role of VEGF-A variants in adolescent cardiometabolic profile; and vi) the creation of the first PRS for BMI in a Greek population of adults.

1.5. Thesis Aims and Objectives

The **aim of the present Dissertation** is to investigate the influence of genetic background and lifestyle factors (e.g. dietary habits) and their respective synergistic effect on obesity-related traits, such as body weight regulation and body composition measurements and levels of glycemic and lipidemic indices, in several age groups. For those purposes, the objectives of the present Dissertation are hereby summarized in the following:

i. *To conduct* a dietary intervention for weight loss using hypocaloric dietary regimes with difference macronutrient content, in adults with overweight or obesity, in the context of the iMPROVE Study [112];
ii. *To investigate* the effect of dietary habits and the dietary intervention for weight loss on anthropometric, cardiometabolic and lifestyle indices in adults with overweight or obesity, in the context of the iMPROVE Study [112];
iii. *To investigate* the effect of BMI-related genetic variants and their interactions with lifestyle characteristics on anthropometric, cardiometabolic and lifestyle

indices in adults with overweight or obesity, in the context of the iMPROVE Study;

iv. *To investigate* the effect of dietary habits on the levels of glycemic, lipidemic and inflammatory indices in adolescent populations using data from the Greek TEENAGE and the French STANISLAS Family Study, in the context of the 2018 Chair Gutenberg project [311];

v. *To investigate* the effect of VEGF-A-related variants and their interactions dietary habits on the levels of glycemic, lipidemic and inflammatory indices in adolescents from the Greek TEENAGE study, in the context of the 2018 Chair Gutenberg project [312];

vi. *To develop* a PRS for BMI, using data from the Greek studies of NAFLD, THISEAS and OSTEOS Studies [208];

For these reasons, the aims and objectives of the present Dissertation are summarized in greater detail according to each of the three projects, in the sections below.

2. Methodology

2.1. The iMPROVE Study

2.1.1 Ethics, Study Registration and Study Aims and Objectives

The **iMPROVE study** constitutes a clinical trial, a dietary intervention created to investigate the synergistic effect of genetic background and lifestyle characteristics on body weight regulation, body composition, biochemical profile and lifestyle parameters. The study was conducted according to the principles laid out in the Declaration of Helsinki and was approved by the Ethical Review Board of Harokopio University of Athens, with a protocol number of 1800/13-06-2019 (see Appendix B1). The iMPROVE study design has been registered to the ClinicalTrials.gov database of clinical studies (ClinicalTrials.gov Identifier: NCT04699448).

Accordingly, **the aim of the study** was to investigate the effect of genetic background and adherence to hypocaloric dietary regimes with difference macronutrient content on the body weight regulation, the body composition, the glycemic and lipidemic profile and lifestyle characteristics of an adult, Greek population with overweight or obesity.

The objectives of the study were, thus, shaped as follows:

i. *To investigate* the effect of adherence to two different hypocaloric dietary regimes on the body weight regulation of Greek adults with overweight or obesity;
ii. *to investigate* the effect of genetic makeup on the body weight regulation, biochemical profile and lifestyle parameters of Greek adults with overweight or obesity, by examining BMI-related variants and constructing GRSs for BMI; *and*
iii. *to investigate* the synergistic effect of genetic makeup and dietary intake on the body weight regulation, biochemical profile and lifestyle parameters of Greek adults with overweight or obesity, by examining interactions between BMI-related variants and GRSs and dietary factors.

As of 2023, relevant project proceedings included in the present thesis have already been published in a peer-reviewed article [112, Appendices].

2.1.2. Study Design and Study Population

In this context, the iMPROVE study was designed as a three-month dietary intervention, with a maximum participation of six months, including Greek adults with overweight or obesity, who fulfilled the following eligibility criteria for inclusion in the study:

i. Age in the spectrum of 18 to 65 years old;
ii. Existence of overweight or obesity (i.e. BMI of more than 25 kg/m^2);
iii. No extreme weight loss in the 3 to 6 months prior to the beginning of the intervention; *and*
iv. Maintenance of a stable level of physical activity for the duration of the intervention

Exclusion criteria for participation in the study included:

i. Presence of pregnancy, lactation or desire to become pregnant in the near future (i.e. during the intervention period);
ii. Presence of unregulated comorbidities or chronic or other diseases, which can influence dietary intake (i.e. existence of type 1 or type 2 diabetes, cardiovascular disease, gastrointestinal disorders, mental illness, dietary disorders, cancer of any form);
iii. Parallel intake of supplements targeted at weight loss; *and*
iv. Parallel participation in a different research study targeted at weight loss.

Eligible volunteers were recruited for participation in the study. **Oral information** on the study protocol and the research aims and objectives was provided to all volunteers by health professionals (i.e. dietitians and nutritionists) and nutrition science undergraduate and/or graduate students, prior to the volunteers' providing written consent for their participation in the study. All volunteers included in the intervention and the analyses present hereby provided **written consent** prior to enrolling in the intervention.

Following their enrolment, eligible volunteers participated in the baseline **in-person meeting** with trained health professionals (i.e. dietitians and nutritionists) and nutrition science students. Volunteers further participated in at least one more in-person follow-up meeting at the end of the 3 months after intervention onset. More specifically, at baseline, participants were randomly allocated to one of the two groups adhering to **hypocaloric dietary regimes with different macronutrient content** (Figure 20), as follows:

i. *Regimen 1*: **60%** of daily dietary intake provided from **carbohydrates**, **18%** of daily dietary intake provided by **protein** and **22%** of daily dietary intake provided by **fat**.

ii. *Regimen 2*: **40%** of daily dietary intake provided from **carbohydrates**, **30%** of daily dietary intake provided by **protein** and **30%** of daily dietary intake provided by **fat**.

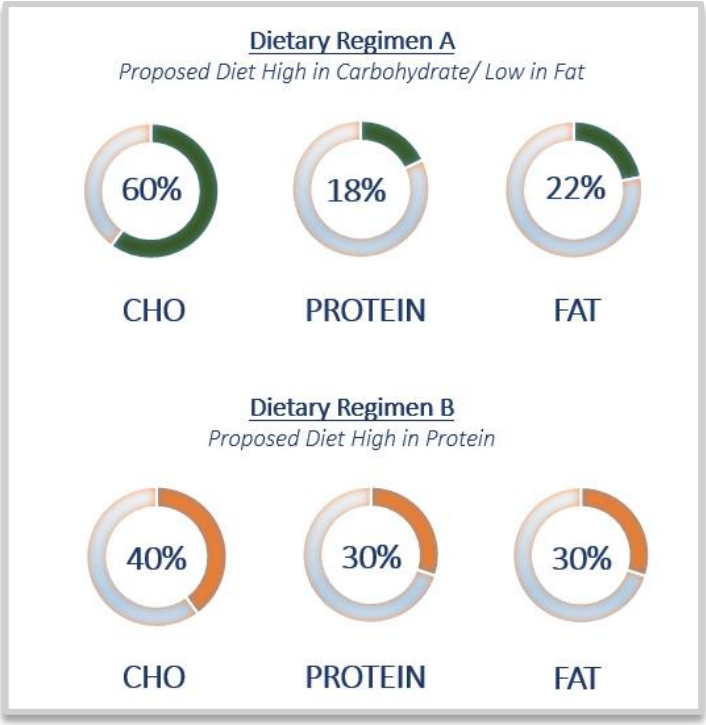


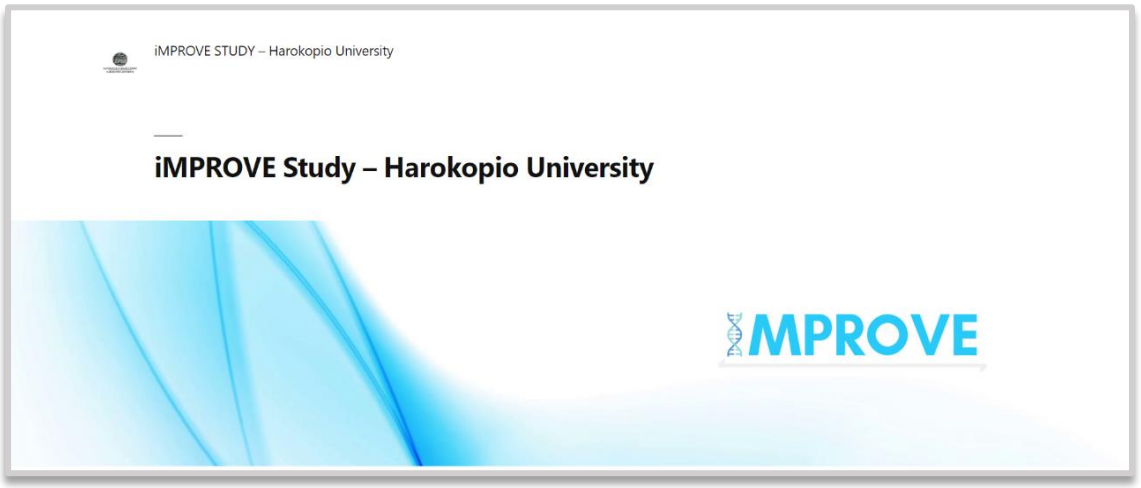
Figure 20. Macronutrient distribution of the proposed diets [112].

Daily dietary requirements were calculated for each volunteer based on their BMR and physical activity level (PAL). Individual daily needs were calculated with a target of achieving an anticipated weight loss of 0.5 to 1kg per week, which was translated to a 500kcal reduction in each participant's daily dietary intake. Three types of diets were created for each macronutrient group, with a daily caloric content of 1500 kcal/day, 1800kcal/day or 2000kcal/day. All proposed diets adhered to the principles of the Mediterranean diet (i.e. proposing consumption of red meat once a week; consumption of fish once or twice per week; consumption of grains two or three times per week and consumption of three portions of fruits and three portions of vegetables per day) and were composed of 5 or 6 meals throughout the day, including easy-to-make and traditional-for-the-Greek-population food combinations and recipes. Each participant was provided with the diet of caloric content most closely corresponding to their calculated caloric needs.

At baseline, participants provided fasting blood samples (23mL) and underwent assessment of body composition (see below). Moreover, all volunteers were required to fill in questionnaires regarding anthropometric and lifestyle data during the entirety of the intervention period. For the purposes of facilitating the long-distance collection of all relevant data, as well as the provision and renewal of the proposed diets, our team proceeded to the development of an original online assessment tool available at: http://83.212.122.254/ (Figure 21). All participants were informed on the use of the online platform during the baseline meeting. Access to the online tool was granted with unique usernames and passwords for each volunteer. During the baseline session, each volunteer was informed on the use of the platform by the healthcare professional and by being taken on a virtual tour and explicitly shown how to access and use it.

At baseline, participants were called to provide information on: i) their medical history; ii) demographic characteristics; iii) feeling of satiety, by completing a 5-scale

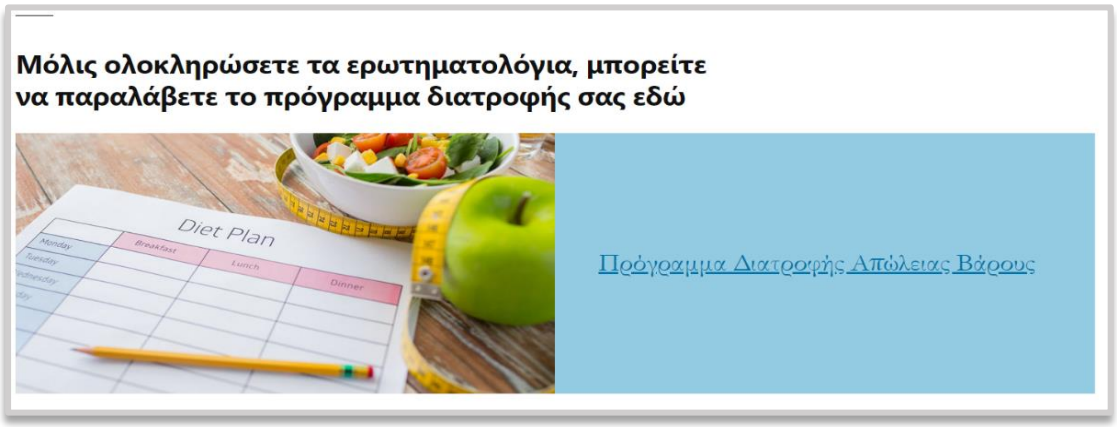
short questionnaire; iv) adherence to the Mediterranean dietary pattern, by completing the questionnaire of the MedDiet Score [313]; v) depression characteristics, by completing the CESD-R-10 Questionnaire [314]; (vi) characteristics of quality of life and health status, by completing the short version of the SF-12 Questionnaire which results in the estimation of a physical (SF PCS 12) and a mental (SF MCS 12) component [315]; vii) characteristics of quality of sleep, by completing the Athens Insomnia Scale Questionnaire [316]; viii) dietary habits, by completing a 69-item Food Frequency Questionnaire (FFQ) [317]; and ix) physical activity habits, by completing the short version of the IPAQ Questionnaire [318] (see Appendix F). Completion of the SF-12, AIS and IPAQ questionnaires and completion of information on the participants' anthropometric measurements (i.e., current weight, waist and hip circumference measurements), feeling of satiety and self-reported adherence to the proposed diet on a monthly basis was also a prerequisite prior to the renewal of the proposed diet. The latter was administered to the participant via access to the platform and was



Α.


Μηνιαία Ερωτηματολόγια

Α.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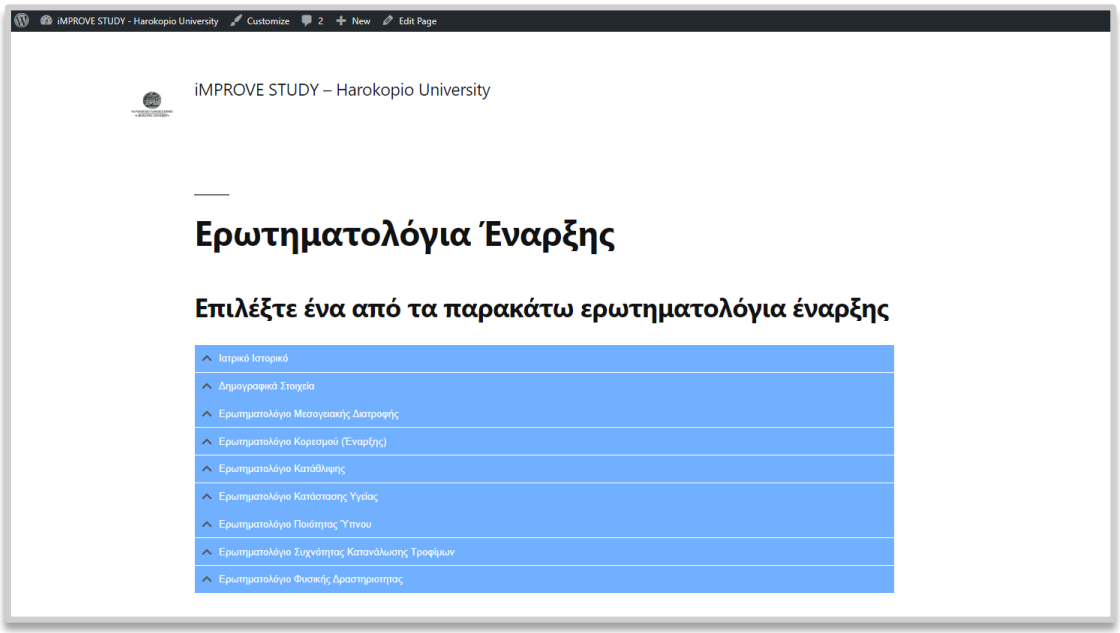
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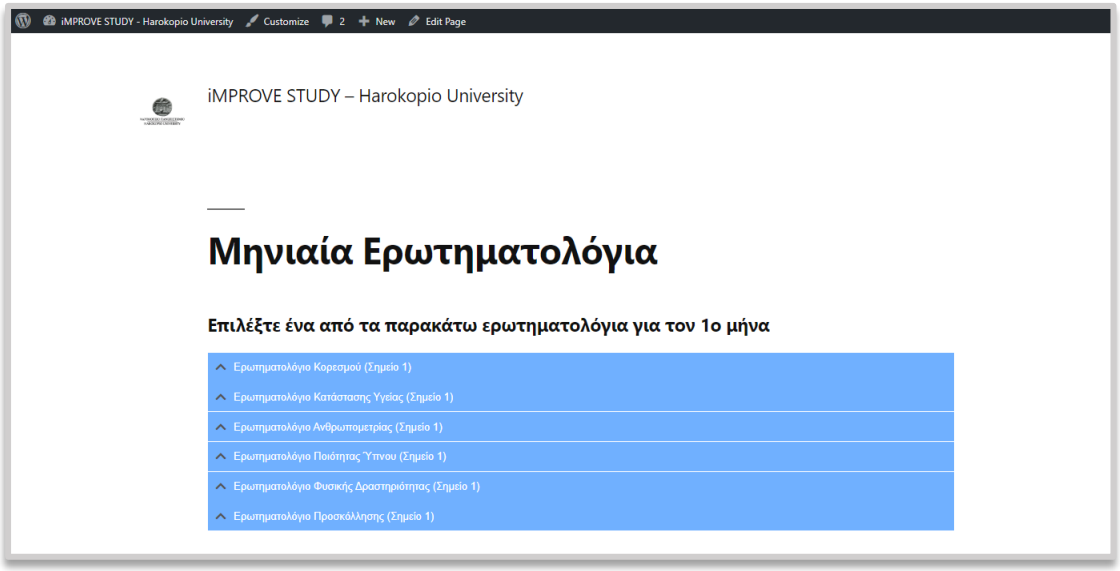
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Ερωτηματολόγιο Φυσικής Δραστηριότητας (Σημείο 1)
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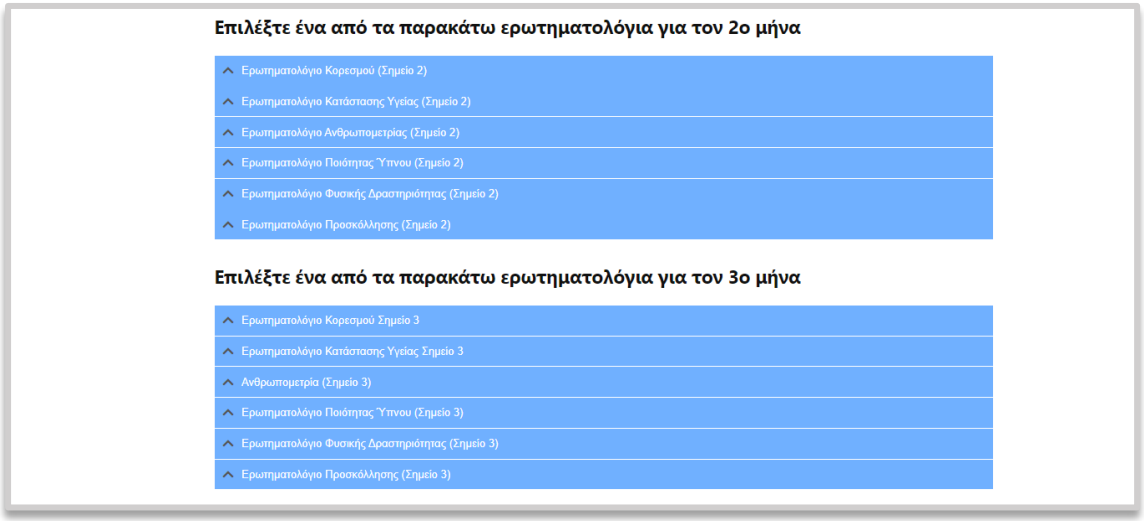
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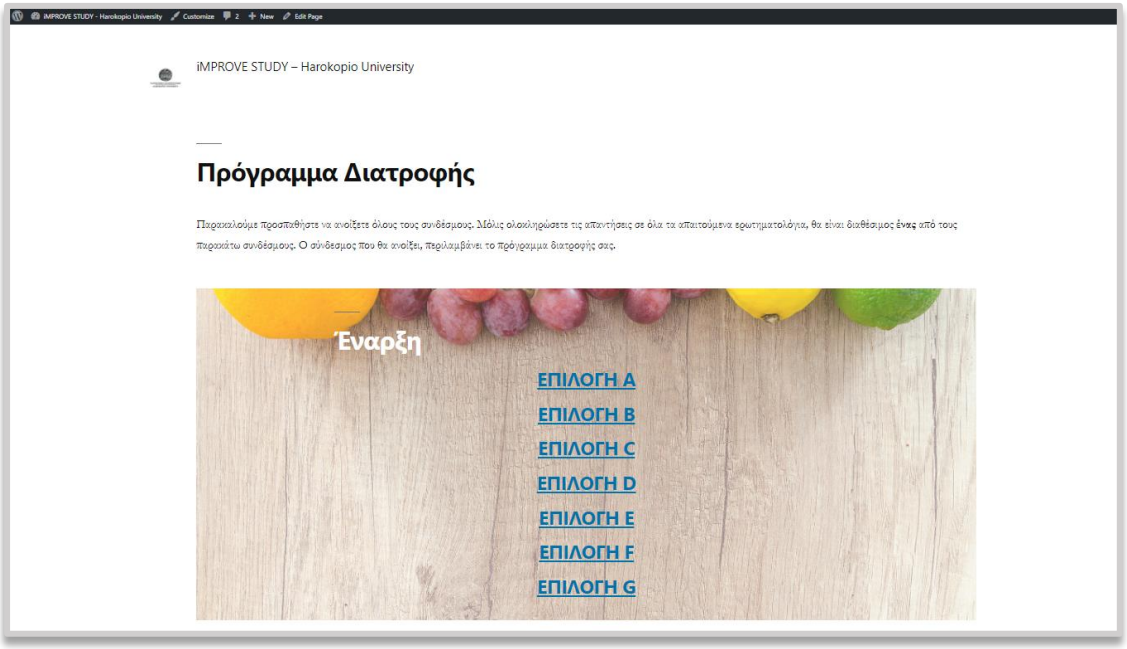


Figure 21. iMPROVE Study Online Questionnaire Tool, found at /http://83.212.122.254/. A. Welcome Page with links to baseline questionnaires, monthly questionnaires and the proposed dietary regimens; B. Page depicting the required baseline questionnaires to be filled out; C. Page showing the monthly questionnaires to be filled out; and D. page showing the links to download the proposed diet (only one link allowed to open per participant).

renewed monthly, once all questionnaires of the preceding month were filled out in the online tool and checked by the nutrition expert.

At the end of the baseline meeting, each volunteer was allocated a nutrition-expert contact who monitored their adherence to the proposed patterns and intervention progress by: i) conducting biweekly follow-up phone calls and monthly 24-h dietary recalls in order to discuss the potential concerns and provide advice; ii) monitoring the monthly completion of all online questionnaires; iii) evaluating the participant's self-reported monthly body measurements; and iv) renewing and allowing access to the proposed diet in the online tool.

2.1.3 Project Outcomes

The **primary outcome** of the study lied in the **change in body weight** after the proposed dietary intervention (minimum of three months). Its **secondary outcomes** are summarized in modifications of the following:

i. Changes in anthropometric measurements and body composition (i.e. WC, WHR, body fat percentage and visceral fat);
ii. Changes in biochemical profile (e g. TC, TG, glucose, HDL-C, LDL-C)
iii. Change in lifestyle parameters (i.e. sleep quality, overall health status and depression symptoms).

2.1.4 Anthropometric, Body Composition and Dietary Assessment

In all in-person meetings, basic anthropometric measurements were collected for each participant, by a trained health professional (i.e. dietitian or nutritionist) or a nutrition science student. Blood pressure was also measured, using an electrical sphygmomanometer, with the volunteer in a sitting position, without crossed legs, at the left-hand side, where the left arm stands relaxed on a surface at the height of the position of the heart. Body weight was measured in kg, via use of electronic scales with the participants in light clothing (clothing weight calculated at 0.5kg). Height was measured in cm using a stable stadiometer, where the participants were barefoot, with their shoulders in a relaxed position and while looking ahead. Waist circumference (WC) and hip circumference (HC) were measured in cm and in the nearest 0.1 cm, using a non-retractable body soft tape measure. The former was measured between the iliac crest and the twelfth rib and the later in measured at the point of the hips presenting the widest extension. Both measurements were taken when participants were standing straight, relaxed and with their hands parallel to their body. During all in-person measurements, guidance and reminders were provided in the participants so as to be able to conduct the measurements themselves on a monthly basis and report them in the online monthly questionnaires.

Body composition was analysed using a bioelectrical impendence analysis machine (*Tanita Body Composition Analyzer BC-418*), where the participants were in light clothing and without any metal objects on them. As described above (see *1.1.2. Body Weight and Composition Assessment Methods*) he method of analysing body composition via use of bioelectrical impedance analysis is based on the principle of measuring the body resistance to circulating electrical currents and the fact that, contrary to muscle tissue containing water, adipose tissue presents high electrical resistance. Participants were informed not to consume any food or drink and not undergo mediocre or intense physical activity/exercise for at least 2 hours prior to the analysis. Body composition analysis provided data on the individual's BMI as well as total and departmental body fat, muscle and water percentage.

Assessment of dietary intake at baseline took place via online completion of a the validated 69- item Food Frequency Questionnaire (FFQ). Dietary assessment and evaluation of the adherence to the proposed diet took place monthly via: i) a 24-h dietary recall, carried out by the nutrition expert; and ii) the online completion of the 5-scale self-reported adherence questionnaire.

2.1.5 Laboratory Analyses

Blood samples (23mL) were collected for each participant in all in-person meetings. Volunteers had been instructed to come to the meeting following a 12-hour, overnight fast. All blood samples were collected in daytime until 10.30 am; two of them in EDTA blood collection tubes and one in a non-EDTA containing vacutainer. Part of the samples (~ 250μL) was immediately sent for blood tests (i.e. red and white blood cells, platelets, hemoglobin and hematocrit). The other EDTA-containing tube and the non-EDTA containing tube underwent centrifuging at 1500rpm, at a temperature of 4^{o}C for 10 minutes. Plasma, buffy coat and red blood cells were collected from the former and serum was collected from later. Samples were stored

at -80^{o}C until being sent for further biochemical testing (i.e. measurements for TC, TG, HDL-C, LDL-C, glucose etc) by an external collaborator.

The buffy coat samples collected after centrifuging of the blood samples were used for DNA extraction. Part of the samples were extracted using the Invitrogen iPrep Purification Instrument and the Invitrogen iPrep PureLink gDNA Blood Kit [319], in the following steps:

i. The instrument was cleaned using a clean pad with 96% ethanol. The corresponding disc for DNA extraction from buffy coat samples was inserted in the machine and the "On" button was pressed.
ii. One iPrep™ PureLink® gDNA Blood Cartridge per sample was inserted in each one of the instrument positions.
iii. One iPrep™ Sample and Elution Tube containing the sample in the heated tube position of cartridge and one empty collection tube was inserted of each of the Cartridges used.
iv. Each one of the empty collection tubes samples' caps was placed in the instrument's T2 position.
v. The instrument was closed and the extraction was ran for approximately thirty minutes.
vi. After the end of the extraction, samples and cartridges were collected and removed from the machine. The instrument was cleaned using a clear pad with 96% ethanol and turned off. The disc for DNA extraction from buffy coat samples was removed and properly stored in a separate case.
vii. The DNA extracted samples were placed in room temperature (25^{o}C) for 24 hours.
viii. After the incubation, sample were placed in the magnetic instrument to remove remaining magnets and the content of the sample was transferred to a clean collection tube prior to measuring DNA concentration using a spectrophotometer.

Furthermore, DNA extraction took place manually for a small part of the samples, using the PureLink® Genomic DNA kits in the following steps:

i. 200μL of each sample were collected followed by addition of 20μL Proteinase K and 20μL RNaseA. The sample underwent short vortexing and was then left to incubate at room temperature (25^{o}C) for two minutes.
ii. 200μL of PureLink® Genomic Lysis/Binding Buffer was added to each sample, followed by a short vortexing and a 10-minute incubation at 55 ^{o}C.
iii. 200μL 96% ethanol was added to each sample, followed by short vortexing.
iv. The entire content of each sample (~640μL) was transferred to a PureLink® Spin Column Collection Tubes and was further centrifuged at 10.000 rpm for one minute at room temperature (25^{o}C)
v. After centrifugation, the Collection Tube for each sample was discarded and replaced with a new PureLink® Collection Tube.
vi. 500μL of the Wash Buffer 1 was added to each tube, followed by another centrifugation at 10.000 rpm for one minute, at room temperature (25^{o}C)
vii. After centrifugation, the Collection Tube for each sample was discarded and replaced with a new PureLink® Collection Tube.

viii. 500μL of the Wash Buffer 2 was added to each tube, followed by another centrifugation at 10.000 rpm for three minutes, at room temperature (25^{o}C).

ix. After centrifugation, the column containing the DNA sample was transferred to a new microcentrifuge tube, followed by addition of 50μL of the PureLink® Genomic Elution Buffer and another centrifugation at maximum speed (11000 rpm) for one minute at room temperature (25^{o}C).

Quantification of the extracted DNA took place via use of a spectrophotometer in the following steps:

i. The instrument was cleaned using a clean pad with 96% ethanol and, then opened.

ii. The option for measuring Nucleic Acids and ssDNA was selected.

iii. Using a 10μL pipette, one microliter of the black solution was used to μηδενίζω the device prior to measuring sample DNA concentrations.

iv. Using a 10μL pipette, one microliter of each sample was placed in the head of the photometer and the "Sample" button was pressed.

v. Sample absorbance at 260nm, 280 nm and 230nm (ratios 260/280 and 230/260) was recorded in a lab book for each of the samples. The 260/280 ratio is used to assess DNA sample purity and a ratio of 1.8-2.0 is considered to declare good sample quality. Lower 280/260 ratios indicate presence of other substances such as protein, phenols or other contaminants absorbing at or near 280nm [320]. The 260/230 ratio is used as a secondary measure to indicate sample clarity, with the range of 2 to 2.2. considered to be indicating a sample of good purity. Higher 260/230 ratios are considered to indicate the presence of contaminants absorbing at higher nm. Accordingly, lower ratios indicate the presence of substances absorbing at or lower than 230nm, such as carbohydrates and phenols [320].

All DNA samples were then stored at -20^{o}C for a maximum duration of 2 months up to being sent for further analyses and genotyping. If needed to be stored for more than 2 months, samples were stored at -80^{o}C.

Genotyping of at least 50ng/μL of the extracted DNA samples took place via: i) use of the Axiom Precision Medicine Diversity Research Array (PMD Research Array), containing more than 800.000 SNPs, deletions and copy number variations from the 1000Genomes Project Phase III [321], for a part of the extracted samples ; ii) use of the Axiom Precision Medicine Diversity Research Array (PMRA), containing more than 800.000 markers, for a different groups of extracted samples; and iii) GSA Erasmus MC. Following completion of the genotyping, imputation analyses took place using the 1000 genomes Phase 3 panel and the IMPUTE2 software.

2.1.6 Statistical Analyses

The entirety of phenotypic data analyses was conducted using the Statistical Package for Social Sciences (SPSS), version 23 [322], the R statistical package [323] and the STATA statistical software [324]. Variable distribution was evaluated using the Shapiro–Wilk, Kolmogorov–Smirnov tests and Q-Q plots. Mean values and standard deviation are presented for variables following the normal distribution, while median and interquartile range are presented for variables not normally distributed. Body

Mass Index (BMI) was calculated as weight divided by height (kg/m2) and LDL-C was calculated using the Friedewald Equation, where LDL − C = (TC) − (HDL − C) − (TG/5) [325]. Differences in mean/median values of variables within different groups (i.e. BMI groups, sex or diet groups) were evaluated using the non-parametric Mann–Whitney and Kruskal-Wallis tests. Multivariate linear regressions were employed to test for potential associations between the various phenotypic variables and are presented as beta coefficients (β) and standard error (SE). Variables not following the normal distribution were log-transformed.

Information on dietary habits deriving from the collected FFQs and 24-hour dietary recalls were analyzed using the Nutritionist Pro software [326]. The dietary patterns for the iMPROVE cohort were extracted by conducting principal component analysis (PCA) on 32 food groups deriving from the FFQ information. The Varimax orthogonal rotation was used and the Kaiser-Mayer-Olkin KMO and Bartlett's test was implemented to evaluate data adequacy. Five dietary patterns were set to be extracted with Eigen values bigger than 1. Potential associations between the extracted patterns and several indices were examined by separating them into tertiles and testing for associations using the parametric ANOVA test and the non-parametric Kruskal–Wallis test, depending on the distribution of the examined variable. Multivariate linear regressions were used to examine relations with anthropometric, biochemical and lifestyle indices, adjusting for three models of confounding factors (i.e. Model 1: age, sex; Model 2: age, sex, smoking habits, physical activity level and logBMI; and Model 3: age, sex, smoking habits, physical activity level, logBMI, education years, family and professional status). The level of statistical significance for all analyses was set at $\alpha = 0.05$ and results were also interpreted for the adjusted cut-off value of a = 0.05/number of patterns extracted (i.e., a = 0.05/5 = 0.01).

Following the extraction of the dietary patterns, we included them in using Pearson's chi-square test values to investigate all phenotypic variables for potential correlations to logBMI and/or body fat percentage. We used statistically significantly correlated variables to construct a novel Lifestyle Index (LI) where higher values indicated favorable effects. We further proceeded to test for potential associations with anthropometric and biochemical indices using multivariate linear regressions adjusting for age and sex (Model 1), as well as age, sex and BMI (Model 2).

We further proceeded to using the information on baseline physical activity levels and the calculated MedDiet Score into creating 4 groups of high or low PAL and high or low adherence to the Mediterranean diet. We subsequently used the Kruskal-Wallis test to assess within-group differences in anthropometric characteristics and conducted multivariate linear regressions to examine associations between the groups and anthropometric, biochemical and lifestyle indices, adjusting for age and sex (Model 1), as well as age, sex and BMI (Model 2).

Proceeding to the observed results of the dietary intervention, we used the non-parametric Mann-Whitney test to assess differences between the two sex and diet groups and the non-parametric Wilcoxon signed-rank test to assess differences pre- and post-intervention, as well as differences between baseline and the end of each examined month. In order to account for the missing values observed post-intervention, we used the STATA software to perform imputation of missing values, using the multiple imputation method conducted in the three following steps: i) the imputation step, where n completed imputed datasets are generated; ii) the

estimation step, where the desired analysis is performed separately to each of the imputations; and iii) the pooling step, where the results obtained from the estimations of *Step 2* are combined into a single multiple-imputation model. Hereby, the missing data mechanism was assumed to be missing-at random (MAR), as the loss to follow up did not differ between the two diet groups and no other known parameter (across the variables) was correlated with loss to follow-up. Additional performed several sensitivity analyses were performed in order to verify the stability of inferences produced at the pooling step. The correlation of body weight at baseline and the change in body weight at 3 months with categorical variables was investigated with Student's t-test or Mann-Whitney test and the non-parametric Kruskal Willis test. The correlation of body weight at baseline and the change in body weight at 3 months with numerical variables was investigated with Spearman's rho. The imputation step was performed for the body weight at 1st, 2nd and 3rd month of follow-up simultaneously using a multivariate normal regression model. When more than one variable contain missing values, it is advisable to impute the missing values in one analysis. As weight did not follow the normal distribution in the imputation model the logarithm of weight at month 1, 2 and 3 was imputed for the following scenarios: i) Weight Baseline, Sex, Age, Diet Group; ii) Weight Baseline, Sex, Age, Diet Group, Live alone; iii) Weight Baseline, Sex, Age, Diet Group, Live alone, education years; and iv) Base case: Weight Baseline, Sex, Age, Diet Group, Live alone, Fat (%) baseline. For each case, 20, 50 and 100 imputations were estimated, with the latter presenting robust results. The imputation model was a multivariate normal regression model using an iterative Marcov chain Monte Carlo (MCMC) procedure to generate impute values. For all cases, we assumed a burn-in period of 10,000 iterations, 1,000 iterations between 100 imputations, and a non-informative prior distribution (Jeffreys). The noninformative priors provide no extra information about model parameters beyond that already contained in the data. This model should be checked for convergence (i.e., if the produced distribution converges to a stationary distribution). For each one of the 100 datasets that were produced, we converted back the logarithm of weight in its normal scale for each month. Next, the change in weight was computed as the difference of the weight at 3rd month (for every one of the 100 imputations) minus the baseline weight. Model convergence was checked with trace and autocorrelation plots (Supplementary table A1 in Appendix). At the pooling step, a null linear regression model was used to estimate the mean (95% CI) change in body weight at 3 months of the total sample. An additional linear regression model was fitted to investigate the difference of mean change in body weight at 3 months between the two diet groups. The results of this step are presented as beta coefficients (95% CIs).

In order to investigate the effect of genetic predisposition in the examined indices at baseline and post-intervention, we set out to explore the effect of candidate genetic variants in two ways: i) via the construction of an unweighted and a weighted genetic risk score (uGRS and wGRS) using the well-known SNPs related to BMI published by Locke et al [326] (Supplementary Table S1); ii) via use of 10 target variants known for their associations with BMI, based on current literature (Table 11); and iii) via use of 10 target variants known for their associations with body fat indices

(Table 12), based on current literature. Regarding the changes in weight observed post-intervention, we proceeded to calculating an uGRS and a wGRS for BMI, using the BMI-related SNPs first identified In the cornerstone analyses of 2015 by Locke et al [326].

We used the available imputed data for the iMPROVE cohort. For missing SNPs, we included relevant proxies with an observed R^2 of above 0.8, using LDlink. Details on the SNPs and SNP proxies included in the wGRS and uGRS are presented in Supplementary Table S1. We used a threshold of 0.8 for the imputation INFO score for all SNPs included in the analyses. Quality control for sample and SNP exclusion criteria consisted of: i) Sample call rate at 95%; ii) genotyping call rate at 98%; iii) Hardy Weinberg Equilibrium (HWE) exact p<0.0001; and iv) minor allele frequency at 1%. After QC, we used the available data for 84 out of the 97 primary SNPs. Construction of the GRSs took place after coding each SNP genotype with 0,1 or 2 based on the copies of the effect allele (i.e. 0 for a homozygote without a copy of the effect allele, 1 for a heterozygote and 2 for a homozygote with 2 copies of the effect allele). Creation of the uGRS included the aggregation of all coded SNPs in an additive variable, while for the wGRS a subsequent multiplication took place using the SNPs' respective effect size according to the European sex-combined results provided from Locke et al [326]. The final, cumulative wGRS variable was, thus, presented in the form of:

$$wGRS = SNP1\ x\ \beta 1 + SNP2\ x\ \beta 2 + \cdots + SNP84\ x\ \beta 84.$$

Table 11. List of the BMI-related SNPs (n=10) investigated for associations in the iMPROVE cohort.

Consortial Summary Statistics								iMPROVE Cohort	
SNP	**Gene**	**Chr**	**Position (bp)**	**Alleles**	**MAF**	**Effect Allele**	**Direction of effect for BMI**	**MAF**	**Ref**
rs6548238_T	LINC01875, TMEM18	2	2:634905	T/C/G	0.12 (T)	C	Positive	T: 0.095	GIANT Consortium
rs1801282_G	PPARG	3	3:12351626	C/G	0.07 (G)	G	Positive	G: 0.053	GIANT Consortium
rs2241766_G	APIPOQ	3	186853103	T/A/C/G	0.15 (G)	G	Positive	G: 0.45	GIANT Consortium
rs925946_T	BDNF	11	11:27645655	T/A/C/G/	0.25 (T)	T	Positive	T: 0.103	GIANT Consortium
rs1421085_C	FTO	16	16:53767042	T/C	0.23 (C)	C	Positive	C: 0.26	GIANT Consortium
rs1121980_A	FTO	16	16:53775335	G/A/C	0.37 (A)	A	Positive	A: 0.28	GIANT Consortium
rs17817449_G	FTO	16	16:53779455	T/A/G	0.31 (G)	G	Positive	G: 0.28	GIANT Consortium
rs3751812_T	FTO	16	16:53784548	G/T	0.22 (T)	T	Positive	T: 0.25	GIANT Consortium
rs9939609_A	FTO	16	16:53786615	T/A	0.34 (A)	A	Positive	A: 0.26	GIANT Consortium
rs17782313_C	MC4R	18	18:60183864	T/A/C	0.24 (C)	C	Positive	C: 0.13	GIANT Consortium

SNP: Single Nucleotide Polymorphism**, Chr:** Chromosome, **bp:** base pairs, **MAF:** Minor Allele Frequency (as shown in GWAS Catalog**), Ref:** Reference

Table 12. List of the fat-related SNPs (n=10) investigated for associations in the iMPROVE cohort.

Consortial Summary Statistics								iMPROVE Cohort
SNP	**Gene**	**Chr**	**Position (bp)**	**Alleles**	**MAF**	**Effect Allele**	**Direction of effect for fat-related indices**	**MAF**
rs574367_T	LINC01741, SEC16B	1	1:177904075	G/T	0.15 (T)	T	Positive	T: 0.13
rs2605100_A	LYPLAL1-AS1,ZC3H11B	1	1:219470882	A/G	0.19 (A)	G	Positive	A: 0.22
rs4846567_T	LYPLAL1-AS1,ZC3H11B	1	1:219577375	G/T	0.22 (T)	G	Positive	T: 0.15
rs10195252_T	COBLL1	2	2:164656581	T/C	0.40 (C)	T	Positive	T: 0.25
rs206936_G	NUDT3,RPS10-NUDT3	6	6:34335092	A/G	0.43 (G)	G	Positive	G: 0.10
rs4994_G	ADRB3	8	8:37966280	A/G	0.12 (G)	G	Positive	G: 0.04
rs11191548_C	CNNM2	10	10:103086421	T/C	0.15 (C)	C	Positive	C: 0.06
rs6265_T	BDNF-AS,BDNF	11	11:27658369	C/T	0.20 (T)	T	Negative	T: 0.19
rs1443512_A	HOXC12,HOXC13	12	12:53948900	A/C/G/T	0.34 (A)	A	Positive	A: 0.14
rs12970134_A	RNU4-17P	18	18:60217517	G/A	0.21 (A)	A	Positive	A: 0.20

SNP: Single Nucleotide Polymorphism**, Chr:** Chromosome, **bp:** base pairs, **MAF:** Minor Allele Frequency (as shown in GWAS Catalog**), Ref:** Reference

We proceeded to testing for associations between the GRSs and the baseline anthropometric and lifestyle indices post-intervention, using multivariate linear regressions adjusting for age and sex (Model 1) and PAL and smoking (Model 2), in the R statistical package. We further investigated potential associations between the 10 BMI-related and the 10 fat-related target variants and the corresponding examined indices via multivariate linear regressions adjusting for age and sex (Model 1), as well as PAL and smoking (Model 2), using the PLINK toolset version 1.9. In order to examine the effect of gene-diet interactions in the tested indices, we investigated the impact of the interactions between the GRSs (using the R package) or the candidate variants (using the PLINK toolset) and the extracted dietary patterns for the baseline measurements. Again, we used multivariate linear regressions adjusting for age, sex, each GRS and dietary pattern (Model 1), as well as age, sex, PAL, smoking, each GRS and dietary pattern (Model 2). The levels of statistical significance for each examination were set as follows: i) for the baseline interactions between the GRSs and the five extracted patterns α was set at 0.01 (i.e. a = 0.05/5 = 0.01); and ii) for the baseline interactions between the 10 BMI or 10 body fat-related candidate variants and the five extracted patterns α was set at 0.003 (i.e. a = 0.05/15 components = 0.003).

In order to assess the genetic effect on the changes observed post-intervention, we first used the imputed data for the weight loss observed at three months to conduct multivariate linear regressions in the STATA software to examine the effect of the GRSs and the 10 BMI-related SNPs, adjusting for three models of confounding factors (Model 1: Adjusted for age, sex; Model 2: Adjusting for age, sex, PAL, smoking; Model 3: Adjusting for age, sex, PAL, smoking and diet group). Subsequently, we used the observed data for weight loss to assess potential differences within groups of high or low uGRS or wGRS (separated using the sample median) and the different genotypes of the 10-BMI related variants. Differences were examined using Mann-Whitney and the Kruskal-Wallis tests. We further investigated potential associations between the 10 BMI-related and the 10 fat-related target variants and the changes in the examined indices via multivariate linear regressions adjusting for age and sex (Model 1), as well as PAL and smoking (Model 2), using the PLINK toolset version 1.9.

In order to examine the effect of gene-diet interactions in the tested indices, we investigated the impact of the interactions between the GRSs (using the R package) or the candidate variants (using the PLINK toolset) and diet group for the changes observed post-intervention. Once more, we used multivariate linear regressions adjusting for age, sex, each GRS and diet group (Model 1), as well as age, sex, PAL, smoking, each GRS and diet group (Model 2). The level of statistical significance for the associations between the post-intervention changes and the 10 BMI or 10 body fat-related candidate variants was set at 0.005 (i.e. a = 0.05/10 variants = 0.005).

2.2. The 2018 Gutenberg Chair Project: TEENAGE and STANISLAS Cohorts

2.2.1. Ethics and Registration of Studies and Project Aims and Objectives

The aim of the 2018 Gutenberg Chair Project concerned the retrospective analysis of the lifestyle habits of two adolescent European populations and their potential associations with genetic variants and cardiometabolic indices. The project concerned the analysis of data collected for the adolescents of the Greek TEENAGE study and the French STANISLAS Family Study. The objectives of the project were, thus, shaped as follows:

i. To *identify* the dietary patterns followed in the two populations;
ii. to *investigate* the interaction between the dietary habits and indices of cardiometabolic profile;
iii. *to assess* the effect of genetic variants on the formation of adolescent anthropometric and cardiometabolic indices.

For the purposes of investigating gene-diet interactions on anthropometric and biochemical profile, we built on our team's previous expertise and sought to examine the combined effect of VEGF-A-related variants, due to its previously-established impact on the indices.

In this context, data from the Greek TEENAGE and the French (Suivi Temporaire Annuel Non Invasif de la Sante des Lorrains Assures Sociaux (STANISLAS) Family Study were analysed. The TEENAGE study involved adolescent participants from the region of Attica, Greece and was conducted according to the principles laid out by the Declaration of Helsinki. The study was approved by the Institutional Review Board of Harokopio University [protocol number 20/29-05-2008] and the Greek Ministry of Education and Religious Affairs. Prior to study enrolment, all adolescent participants and their parents received information on the study protocol and procedures with teenagers proceeding to providing gave verbal consent, followed by their respective guardians' written consent.

The STANISLAS Family study involved a 10-year longitudinal cohort including families from the regions of Vosges and the South of Meurthe and Moselle, France. The study was approved by the advisory committee for the protection of people in biomedical research in Nancy, France and all study protocols were approved by the institutional ethics committees. All STANISLAS volunteers provided written informed consent prior to study enrolment, while consent for underage participants was provided by their respective parents/legal guardians.

As of 2023, relevant project proceedings included in the present thesis have already been published in peer-reviewed articles [311,312].

2.2.2. Study Designs and Study Populations

The TEENAGE (Teens of Attica: Genes and Environment) study was a cross-sectional study conducted in the Attica region during the period 2008-2010. The study consisted of healthy teenagers, students of schools in Attica aged 13 to 15 years old. **The aim of the study** was the assessment the teenagers' anthropometric measurements, body composition, biochemical profile, dietary and physical activities habits and eating behaviours and investigation of their potential relations with genetic background.

Details on the TEENAGE study design and research have already been described elsewhere [328-330]. For the purposes of the study, all the teenagers and their parents received information on the study protocol and procedures and provided written consent prior to their enrolment. Overall, 857 adolescents from schools of Attica were recruited. All participants underwent a 2-stage, baseline in-person session with a healthcare professional (nutritionist or paediatric health care professional), during which they provided the written consent, followed by collection of anthropometric, dietary, physical activity and clinical assessment data and blood samples, were collected. After a 10-period following the baseline meeting, participants were telephonically contacted by the healthcare professionals, to conduct a secondary dietary and physical activity assessment (i.e. via 24-hour recalls).

Regarding the STANISLAS Family study, details on its design and research have, also, already been described elsewhere [331-333]. The study constituted a 10-year longitudinal study conducted in the region of Vosges and the South of Meurthe and Moselle (East part of France), first recruiting volunteers during the period 1993-1995. The study consisted of healthy nuclear families, with parents up to the age of 65 years old and children older than 6 years of age, living in the above region. Available volunteers participated in three 5-year-follow-ups until the period 2003-2005 for participants living in the region of Nancy, France. All participants were of European-Caucasian origin, without reporting existence of chronic disorders (CVD, cancer, diabetes, hypertension etc.). Overall, 1006 families were recruited.

2.2.3. Project Outcomes

The **primary outcome** of the present study analyses using data from both the TEENAGE and the STANISLAS cohorts, concerns the assessment of dietary habits in the formation of teenage cardiometabolic indices. Furthermore, the study secondary outcomes include: the assessment of genetic variants on adolescent cardiometabolic profile and the subsequent investigation of their combined effect with lifestyle characteristics.

2.2.4. Anthropometric and Lifestyle Assessment

For the nodes of the TEENAGE study, anthropometric data were collected as follows: i) weight measurements, in kg (via use of scales, were the participants were in light clothing and barefoot); ii) height measurements, in cm (using a portable stadiometer, where volunteers barefoot, with relaxed shoulders and while looking ahead); waist and hip circumference measurements, in cm (via use of a soft tape, at the twelfth rib and the iliac crest for the former and at the widest point of the hips for the later). Skinfold measurements for the triceps, subscapular and suprailiac skinfolds were collected using a Lange skinfold calipers.

Dietary habits were assessed via provision of a 24-hour dietary recall during the assessment meeting and a second 24-hour recall in the 10-day period after the in-person session. Dietary data were analysed using the Nutritionist Pro software [326] and mean intakes of energy, macronutrients and micronutrients were provided for each participant.

In the context of the STANISLAS Family Study, all participants underwent an in-person session with trained professionals during which anthropometric, dietary, lifestyle and clinical examination data and blood samples, were collected. Anthropometric data were collected as follows: i) weight measurements, in kg; ii) height measurements, in cm; waist-to-hip ratio measurements. WC was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. All measurements were recorded by trained professionals to the nearest 0.1 cm. Dietary habits were assessed by completion of a 3-day food consumption diary at baseline. Analysis of the dietary data was conducted using the GENI package {52}, nutritional database program and mean intakes of energy, macronutrients and micronutrients were provided. Body Mass Index (BMI) for both studies was calculated as weight divided by height (kg/m^2).

2.2.5. Laboratory Analyses

Regarding the TEENAGE Study, blood samples were collected during the in-person session with each adolescent in every participating school. DNA samples were collected for each participating adolescent and were further genotyped via use of the Illumina HumanOmniExpress BeadChips (Illumina, San Diego, CA, USA) at the Wellcome Trust Sanger Institute, Hinxton, UK [328-330.]. Imputation of the genotyped data was conducted using the Haplotype Reference Consortium (HRC) panel. For the STANISLAS Family Study, blood samples were collected during the baseline meeting and all related measurements were conducted at the laboratory of the Centre for Preventive Medicine (CMP) in Vandoeuvre lès Nancy, France [331-333]

2.2.6. Statistical Analyses

All data phenotypic analyses were carried out via use of the Statistical Package for the Social Sciences (SPSS), version 23 [322] and the R Packages [323], while genetic data were analyzed using the PLINK: Whole genome data analysis toolset version 1.9 [333]. Assessment of phenotypic variables' distribution was conducted via use of the Shapiro–Wilk and Kolmogorov-Smirnov tests. Variables displaying normal distribution are presented as mean and standard deviation (SD), whereas the median and interquartile range (IQR) is shown for all variables not following the normal distribution (Shapiro–Wilk/ Kolmogorov-Smirnov $p > 0.05$). Non-normal variables were log-transformed for normal distribution. We used the Student's t-test for parametric and the Mann–Whitney test for all non-parametric hypotheses testing for continuous variables. LDL-C was calculated for the STANISLAS adolescents using the Friedeweld Equation [LDL − C = (TC) − (HDL − C) − (TG/5)] [324] and pulse pressure (PP) was calculated for the TEENAGE subjects as systolic blood pressure (SBP in mmHg) -D diastolic blood pressure (DBP in mmHg).

We used dietary data from the 24-hour dietary recalls of 766 teenagers from the TEENAGE Study and 3-day food diaries from 287 adolescents from the STANISLAS Family Study, to proceed to identification of food groups and conduct PCA to extract all dietary patterns for both populations. PCA is a widely-used

epidemiological tool, implemented in the assessment of dietary data and the subsequent extraction of dietary patterns [35], having been previously tested in large young populations [36]. PCA was conducted on 15 food groups for the TEENAGE study population and 15 food groups for the STANISLAS Family study population, based on the available data for the cohorts. The varimax orthogonal rotation was used for the extraction of the patterns and the Kaiser criterion was set at retaining 5 components with Eigen values bigger than 1

Testing for phenotypic associations between the extracted patterns and the cardiometabolic indices took place via multiple linear regressions in the TEENAGE cohort and linear mixed modeling in the STANISLAS cohort, due to the latter consisting of family members and the subsequent need for correction of family bias of siblings included in the analyses. Associations were investigated, adjusting for 4 different models of confounding factors. Model 1 included adjustment solely for the age and sex of the participants; Model 2 included adjustment for sex, age and level of physical activity; Model 3 consisted of adjustment for their age, sex, level of physical activity and BMI; and, finally, Model 4 included adjustment for age, sex, physical activity, BMI and energy intake. Multiple linear regression results are presented as beta coefficients (β) and standard error (SE). Linear mixed model results are presented as estimates and standard error (SE). All statistical analyses included the level of nominal significance set at $\alpha = 0.05$ and the adjusted threshold after multiple testing was set to (0.05/5 components examined, i.e., dietary patterns = 0.01).

Based on our team's previous expertise in VEGF-A research, in order to investigate gene-diet interactions, we chose to examine the effect of 11 previously VEGF-A-associated variants. We separately examined associations between each variant and the indices of interest. We used a threshold of 0.7 for the imputation INFO score for all SNPs included in the analyses. Quality control for sample and SNP exclusion criteria consisted of: i) Sample call rate at 95%; ii) Hardy Weinberg Equilibrium (HWE) exact $p<0.0001$; and iii) genotyping call rate at 99%. We investigated potential relations between the 11 target SNPs and the cardiometabolic parameters, via linear regression analyses. Associations were examined after adjusting for 3 different models of confounding factors, namely: i) Model 1, which consisted of adjustment for age and sex; ii) Model 2, which further included exercise level; and iii) Model 3 additionally incorporating the adjustment for the five previously extracted dietary patterns [19]. Multiple linear regression results for each SNP are presented as betas [regression coefficients (β)] and p-values. The threshold for statistical significance was set at 0.05. The adjusted threshold for multiple testing was set at 0.005 (0.05/11 components examined).

We further proceeded to constructing an unweighted genetic risk score (uGRS) for VEGF-A using the target-SNPs identified by Choi et al. For the purposes of the present analyses, we used the SNPs with available data in the TEENAGE cohort (i.e. 9 out of 10 variants). The uGRS was constructed via scoring the risk alleles positively associated with VEGF-A levels in the following steps:

i. Each SNP received a score of 0,1 or 2, according to the number of effect alleles of the participants' genotype (i.e. a homozygote for the effect allele received a score of 2, a heterozygote received a score of 1 and a homozygote for the non-effect allele received a score of 0);

ii. Creation of the uGRS took place by adding all coded genotypes in a summarized variable of the form uGRS= SNP_1 (coded G_1) + SNP_2 (coded G_2) + … + SNP_9 (coded G_9).

Following the associations explored for each SNP separately, we further used multiple linear regressions to examine associations between the uGRS and the metabolic indices, as well as the potential effect of the interactions between the uGRS and the formerly extracted dietary patterns (eg. uGRS*Dietary pattern 1). Multiple linear regression results are presented as estimates [beta coefficients (β)] and standard error (SE). In the case of examining the interactions, the adjusted threshold for statistical significance was set at 0.01 (i.e. 0.05/5 components examined).

2.3. Polygenic Risk Score for Body Mass Index on the NAFLD, THISEAS and OSTEOS studies

2.3.1. Ethics, Registration for the Studies and Project Aims and Objectives

The present project constitutes a retrospective analysis using data from three previous studies, namely the case-control Greek Non-Alcoholic Fatty Liver Disease (NAFLD) study [334,335], the cross-sectional OSTEOS study [336] and the case-control THISEAS (The Hellenic Study of Interactions between Single Nucleotide Polymorphisms and Eating in Atherosclerosis Susceptibility) [337,338] studies. All three studies were approved by the Research Ethics Committee of Harokopio University of Athens: NALFD protocol number: 38074/13-07-2012; OSTEOS protocol number: 15/8-12-2005, 8/12/2005 and THISEAS protocol number: 10/9-6-2004, 14/6/2004 and all participants provided written consent prior to enrolment.

Hereby, use of part of the studies' data (i.e. BMI measurements) took place with the **overall aim** of creating a PRS for adult BMI, using a novel method created in the nodes of our team's activities. **The project objectives** were, thus, shaped as follows:

i. *To create* a PRS for BMI using data from the NAFLD, OSTEOS and THISEAS studies; *and*
ii. *to investigate* the effect of the generated PRS for BMI on the BMI levels of our pooled cohort.

As of 2023, relevant project proceedings included in the present thesis have already been published in a peer-reviewed article [208].

2.3.2. Study Designs and Study Populations

The NAFLD study constituted a cross-sectional study conducted in the region of Attica. The study recruited adult participants without liver disease/injury and reporting absence of excess alcohol drinking at the time of induction to the study. The aim of the study was to assess the effect of genetic background and gene-diet interactions in NAFLD presence. Details on the study protocol have already been published elsewhere [334, 335]. Briefly, volunteers were recruited from the Outpatient Clinics of the First Department of Propaedeutic and Internal Medicine in Laiko General Hospital, during the period 2012 to 2015 [334,335]. Recruits were further screened for NAFLD through abdominal ultrasound, and deemed as controls in the absence of hepatic steatosis or in the presence of mild stage, or cases in the presence of moderate or severe hepatic steatosis [334,335]. Overall, 342 participants were recruited and proceeded to enrolling in the study.

The OSTEOS project also constituted a cross-sectional study conducted throughout Greece during the 2010–2012 period. The project included community-dwelling adults from rural and urban areas of Greece. The aim of the study was to assess parameters of bone health in adults and investigate the potential effect of genetic predisposition. Details on the study protocol have already been published elsewhere [336]. Briefly, volunteers were recruited in cooperation with the Hellenic Society for the Support of Patients with Osteoporosis and the Laboratory for the Research of Musculoskeletal System "Th. Garofalidis", School of Medicine, National and Kapodistrian University of Athens and underwent assessment of bone health

status using quantitative ultrasound (QUS). Overall, 970 adults were recruited and proceeded to enrolling in the study.

Finally, the THISEAS study was another project of cross-sectional design, conducted in the region of Attica during the years 2006–2010. The aim of the study was to assess the effect of genetic and lifestyle determinants in CVD and atherosclerosis. Details on the study protocol have already been published elsewhere [337,338]. Briefly, recruits were mainly assessed using coronary angiography information and were categorized as controls if they presented negative coronary findings or a negative stress test, or did not report any related clinical symptoms. Volunteers were categorized as cases in the presence of acute coronary syndrome or stable coronary artery disease (> 50 % stenosis in ≥ 1/3 main coronary vessels) [337,338]. Overall, a total of 2565 participants were recruited from three Athenian hospitals, open protection centres and municipalities during the project period.

2.3.3. Project Outcomes

The **primary outcome** of the present study analyses using data from all three studies, concerns the creation of a PRS for BMI. Furthermore, as it will be explicitly described below, the effect of the developed PRS on the BMI measurements of the pooled data was further examined.

2.3.4. Anthropometric Measurements

Anthropometric measurements of body weight and body height were measured for all three studies. Body weight was measured using the TANITA Segmental Body Composition Analyzer BC-418 and a calibrated scale to the nearest 0.1 kg. Height was calculated to the nearest 0.5 cm using a mounted stadiometer. Participants were barefoot and maintained light clothing and the measurements occurred twice and average values were kept as final in all projects. All measurements were conducted by trained professionals. Body Mass Index (BMI) for all studies was calculated as weight divided by height (kg/m2).

2.3.5. Laboratory Analyses

Blood samples were collected at the baseline in-person meetings for all volunteers of the three studies. For the NAFLD study, DNA samples were isolated using peripheral blood lymphocytes and genotyped via use of the Infinium CoreExome-24 BeadChip, Illumina genome-wide SNP array (with 567,218 fixed markers). OSTEOS' DNA samples were isolated from buffy coats and genotyped using the Axiom Precision Medicine Diversity Research Array [with over 850,000 SNPs, insertions, deletions and copy number variations (CNVs)]. DNA samples from the THISEAS study were extracted from whole blood and genotyped using the Illumina Metabochip (with about 200.000 SNPs).

2.3.6. Statistical analyses

Data analysis was conducted using the R, Plink version 1.9, IMPUTE2 and PRSice2 packages. For the genetic data, Plink 1.9 [333] toolset was used to convert all relevant files into plink file format (BED+BIM+FAM files). Imputation of missing SNPs

for the pooled cohort was conducted using the snpStats and the R package scrime, version 1.3.5 packages and extension of the dataset was conducted using the IMPUTE2 software [339] (1000 Genomes Project reference panel).

A threshold of 0.9 for the imputation INFO score was used for all SNPs included in the analyses. Quality control for sample and SNP exclusion criteria consisted of: i) Sample call rate at 95%; ii) Hardy Weinberg equilibrium exact p-value at < 10−9; iii) Minor Allele Frequency at 5%; and iv) genotyping call rate at 90%. To account for population stratification, PCA was conducted using the SNPRelate, version 1.30.1 R package and the Tracy-Widom statistic was used to assess significant components based on eigen values [340]. In an effort to maximise the quality of the extracted PRS and simultaneously examine the efficacy of already implemented approaches, summary statistics were extracted using General Linear Models (GLM, R version 4.2.0), statgenGWAS version 1.0.8. [341], SNPTEST version 2.5.4 [342] and Plink 1.9.

PRS extraction proceedings took place via use of the PRSice2 package and after a repetitive process of the following steps for a total of 100 times:

i. the overall cohort was split into a training (80% of the samples) and a testing set (20% of the samples);
ii. association tests for each SNP and BMI were performed using all 4 aforementioned methods, while correcting for confounding factors (i.e. sex, age, NAFLD status and the significant PCA-derived components);
iii. the target set was imported to PRSice2 along with the summary statistics from the application of each methodology, where the package extracted the optimal number of SNPs to be included in a candidate PRS, along with performance metrics on the PRS statistical significance and the percentage of additional variance explained (R2), in a variable of the form $\mathrm{PRS} = \sum_{i=1}^{k} \frac{\beta_i G_i}{N}$;

Following the extraction of the candidate PRSs, the frequency of each included SNP in the PRSs was measured, using a minimum threshold of appearing 5 times to further proceed to the downstream procedures. Subsequently, a PRS was constructed for each frequency, comprising the multiplication of the SNPs appearing equally or above this frequency with their corresponding weights, averaged over the iterations where each SNP appears. This was repeated for all observed frequencies creating a distribution of PRS R^2 value, which were further penalized based on the number of SNPs in the PRS. As a result, the PRS with the best performance metrics (R^2) was chosen for further analysis. For the purposes of present the data, PRS reported values were normalized to a range of 0 to 1.

3. Results and Discussion

3.1. The iMPROVE Study

3.1.1. Baseline Characteristics of the Study Population

Parts of the following information in 3.1.1. constitute information published under the publication Nutrients 2021, 13, 3495. https://doi.org/10.3390/nu13103495 and can be further found in Appendix C.

Out of the 235 volunteers expressing interest to participate in the study, data are shown for 202 eligible subjects who successfully attended the baseline meeting, completed the majority of the baseline questionnaires using the online tool, and were recruited in one of the two intervention arms. The sample size of the 202 individuals assures adequate power to detect statistical significance. Table 13 shows that the vast majority of the participants were reported as non-smokers (151 non-smokers vs. 50 smokers, out of 201 participants with available data), with women representing the majority of the observed smokers (38 out of 50 overall smokers, 76%). The estimated baseline physical activity level showed that roughly half of the subjects were leading a moderately active way of life (104 out of the 199 participants with available data), with about 32% reporting a sedentary lifestyle (64 out of 199 participants). Volunteers reporting vigorous activity represented the smallest sample with only 31 out of 199 participants. All 202 eligible volunteers were randomly allocated to the intervention groups, with 46.5% following the high-carbohydrate/low-fat diet and 53.5% adhering to the high-protein diet.

Table 13. Baseline characteristics of the iMPROVE cohort, by sex.

Variable	Total	Men	Women
Smoking	201	59	142
Smokers	50	12	38
Non-smokers	151	47	104
PAL	199	59	140
Low	64	20	44
Moderate	104	28	76
Vigorous	31	11	20
Diet Group	202	59	143
High Carb/Low Fat	94	19	75
High Protein	108	40	68

The entirety of the study population's anthropometric, clinical, dietary, and lifestyle characteristics in the whole sample, as well as per weight group (with overweight vs with obesity), is presented in Table 14. Median ± IQR is presented for all non-normally distributed variables and mean ± standard deviation (SD) is presented for the variables following the normal distribution. Our baseline sample consisted of 143 women (70.8%) and 59 men (29.2%), with a median age of 47 years old. The majority of participants were married (60.9%), with more than half of our sample reporting having higher education (61.9%) and less than 3% reporting having no acquired education at all. Contrary to the existence of overweight and obesity, measurements on the entire sample indicated an overall relatively "healthy" profile, given the within-normal-range values for most biochemical and lifestyle indices measured.

Compared to volunteers with overweight, individuals with obesity presented statistically significant increased levels of blood pressure measurements (SBP of 123 vs 119.19, p-value=0.025, DBP of 82.74 vs 80, p-value=0.001). The dominating

difference between the two groups was mostly apparent across the spectrum of anthropometric indices, where, naturally, individuals with obesity presented higher levels of weight and BMI (96kg vs 78.24kg, p<0.001 and 34.3kg/m^2 vs 27.82kg/m^2, p<0.001, respectively). The difference between the two groups was vastly apparent on body composition indices where all measured markers presented higher levels in the obesity group, with, namely: higher levels of body fat percentage (40.82% vs 35.45%, p-value<0.001), body fat in kilograms (37.8kg vs 25.85kg, p<0.001), fat-free mass (54kg vs 48kg, p-value<0.001), total body water (54kg vs 48kg, p<0.001), visceral fat (level 12 vs level 8, p<0.001), percentage of upper body fat (39.52% vs 33%, p<0.001), kilograms of upper body fat (20kg vs 14kg, p<0.001), kilograms of fat-free mass (30kg vs 27kg, p<0.001), WC (104.5cm vs 93cm, p<0.001), HC (120cm vs 110cm, <0.001) and WHR (0.87 vs 0.85, p<0.040). On the contrary, the two groups did not present statistically significant different levels of biochemical indices or lifestyle characteristics, denoting a relative homogeneity for those markers across the entirety of the cohort.

Table 14. Anthropometric, clinical, dietary characteristics and characteristics of depression, quality of sleep, and health status in the iMPROVE cohort, by weight group (i.e. with overweight vs obesity).

Variable	Total		With Overweight		With Obesity		
	N	Median (IQR)	N	Median(IQR)	N	Median (IQR)	*p**
Age	202	47 (15)	78	47 (18)	124	47.5(13)	0.855
SBP (mmHg)	198	121.00 (21)	77	119.19 (14.87)**	121	123(24)	**0.025**
DBP (mmHg)	198	80.8 (9.8)**	77	80 (11)	121	82.74 (10.19)**	**0.001**
Pulse Rate (Beats per minute)	198	74 (15)	77	73.03 (9.66)**	121	74 (20)	0.125
Anthropometric Characteristics							
Weight (kg)	202	87 (26)	78	78.24 (9.52)	124	96(24)	**<0.001**
BMI (kg/m^2)	202	31.35 (6.9)	78	27.82 (1.32)**	124	34.3 (6.5)	**<0.001**
Body fat (%)	202	37.95 (10.8)**	78	35.45 (11)	124	40.82 (10.2)	**<0.001**
Body fat (kg)	202	32.95 (13.3)	78	25.85 (4.96)**	124	37.8 (15.1)	**<0.001**
Fat free mass(kg)	202	52 (18)	78	48 (17)	124	54 (19)	**<0.001**
Total body water (kg)	202	38 (13)	78	35 (13)	124	39.5 (14)	**<0.001**
Visceral fat	202	10.00 (6)	78	8 (3)	124	12 (5)	**<0.001**
Upper body fat (%)	201	36.7 (6.98)	78	33 (8)	123	39.52 (6.37)**	**<0.001**
Upper body fat (kg)	201	18 (7)	78	14 (2)	123	20 (8)	**<0.001**
Upper body fat-free mass (kg)	201	29 (9)	78	27 (8)	123	30 (9)	**<0.001**
Waist circumference (cm)	183	99.00 (17)	75	93 (12)	108	104.5 (16)	**<0.001**
Hip circumference (cm)	183	115 (13)	75	110 (7)	108	120 (15)	**<0.001**
WHR	183	0.85 (0.08)**	75	0.85 (0.11)	108	0.87 (0.09)	**0.040**
Biochemical Biomarkers							
Fasting glucose (mg/dL)	193	92.00 (11)	76	92 (10)	117	93 (12)	0.128
Urea (mg/dL)	193	28.00 (9)	76	28.9 (7.09)**	117	27 (8)	0.332
Creatinine (mg/dL)	193	0.68 (0.21)	76	0.68 (0.21)	117	0.68 (0.21)	0.765
Uric acid(mg/dL)	193	4.70 (1.5)	76	4.49 (0.97)**	117	5.02 (1.19)**	0.001
Total cholesterol (mg/dL)	193	177.96 (33.58)**	76	177.69 (31.57)**	117	178 (47)	0.967
HDL-C (mg/dL)	193	49.00 (17)	76	52.79 (11.82)**	117	47 (14)	0.018
LDL-C (mg/dL)	193	105.20 (38.7)	76	107.05 (28.27)**	117	105.6 (31.47)**	0.963
Triglycerides (mg/dL)	193	90.00 (65)	76	78.5 (43)	117	96 (80)	0.002
Total bilirubin (mg/dL)	193	0.37 (0.23)	76	0.39 (0.25)	117	0.37 (0.23)	0.904
Direct bilirubin (mg/dL)	193	0.16 (0.08)	76	0.16 (0.08)	117	0.15 (0.08)	0.762
Serum protein (g/dL)	193	6.70 (0.5)	76	6.65 (0.5)	117	6.7 (0.5)	0.755
Serum albumin (g/dL)	193	4.20 (0.3)	76	4.29 (0.27)**	117	4.2 (0.4)	0.013
SGOT/AST (IU/L)	193	16.00 (6)	76	16 (5)	117	16 (7)	0.314

SGPT/ALT (IU/L)	192	15.00 (12)	76	14.5 (9)	116	15 (15)	0.124
Lifestyle Characteristics							
MedDiet Score **	147	30.85 (3.86)**	59	31.53 (3.64)**	88	30.40 (3.98)**	0.072
AIS Score	140	5 (7)	49	6 (7.5)	91	5 (6)	0.584
CESD-R-10 Scale	201	6 (5)	78	6 (5)	123	6 (5)	0.586
SF PCS 12 Score	145	52 (12)	53	52 (8)	92	51 (12)	0.196
SF MCS 12 Score	145	46 (16)	53	49 (19)	92	49 (13)	0.538

*p: p-value of Mann-Whitney within-group tests

** The selected variables follow the normal distribution and are presenting as mean ± SD

Concerning the lifestyle characteristics, calculation of the MedDiet score demonstrated mediocre adherence of the overall sample to MD with a mean of 30.85 out of a scale indicating greatest adherence at a maximum score of 55. At baseline and throughout the study period, the 8-item AIS score on evaluation of sleep qualities was calculated for participants who reported the selected outcomes more than three times per week in the month leading to the score measurement. At baseline and for the overall sample, the AIS score presented a median of 5 out of the scale maximum scoring of 24 highlighting that, overall, participants did not express significant irregularities neither in sleep quality, including sleep induction, total sleep duration, and awakenings at night and expressed delayed sleep induction, nor in effects of sleep on aspects of the next day (i.e., well-being, overall functioning, and sleepiness). Similarly and as evaluated via the CESD-R-10 scale, the majority of the participants did not display depression characteristics, such as feelings of fear and helplessness, with the overall sample presenting a mean score of 6 and the scale maximum scoring calculated at 18. Concerning the quality-of-life measurements evaluated with the SF-12 short questionnaire, its physical component (SF PCS 12) on self-reported quality of life showed a median score of 52 out of a maximum of 100 for the overall sample. As the cut-off for a satisfactory physical state is set at 50, the observed median of 52 denotes a mediocre level of physical functioning. The questionnaire's mental component median of 46 showed that the participants presented a mediocrely satisfactory level of mental functioning, as its cut-off of 42 or less implies signs of mediocre-to-deteriorating mental functioning state.

Overweight participants constituted 38.6% of our overall sample, with the remaining 61.4% spreading across the three different obesity categories (35.1%, 14.9%, and 11.4% of the participants classified as Class I, II, or III obese, respectively). After investigating within-group potential differences in the lifestyle measurements between the extensive categorizations of BMI levels, no statistically significant differences were shown (Figure 22).

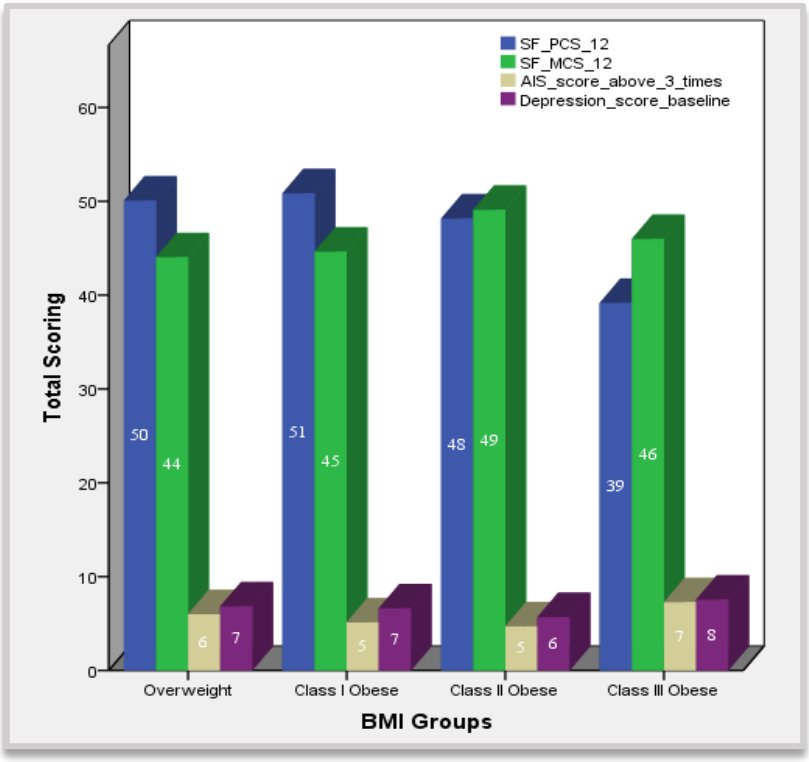


Figure 22. Baseline scoring of the four lifestyle questionnaires, based on extensive BMI categories.

Moreover, we moved to conducting multivariate linear regressions to further examine the potential effect of the aforementioned lifestyle aspects on the baseline logBMI and body fat percentage of the entre cohort. After adjusting for age, sex, smoking habits, physical activity level and education years, a statistically significant association was shown only for the SF-PCS-12, displaying a negative effect on both logBMI and body fat percentage values ($\beta = -0.003$, $p < 0.001$ and $\beta = -0.204$, respectively).

Table 15. Multivariate linear regression analyses on the relation between lifestyle characteristics and BMI and body fat baseline values.

Variable	Model 1 *		
	β	*SE*	*p*-Value
logBMI			
AIS Score	0.001	0.001	0.609
CESD-R-10 Scale	0.002	0.001	0.188
SF PCS 12 Score	−0.003	0.001	**<0.001**
SF MCS 12 Score	0.001	0.001	0.232
Body fat (%)			
AIS Score	0.174	0.125	0.167
CESD-R-10 Scale	0.163	0.099	0.102
SF PCS 12 Score	−0.204	0.057	**<0.001**
SF MCS 12 Score	0.062	0.053	0.249

Model 1: Adjusted for age, sex, smoking, physical activity level and education years

Baseline Dietary Patterns for the iMPROVE cohort

Following the collection of data on the 202 participants' dietary intake using the 69-item FFQ, we proceeded to conducting PCA on 32 identified food groups. The analysis resulted in the identification of five dietary patterns accounting for 40.34% of the sample's total variance. The KMO and Bartlett's Test ($p < 0.001$) presented a KMO Measure of 0.726, indicating sufficient data adequacy. All factor loadings for each component were above or approaching a value of 0.3. As shown in Table 16, the 32 food groups reflected the variety of foodstuffs consumed by the sample population, including both widely consumed food categories such as meat and cereals, as well as traditional Greek recipes (i.e., pastitsio, spinach rice, and homemade pies). Alcohol and beer reported servings were included in the analysis, due to the sample's low median values (2 and 16 mL/d, respectively) and the lack of heavy drinkers.

Table 16. Mean consumption and PCA factor loadings of the 32 FFQ-derived food groups.

			Components				
	Mean Consumption (Median. IQR)	Food Group	1	2	3	4	5
Croissant (g/d)	5.2 (11.56)	Sweets	0.705				
Chocolate (g/d)	12.85 (8.85)						
Tarts (g/d)	10.00 (10.00)						
Ice cream (g/d)	7.66 (24.64)						
Mayonnaise (g/d)	1.11 (2.02) *	Mayonnaise	0.664				
White bread (g/d)	19.28 (17.28)	Refined Cereals	0.643				
Cereals (g/d)	4.28 (4.28)						
White rice (g/d)	10.53 (23.32)						
Barley (g/d)	9.33 (30.00)						
Burger bread (g/d)	3.00 (10.44) *						
Chips (g/d)	4.66 (4.66)	Salty Snacks	0.628				
Crackers (g/d)	1.33 (4.28)						
Honey (g/d)	1.07 (4.66)	Sugary Snacks	0.596				
Soft drinks (mL/d)	28.69 (72.42) *						
Fruit compost (g/d)	7.58 96.66) *						
Tray Sweets (g/d)	10.00 (10.00)	Tray Sweets	0.584				
Pastitsio (g/d)	10.00 (10.00)	Pastitsio	0.493				
Potatoes (boiled. cooked. not fried) (g/d)	11.53 (25.53)	Potatoes (boiled, cooked, not fried)	0.469				
Chicken (g/d)	32.14 (0.00)	Chicken	0.388				
Seed oil (g/d)	3.23 (8.09) *	Seed oil, margarine, butter	0.374				
Margarine (g/d)	1.03 (2.46) *						
Butter (g/d)	0.50 (1.00)						
Light mayonnaise (g/d)	0.71 (1.84) *	Light Products	0.367				
Light cold cuts (g/d)	2.00 (6.42)						
Light soft drinks (g/d)	22.00 (70.71)						
Sausage (g/d)	1.08 (1.45)					−0.342	
Tomatoes (g/d)	64.28 (42.85)	Vegetables		0.640			
Lettuce (g/d)	34.28 (34.28)						
Broccoli (g/d)	21.42 (14.76)						
Spinach (g/d)	6.00 (13.28)						
Full fat milk (mL/d)	43.46 (71.26)	Dairy		0.568			
Low fat milk	51.42 (154.28)						
White cheese (g/d)	6.42 (17.28)						
Eggs (g/d)	10.71 (7.38)	Eggs		0.562			
Oranges (g/d)	36.42 (97.95)	Fruits		0.525			
Apples (g/d)	30.00 (80.66)						
Bananas (g/d)	21.42 (57.61)						
Winter fruit (g/d)	32.14 (86.42)						
Summer fruit	32.14 (64.28)						
Whole bread (g/d)	19.28 (17.28)	Non-refined cereals		0.443			
Brown rice (g/d)	6.72 (14.73) *						
Whole pasta (g/d)	8.54 (9.33)						
Large fish (g/d)	10.00 (22.14)	Large fish		0.432			
Olive oil (g/d)	45.00 (45.00)	Olive oil		0.345			
Dried fruit (g/d)	3.35 (6.88) *	Dried fruit		0.330			
Coffee (mL/d)	240.00 (240.00)	Caffeinated				−0.504	
Tea (mL/d)	16.00 (51.42)	Beverages					
Seafood (g/d)	10.00 (10.00)	Seafood			0.685		
French Fries (g/d)	4.83 (15.53)	French Fries			0.648		
Homemade pies (g/d)	10.00 (0.00)	Pies			0.510		

Other pies (g/d)	10.00 (10.00)						
Beef (g/d)	10.00 (22.14)	Red Meat			0.499		
Minced beef	25.71 (17.71)						
Pork (g/d)	10.00 (22.14)						
Lamb (g/d)	5.83 (13.84)						
Alcohol (mL/d)	2.00 (6.42)	Alcohol and Beer			0.398		
Beer (mL/d)	16.00 (51.42)						
Legumes (g/d)	64.28 (44.28)	Legumes				0.698	
Spinach and Rice (g/d)	16.66 (53.57)	Traditional, Greek				0.695	
Green Peas (g/d)	42.85 (29.52)	recipes					
Olives	1.00 (3.21)	Olives					0.645
Small fish (g/d)	10.00 (32.14)	Small fish					0.584
Nuts (g/d)	3.33 (28.81)	Nuts					0.343
Fruit Juice (g/d)	16.00 (51.42)	Fruit Juice					0.311
Total Variance Explained (%)			**14.74%**	**9.87%**	**6.26%**	**4.96%**	**4.49%**

* The selected variables follow the normal distribution and are presenting as mean ± SD

Based on the inclusion of corresponding food groups in the PCA, the five dietary patterns identified were categorized as follows:

i. a “Mixed” dietary pattern, consisting of light products and processed products high in fat and sugars (i.e., sweets, mayonnaise, refined cereals, salty snacks, sugary snacks, tray sweets, the Greek pastitsio, potatoes, chicken, seed oil, margarine, butter, light products, and sausage), (explaining 14.74% of the total variance);
ii. a “Mediterranean-proxy” (or Med-proxy) pattern including the consumption of food groups usually found in the Mediterranean diet, such vegetables, dairy, eggs, fruits, non-refined cereals, large fish, olive oil, dried fruit, and caffeinated drinks, such as coffee and tea (explaining 9.87% of the total variance);
iii. an “Eating out” pattern, consisting of food group combinations frequently consumed outside the household, i.e., seafood, French fries, pies, red meat and alcohol (explaining 6.26% of the total variance);
iv. a “Traditional, vegetarian-alike” pattern reporting consumption of legumes and traditional Greek recipes (i.e., spinach rice and cooked green peas) (explaining 4.96% of the total variance); and
v. a “High in unsaturated fats and fruit juice consumption” pattern, including olives, small fish, nuts and fruit juice, with the first, high in unsaturated fats and fruit juice consumption (explaining 4.96% of the total variance).

Following the extraction of the dietary patterns, we proceeded to conducting multiple linear regressions adjusting for the 3 models of the confounding factors [i.e. Model 1: Adjusting for age and sex; Model 2: Adjusting for age, sex, smoking habits, physical activity level and logBMI (except for logBMI values); Model 3: Adjusting for age, sex, smoking habits, physical activity level, logBMI, education years, family and professional status]. As shown in Table 17: i) the “Mixed” pattern was associated with increased logBMI (Model 1: $\beta = 0.018$, $p < 0.001$, Model 2:$\beta = 0.017$, $p < 0.001$, Model 3: $\beta = 0.015$, p-value = 0.009); increased body fat percentage (Model 1: ($\beta = 1.191$, $p = 0.002$); increased logVisceral fat values (Model 1: $\beta = 0.031$, $p = 0.001$); increased logTriglycerides (Model 1: $\beta = 0.038$, $p = 0.008$); increased logSGPT values (Model 1: $\beta = 0.052$, $p < 0.001$, Model 2: $\beta = 0.048$, $p = 0.002$, Model 3: $\beta = 0.069$, $p < 0.001$); and decreased logHDL cholesterol values (Model 1: $\beta = -0.020$, $p = 0.005$); and ii) the “Med-proxy” pattern was associated with decreased levels of logTotal Bilirubin (Model 2: $\beta = -0.044$, $p = 0.009$). Moreover, nominal associations ($p < 0.05$) are were observed for the following: i) the “Mixed” pattern and increased levels of logSerum protein (Model 1: $\beta = 0.004$, $p = 0.037$, Model 3: $\beta = 0.005$, $p = 0.029$); as well as increased levels of logSGOT/AST (Model 1: $\beta = 0.024$, $p = 0.028$, Model 2: $\beta = 0.024$, $p = 0.042$, Model 3: $\beta = 0.028$, p-value = 0.022); ii) the “Med-proxy” pattern and lower logCreatinine, (Model 3: $\beta = -0.014$, $p = 0.020$), as well as lower values of logTotal Bilirubin (Model 1: $\beta = -0.037$, $p = 0.021$, Model 3: $\beta = -0.043$, $p = 0.016$); iii) the “Traditional, vegetarian-alike and reduced levels of body fat percentage (Model 1: $\beta = -0.795$, $p = 0.039$, Model 2: $\beta = -0.495$, $p = 0.021$); decreased logVisceral fat values, (Model 2: $\beta = -0.008$, $p = 0.033$); as well as increased levels of logHDL (Model 2: $\beta = 0.017$, $p = 0.018$, Model 3: $\beta = 0.016$, $p = 0.040$).

Table 17. Multivariate linear regressions between the extracted dietary patterns and indices of anthropometric and biochemical characteristics.

	Model 1			Model 2			Model 3		
	β	*SE*	*p*	*B*	*SE*	*p*	*β*	*SE*	*p*
LogBMI									
Mixed Pattern	0.018	0.005	**<0.001**	0.017	0.005	**0.001**	0.015	0.005	**0.009**
Med-proxy Pattern	−0.002	0.005	0.752	<0.001	0.005	0.927	−0.001	0.006	0.844
Eating-out Pattern	0.004	0.005	0.363	0.004	0.005	0.429	0.001	0.005	0.772
Traditional, vegetarian-alike Pattern	−0.005	0.005	0.272	−0.008	0.005	0.131	−0.008	0.005	0.132
High in unsaturated fats and fruit juice consumption Pattern	−0.005	0.005	0.338	−0.006	0.005	0.266	−0.006	0.005	0.275
Body Fat (%)									
Mixed Pattern	1.191	0.387	**0.002**	−0.124	0.222	0.576	−0.163	0.247	0.512
Med-proxy Pattern	−0.057	0.416	0.892	0.316	0.225	0.162	0.410	0.242	0.092
Eating-out Pattern	0.334	0.395	0.399	0.019	0.209	0.929	−0.039	0.222	0.861
Traditional, vegetarian-alike Pattern	−0.795	0.382	0.039	−0.495	0.213	0.021	−0.416	0.235	0.067
High in unsaturated fats and fruit juice consumption Pattern	−0.275	0.396	0.489	0.114	0.213	0.594	0.247	0.226	0.275
LogVisceral Fat									
Mixed Pattern	0.031	0.009	**0.001**	−0.002	0.004	0.566	<0.001	0.004	0.898
Med-proxy Pattern	−0.003	0.010	0.733	0.001	0.004	0.745	0.004	0.004	0.390
Eating-out Pattern	0.010	0.010	0.274	0.002	0.004	0.581	0.002	0.004	0.648
Traditional, vegetarian-alike Pattern	−0.017	0.009	0.062	−0.008	0.004	**0.033**	−0.007	0.004	0.088
High in unsaturated fats and fruit juice consumption Pattern	−0.008	0.010	0.398	0.002	0.004	0.662	0.005	0.004	0.213
logGlucose(mg/dL)									
Mixed Pattern	0.006	0.004	0.127	-0.001	0.004	0.810	0.001	0.004	0.809
Med-proxy Pattern	<0.001	0.004	0.982	0.002	0.004	0.627	0.002	0.004	0.545
Eating-out Pattern	0.002	0.004	0.651	0.002	0.003	0.596	0.001	0.004	0.885
Traditional, vegetarian-alike Pattern	0.003	0.004	0.452	<0.001	0.004	0.995	0.001	0.004	0.861
High in unsaturated fats Pattern	−0.003	0.004	0.362	−0.004	0.004	0.262	−0.005	0.004	0.204
logUrea (mg/dL)									
Mixed Pattern	0.004	0.007	0.566	0.002	0.008	0.818	0.001	0.009	0.937
Med-proxy Pattern	0.010	0.008	0.203	0.012	0.008	0.141	0.012	0.009	0.189
Eating-out Pattern	−0.005	0.007	0.479	−0.006	0.007	0.448	−0.008	0.008	0.333
Traditional, vegetarian-alike Pattern	<0.001	0.007	0.974	−0.001	0.008	0.859	−0.004	0.008	0.629
High in unsaturated fats Pattern	−0.003	0.007	0.733	−0.002	0.008	0.764	−0.004	0.008	0.609
logCreatinine(mg/dL)									
Mixed Pattern	0.008	0.005	0.141	0.008	0.006	0.156	0.012	0.006	0.054
Med-proxy Pattern	−0.009	0.005	0.110	−0.011	0.006	0.049	−0.014	0.006	0.020
Eating-out Pattern	−0.001	0.005	0.871	0.001	0.005	0.870	0.001	0.005	0.876
Traditional, vegetarian-alike Pattern	−0.003	0.005	0.585	−0.007	0.005	0.163	−0.008	0.006	0.153
High in unsaturated fats and fruit juice consumption Pattern	0.002	0.005	0.746	0.001	0.005	0.908	<0.001	0.006	0.996
logUric Acid(mg/dL)									
Mixed Pattern	0.005	0.006	0.396	−0.006	0.007	0.337	−0.004	0.007	0.589
Med-proxy Pattern	−0.005	0.007	0.480	−0.002	0.007	0.746	-0.001	0.007	0.919
Eating-out Pattern	0.003	0.006	0.595	0.002	0.006	0.741	−0.001	0.006	0.831
Traditional, vegetarian-alike Pattern	−0.002	0.006	0.762	−0.004	0.006	0.519	−0.004	0.007	0.513

Total Cholesterol (mg/dL)									
Mixed Pattern	−0.247	2.402	0.918	1.304	2.575	0.613	0.953	2.916	0.744
Med-proxy Pattern	−3.167	2.521	0.211	−2.753	2.612	0.293	−3.480	2.847	0.224
Eating-out Pattern	−0.099	2.395	0.967	−0.302	2.415	0.901	−0.668	2.601	0.798
Traditional, vegetarian-alike Pattern	2.295	2.320	0.324	3.770	2.465	0.128	4.722	2.619	0.073
High in unsaturated fats Pattern	−0.185	2.447	0.940	0.837	2.521	0.740	0.139	2.715	0.959
logHDL-C (mg/dL)									
Mixed Pattern	−0.020	0.007	**0.005**	−0.013	0.007	0.081	−0.016	0.008	0.060
Med-proxy Pattern	−0.004	0.008	0.552	−0.009	0.008	0.265	−0.010	0.008	0.257
Eating-out Pattern	0.009	0.007	0.214	0.011	0.007	0.124	0.013	0.008	0.077
Traditional, vegetarian-alike Pattern	0.013	0.007	0.056	0.017	0.007	0.018	0.016	0.008	0.040
High in unsaturated fats and fruit juice consumption Pattern	0.006	0.007	0.440	0.006	0.007	0.401	0.004	0.008	0.625
logLDL-C (mg/dL)									
Mixed Pattern	−0.007	0.010	0.495	0.005	0.010	0.607	0.002	0.011	0.869
Med-proxy Pattern	−0.008	0.010	0.450	−0.004	0.010	0.672	−0.007	0.011	0.593
Eating-out Pattern	−0.003	0.010	0.743	−0.004	0.009	0.648	−0.006	0.010	0.546
Traditional, vegetarian-alike Pattern	−0.006	0.009	0.539	0.002	0.010	0.815	0.005	0.010	0.625
High in unsaturated fats and fruit juice consumption Pattern	−0.010	0.010	0.324	−0.003	0.010	0.761	−0.003	0.011	0.756
logTriglycerides(mg/dL)									
Mixed Pattern	0.038	0.014	0.008	0.021	0.015	0.161	0.032	0.016	0.051
Med-proxy Pattern	−0.009	0.015	0.543	−0.003	0.015	0.819	−0.003	0.016	0.868
Eating-out Pattern	<0.001	0.014	0.997	−0.003	0.014	0.808	−0.007	0.015	0.625
Traditional, vegetarian-alike Pattern	0.009	0.014	0.498	0.006	0.014	0.652	0.015	0.015	0.312
High in unsaturated fats and fruit juice consumption Pattern	0.002	0.014	0.908	<0.001	0.015	0.987	−0.005	0.016	0.746
logTotal Bilirubin(mg/dL)									
Mixed Pattern	−0.002	0.015	0.901	<0.001	0.017	0.988	0.013	0.018	0.490
Med-proxy Pattern	−0.037	0.016	**0.021**	−0.044	0.016	**0.009**	−0.043	0.018	**0.016**
Eating-out Pattern	−0.005	0.015	0.739	−0.001	0.015	0.945	−0.005	0.016	0.781
Traditional, vegetarian-alike Pattern	0.032	0.015	**0.031**	0.030	0.016	0.058	0.033	0.016	**0.046**
High in unsaturated fats and fruit juice consumption Pattern	0.017	0.016	0.274	0.014	0.016	0.380	0.015	0.017	0.385
logSerum protein(g/dL)									
Mixed Pattern	0.004	0.002	0.037	0.002	0.002	0.190	0.005	0.002	0.029
Med-proxy Pattern	−0.001	0.002	0.557	−0.001	0.002	0.710	<0.001	0.002	0.817
Eating-out Pattern	−0.001	0.002	0.603	−0.001	0.002	0.595	−0.002	0.002	0.274
Traditional, vegetarian-alike Pattern	0.002	0.002	0.161	0.002	0.002	0.214	0.003	0.002	0.135
High in unsaturated fats and fruit juice consumption Pattern	0.001	0.002	0.537	0.001	0.002	0.542	<0.001	0.002	0.809
logAlbumin(g/dL)									
Mixed Pattern	0.002	0.004	0.665	0.003	0.004	0.413	0.003	0.002	0.206
Med-proxy Pattern	−0.002	0.004	0.697	−0.002	0.004	0.595	0.002	0.002	0.297
Eating-out Pattern	<0.001	0.004	0.920	0.001	0.004	0.775	<0.001	0.002	0.960
Traditional, vegetarian-alike Pattern	0.003	0.004	0.361	0.003	0.004	0.501	0.003	0.002	0.109
High in unsaturated fats Pattern	−0.002	0.004	0.591	−0.003	0.004	0.427	0.001	0.002	0.789
LogSGOT/AST(IU/L)									
Mixed Pattern	0.024	0.011	**0.028**	0.024	0.012	**0.042**	0.028	0.012	**0.022**

Med-proxy Pattern	0.003	0.011	0.781	0.004	0.012	0.724	0.002	0.012	0.881
Eating-out Pattern	−0.011	0.011	0.288	−0.010	0.011	0.357	−0.014	0.011	0.191
Traditional, vegetarian-alike Pattern	0.003	0.010	0.784	−0.003	0.011	0.820	−0.003	0.011	0.751
High in unsaturated fats and fruit juice consumption Pattern	0.005	0.011	0.642	0.003	0.011	0.780	0.001	0.011	0.895
logSGPT/ALT (IU/L)									
Mixed Pattern	0.052	0.014	**<0.001**	0.048	0.015	**0.002**	0.069	0.016	**<0.001**
Med-proxy Pattern	−0.009	0.015	0.567	−0.004	0.016	0.788	−0.005	0.016	0.756
Eating-out Pattern	−0.004	0.015	0.766	−0.006	0.015	0.660	−0.010	0.015	0.509
Traditional, vegetarian-alike Pattern	0.002	0.014	0.876	0.001	0.015	0.966	<0.001	0.015	0.954
High in unsaturated fats and fruit juice consumption Pattern	0.004	0.015	0.783	0.005	0.015	0.762	−0.003	0.015	0.823
logAIS									
Mixed Pattern	0.038	0.029	0.194	0.025	0.032	0.445	0.026	0.034	0.435
Med-proxy Pattern	0.010	0.030	0.738	0.021	0.032	0.509	0.029	0.035	0.401
Eating-out Pattern	-0.039	0.039	0.305	-0.035	0.041	0.390	-0.028	0.044	0.528
Traditional, vegetarian-alike Pattern	-0.017	0.029	0.569	-0.039	0.032	0.231	-0.032	0.035	0.355
High in unsaturated fats and fruit juice consumption Pattern	-0.002	0.031	0.937	-0.011	0.032	0.721	-0.018	0.034	0.604
LogCESD-R-10									
Mixed Pattern	**0.053**	**0.022**	**0.017**	0.047	0.024	0..051	0.053	0.027	0.051
Med-proxy Pattern	0.008	0.023	0.737	0.018	0.024	0.447	2.032e-02	2.604 e-02	0.436
Eating-out Pattern	0.009	0.022	0.657	0.005	0.023	0.829	0.005	0.024	0.851
Traditional, vegetarian-alike Pattern	-0.011	0.022	0.607	-0.009	0.023	0.692	-0.005	0.025	0.851
High in unsaturated fats and fruit juice consumption Pattern	-0.010	0.023	0.644	-0.005	0.023	0.846	-0.002	0.025	0.929
LogSF-PCS-12									
Mixed Pattern	**-0.017**	**0.007**	**0.013**	-0.006	0.007	0.401	-6.972e-03	7.475 e-03	0.353
Med-proxy Pattern	0.010	0.008	0.229	0.006	0.008	0.427	0.002	0.008	0.833
Eating-out Pattern	0.002	0.007	0.761	0.003	0.007	0.679	0.006	0.007	0.374
Traditional, vegetarian-alike Pattern	-0.008	0.007	0.252	-0.004	0.007	0.549	-0.003	0.007	0.647
High in unsaturated fats and fruit juice consumption Pattern	-0.004	0.007	0.557	-0.007	0.007	0.323	-0.006	0.007	0.441
LogSF-MCS-12									
Mixed Pattern	-0.001	0.008	0.887	0.01	0.008	0.942	1.277e-03	9.224 e-03	0.892
Med-proxy Pattern	0.128	0.009	0.187	0.005	0.009	0.586	2.685e-03	1.041 e-02	0.767
Eating-out Pattern	0.009	0.008	0.232	0.013	0.008	0.100	0.014	0.008	0.092
Traditional, vegetarian-alike Pattern	-0.001	0.008	0.890	0.002	0.009	0.773	4.018e-03	9.138 e-03	0.661
High in unsaturated fats and fruit juice consumption Pattern	0.012	0.008	0.140	0..013	0.008	0.122	0.013	0.009	0.159

Model 1: Adjusting for age and sex**; Model 2:** Adjusting for age, sex, smoking habits, physical activity level and logBMI (except for logBMI values)**; Model 3:** Adjusting for age, sex, smoking habits, physical activity level, logBMI, education years, family and professional status

Construction of a Lifestyle Index (LI)

In an effort to further investigate the potential impact of the participants' lifestyle characteristics on their indices of anthropometric and biochemical profile, we proceeded to examining associations between different sets of variables. For this purpose, we investigated potential correlations between the reported lifestyle questionnaire scores, the extracted dietary patterns, and basic aspects, such as smoking and physical activity habits, with anthropometric indices, such as BMI and body fat percentage. All variables under examination were divided into categories, with higher values indicating favorable effects. The association between dietary patterns and investigated indices took place via categorization of attrition to the pattern in tertile groups. Continuous and nominal variables displaying positive correlations were further dichotomized based on the sample's reported median values (attribution of a value of 1 for scores below the sample's median and a value of 2 for scores above the observed median).

Variables displaying statistically significant ($p < 0.05$), positive correlations with either logBMI of body fat percentage values included: the "Mixed" and "Med-proxy" dietary patterns, the CESD-R-10 depression scale score and the physical component of the SF-12 scored questionnaire. Subsequently, a Lifestyle Index (LI) was created, based on the sum of the aforementioned, dichotomized variables and physical activity categories, as shown in below. The LI was calculated for 141 participants and the sample presented a median score of 8 and an IQR of 2, with an overall maximum score calculated at the value of 11.

$$\begin{aligned} &Lifestyle\ Index\ (LI) \\ &\quad = Physical\ Activity\ Category + "Mixed" pattern\ dichotomized\ score \\ &\quad + "Med - proxy" dichotomized\ score + CESD - R \\ &\quad - 10\ dichotomized\ score + SF - PCS\ dichotomized\ score \end{aligned}$$

Consequently, we used multivariate linear regressions adjusting for age, sex and BMI, in order to investigate for potential associations between the newly constructed LI and multiple indices of body composition and biochemical profile. As shown in Table 18, LI presented strong associations, including inverse correlations between: i) logBMI (Model 1: β = −0.010. *p*=0.022); ii) body fat percentage (Model 1: β=-0.903, p-value=0.007); iii) logVisceral fat (Model 1: β=-0.018, p=0.018); iv) logFasting glucose (Model 1: β=-0.009, p=0.004, Model 2: β=-0.007, p=0.023); v) logUric acid (Model 1: β=-0.012, p=0.030); vi) logAlbumin (Model 2: β=-0.008, p=0.032); and vii)logSGPT (Model 1: β=-0.026, p=0.027, Model 2: β=-0.026, p=0.030).

Table 18. Multivariate linear regressions between anthropometric and clinical characteristics and the constructed lifestyle index.

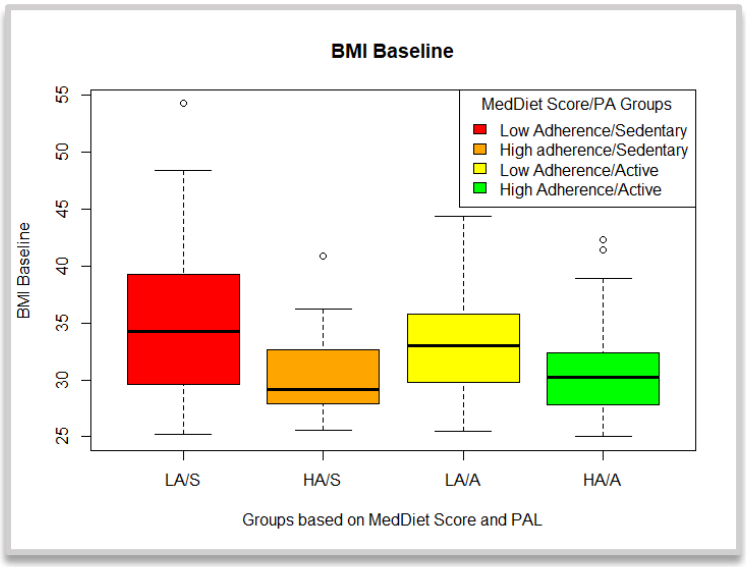
	Model 1			Model 2		
	β	*SE*	*p*	*B*	*SE*	*p*
logBMI						
Lifestyle Index	-0.010	0.004	**0.022**	–	–	–
Body fat (%)						
Lifestyle Index	-0.903	0.328	**0.007**	−0..242	0.186	0.197
LogVisceral Fat						
Lifestyle Index	-0.018	0.008	**0.017**	-0.001	0.003	0.712
logFasting glucose (mg/dL)						
Lifestyle Index	−0.009	0.003	**0.004**	−0.007	0.003	**0.023**
logUrea (mg/dL)						
Lifestyle Index	0.002	0.007	0.728	0.004	0.007	0.608
logCreatinine (mg/dL)						
Lifestyle Index	0.006	0.004	0.150	0.005	0.004	0.210
logUric acid (mg/dL)						
Lifestyle Index	-0.012	0.005	**0.030**	-0.009	0.005	0.103
Total cholesterol (mg/dL)						
Lifestyle Index	-1.210	2.248	0.591	-1.833	2.306	0.428
logHDL-C (mg/dL)						
Lifestyle Index	<0.001	0.006	0.957	-0.001	0.007	0.878
logLDL-C (mg/dL)						
Lifestyle Index	-0.001	0.009	0.937	-0.004	0.009	0.698
logTriglycerides(mg/dL)						
Lifestyle Index	-0.023	0.012	0.062	-0.021	0.013	0.097
logTotal Bilirubin(mg/dL)						
Lifestyle Index	-0.022	0.012	0.069	-0.023	0.013	0.066
logSerum protein(g/dL)						
Lifestyle Index	-0.002	0.001	0.124	-0.002	0.002	0.217
logAlbumin(g/dL)						
Lifestyle Index	-0.007	0.004	0.073	-0.008	0.004	**0.032**
logSGOT (IU/L)						
Lifestyle Index	−0.006	0.009	0.515	−0.008	0.009	0.373
LogSGPT (IU/L)						
Lifestyle Index	-0.026	0.012	**0.027**	-0.026	0.012	**0.030**

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, BMI

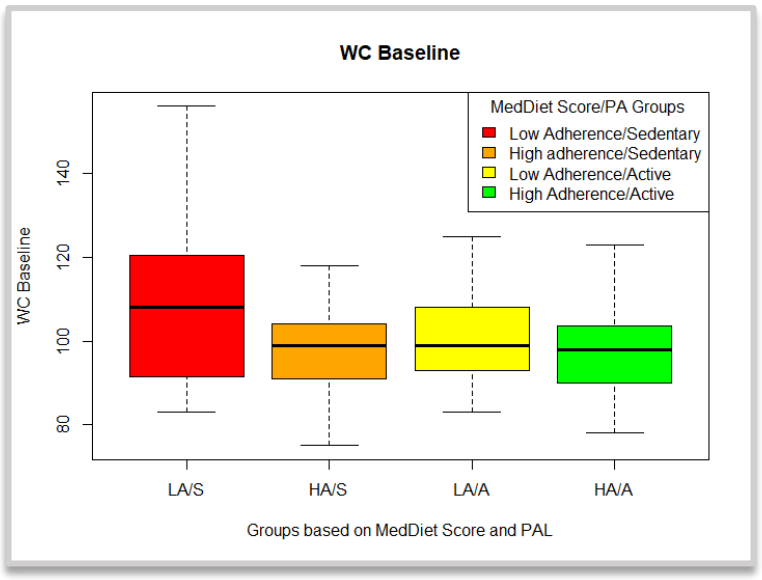
Assessment of adherence to the Mediterranean Diet and Physical Activity Habits

Finally, in the effort to holistically assess the effect of the participants' characteristics on the measured indices, we proceeded to conducting another categorization involving the volunteers' adherence to MD, as measured by the MedDiet score, and their self-reported level of physical activity, as measured by the short IPAQ. We separated the participants on high and low adherence to MD groups, based on the sample MedDiet score median, as well as sedentary versus active physical level, based on their reported PAL (all participants with low PAL were classified as sedentary, whereas participants with moderate or vigorous PAL were classified as "active"). Subsequently, four categories were created, namely: i) the "Low Adherence/Sedentary (LA/S)"; ii) the "High adherence/Sedentary (HA/S)" ; iii)the "Low Adherence/Active (LA/A)"; and v) the "High Adherence/Active (HA/A)" groups. Out of the144 participants with available baseline data, 27 individuals belonged in the first group, 22 in the second, 41 in the third and 54 in the fourth category.

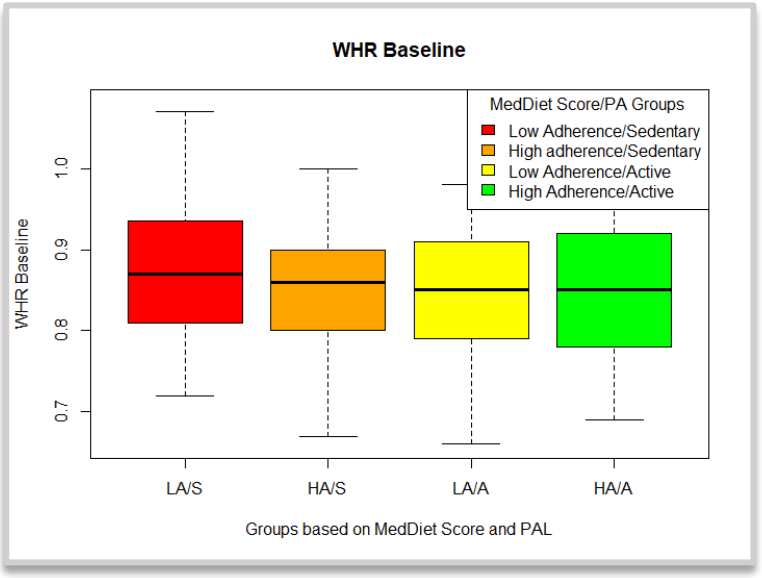
As shown in Figure 23., Kruskal-Wallis tests performed to assess within-group differences showed statistically significant, negative associations between categorization in the higher groups and anthropometric indices, namely BMI levels (p=0.003) and total body fat in kilograms (p=0.004). Categorization to the different groups did not present statistically significant associations for waist measurements, namely WC (p=0.10) and WHR (p=0.67).



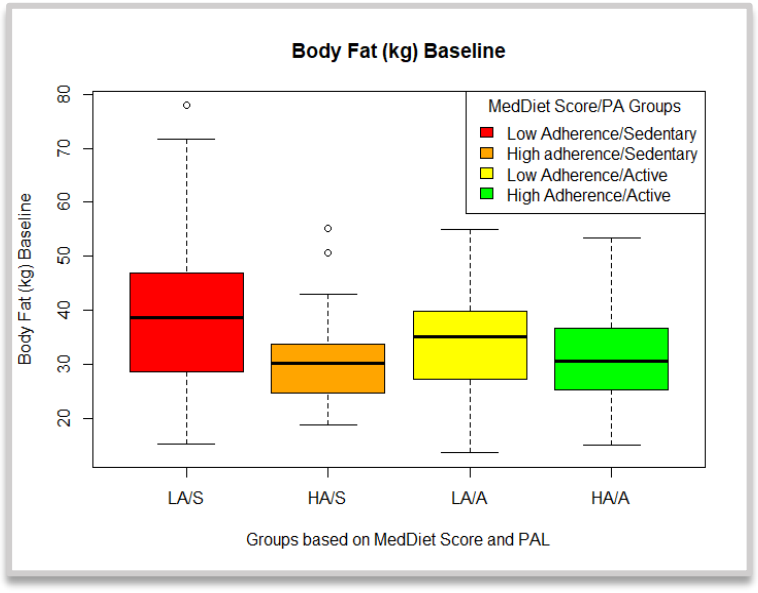
A.


WC Baseline
MedDiet Score/PA Groups
Low Adherence/Sedentary
High adherence/Sedentary
Low Adherence/Active
High Adherence/Active
WC Baseline
80
100
120
140
LA/S
HA/S
LA/A
HA/A
Groups based on MedDiet Score and PAL

B.


WHR Baseline
MedDiet Score/PA Groups
Low Adherence/Sedentary
High adherence/Sedentary
Low Adherence/Active
High Adherence/Active
WHR Baseline
0.7
0.8
0.9
1.0
LA/S
HA/S
LA/A
HA/A
Groups based on MedDiet Score and PAL

C.


Body Fat (kg) Baseline
MedDiet Score/PA Groups
Low Adherence/Sedentary
High adherence/Sedentary
Low Adherence/Active
High Adherence/Active
Body Fat (kg) Baseline
20
30
40
50
60
70
80
LA/S
HA/S
LA/A
HA/A
Groups based on MedDiet Score and PAL

D.

Figure 23. Clustered boxplots depicting A. BMI, B. WC, C. WHR and D. body fat in kg, per MedDiet Score/ PA group, at baseline

As such, we conducted a last set of multivariate regressions to test for the potential effect of adherence to MD and physical activity habits by examining the groups as a factor variable on the levels of the anthropometric, biochemical and lifestyle indices. Table 19 shows the associations with the examined variables, where no additive effect of PA and diet is shown and the associations with the examined indices appear to be mainly driven by the MedDietScore. More specifically, participants in the High Adherence/Active group displayed significantly reduced levels in anthropometric indices such as logBMI (Model 1: β=-0.059, p=0.0002, Model 2: β=-0.060, p=0.0002), logWHR (Model 1: β=-0.019, p=0.019, Model 2: β=-0.018, p=0.02), log of body fat in kilograms (Model 1: β=-0.108, p=0.0008, Model 2: β=-0.107, p=0.0009), log of visceral fat (Model 1: β=-0.103, p=0.001, Model 2: β=-0.102, p=0.001). Out of the biochemical indices examined, participants in this group only displayed statistically significantly lower levels of logglucose (Model 1: β=-0.039, p=0.002, Model 2: β=-0.040, p=0.0015). Interestingly, the HA/A group presented a better profile of mental health, displaying associations with lower levels of CESD-R10 scores (Model 1: β=-0.167, p=0.025, Model 2: β=-0.167, p=0.026) and increased levels of the SF-MCS-12 (Model 1: β=0.071, p=0.01, Model 2: β=0.072, p=0.009).

Table 19. Multivariate linear regressions between anthropometric and clinical characteristics and the constructed MedDiet score/PA groups.

	Model 1			Model 2		
	β	*SE*	*p*	*β*	*SE*	*p*
logBMI						
HA/S Group	-0.067	0.018	**0.0004**	-0.067	0.019	**0.0004**
LA/A Group	-0.029	0.016	0.072	-0.029	0.016	0.078
HA/A Group	-0.059	0.015	**0.0002**	-0.060	0.016	**0.0002**
logWHR						
HA/S Group	-0.018	0.009	0.05	-0.018	0.009	**0.043**
LA/A Group	-0.014	0.008	0.09	-0.016	0.008	0.049
HA/A Group	-0.019	0.008	**0.019**	-0.018	0.007	**0.02**
logBody fat (kg)						
HA/S Group	-0.108	0.037	**0.005**	-0.109	0.038	**0.004**
LA/A Group	-0.06	0.033	0.068	-0.064	0.033	0.05
HA/A Group	-0.108	0.032	**0.0008**	-0.107	0.032	**0.0009**
LogVisceral Fat						
HA/S Group	-0.121	0.037	**0.001**	-0.121	0.037	**0.001**
LA/A Group	-0.054	0.032	0.09	-0.05	0.032	0.09
HA/A Group	-0.103	0.031	**0.001**	-0.102	0.031	**0.001**
logFasting glucose (mg/dL)						
HA/S Group	-0.026	0.015	0.088	-0.024	0.014	0.099
LA/A Group	-0.037	0.013	**0.005**	-0.033	0.013	**0.011**
HA/A Group	-0.039	0.013	**0.002**	-0.040	0.012	**0.0015**
logUrea (mg/dL)						
HA/S Group	-0.013	0.029	0.667	-0.013	0.029	0.6722
LA/A Group	-0.022	0.025	0.389	-0.022	0.026	0.4021
HA/A Group	-0.023	0.025	0.346	-0.024	0.025	0.3458
logCreatinine (mg/dL)						
HA/S Group	0.001	0.021	0.948	2.747e-03	2.128e-02	0.8975
LA/A Group	0.023	0.018	0.214	2.705e-02	1.863e-02	0.1489
HA/A Group	0.014	0.018	0.444	1.237e-02	1.788e-02	0.4904
logUric acid (mg/dL)						
HA/S Group	-0.029	0.024	0.2412	-0.029	0.024	0.240
LA/A Group	-0.018	0.021	0.3882	-0.019	0.022	0.379
HA/A Group	-0.034	0.021	0.098	-0.034	0.021	0.102
Total cholesterol (mg/dL)						
HA/S Group	3.5896	10.170	0.724	3.6494	10.215	0.721
LA/A Group	-9.464	8.843	0.286	-9.296	8.94	0.300
HA/A Group	-1.019	8.541	0.905	-1.084	8.583	0.899
logHDL-C (mg/dL)						
HA/S Group	0.013	0.030	0.648	0.016	0.029	0.604
LA/A Group	0.0001	0.026	0.997	0.005	0.026	0.844
HA/A Group	0.020	0.025	0.407	0.019	0.025	0.449
logLDL-C (mg/dL)						
HA/S Group	0.037	0.04	0.362	0.037	0.041	0.365
LA/A Group	-0.009	0.035	0.792	-0.009	0.036	0.790
HA/A Group	0.002	0.034	0.947	0.002	0.035	0.946
logTriglycerides(mg/dL)						
HA/S Group	-0.080	0.057	0.1685	-0.081	0.058	0.166
LA/A Group	-0.082	0.050	0.105	-0.084	0.051	0.099
HA/A Group	0.075	0.048	0.1235	-0.074	0.049	0.1295
logTotal Bilirubin(mg/dL)						
HA/S Group	-0.046	0.058	0.431	-0.044	0.059	0.458

LA/A Group	0.023	0.051	0.654	0.031	0.051	0.550
HA/A Group	0.016	0.049	0.748	0.012	0.049	0.794
logDirect Bilirubin(mg/dL)						
HA/S Group	-0.004	0.053	0.933	-5.875e-04	5.277e-02	0.991
LA/A Group	0.054	0.046	0.243	6.525e-02	4.618e-02	0.160
HA/A Group	0.042	0.046	0.346	3.824e-02	4.434e-02	0.390
logSerum protein(g/dL)						
HA/S Group	-0.006	0.007	0.3955	-0.006	0.007	0.0408
LA/A Group	-0.009	0.006	0.1643	-0.009	0.007	0.1892
HA/A Group	-0.009	0.006	0.1422	-0.009	0.006	0.1368
logAlbumin(g/dL)						
HA/S Group	4.693e-03	1.717e-02	0.785	5.153e-03	1.721e-02	0.765
LA/A Group	-439e-03	1.493e-02	0.762	-3.247e-03	1.506e-02	0.830
HA/A Group	1.527e-02	1.442e-02	0.292	1.478e-02	1.446e-02	0.309
logSGOT (IU/L)						
HA/S Group	-0.045	0.047	0.341	-0.044	0.047	0.353
LA/A Group	-0.026	0.041	0.528	-0.023	0.041	0.574
HA/A Group	-0.019	0.039	0.637	-0.019	0.039	0.620
LogSGPT (IU/L)						
HA/S Group	-0.044	0.059	0.467	-0.044	0.060	0.4612
LA/A Group	-0.001	0.052	0.9822	-0.003	0.053	0.9482
HA/A Group	-0.056	0.050	0.2635	-0.056	0.050	0.2729
logAIS						
HA/S Group	0.047	0.128	0.714	0.052	0.129	0.688
LA/A Group	0.028	0.101	0.776	0.041	0.104	0.695
HA/A Group	-0.153	0.105	0.150	-0.15	0.106	0.162
LogCESD-R-10						
HA/S Group	-0.069	0.09	0.426	-0.072	0.087	0.4145
LA/A Group	-0.008	0.07	0.907	-0.014	0.075	0.8524
HA/A Group	-0.167	0.07	**0.025**	-0.167	0.073	**0.026**
LogSF PCS 12						
HA/S Group	0.028	0.026	0.28	0.031	0.027	0.2488
LA/A Group	0.005	0.023	0.83	0.0087	0.023	0.7103
HA/A Group	0.038	0.022	0.08	0.038	0.022	0.086
LogSF MCS 12						
HA/S Group	-0.011	0.032	0.7455	-0.006	0.033	0.8477
LA/A Group	0.033	0.028	0.2393	0.039	0.028	0.172
HA/A Group	0.071	0.027	**0.01**	0.072	0.027	**0.009**

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, smoking

3.1.2. Study Results on Body Weight and WHR

Changes observed in collected data for Weight, BMI and WHR

The entirety of changes in weight, BMI and WHR based on observed measurements from baseline up to the end of month 3, is displayed in Tables 20-21. Figure 24 shows the analytical CONSORT flow diagram on the trial's participants.

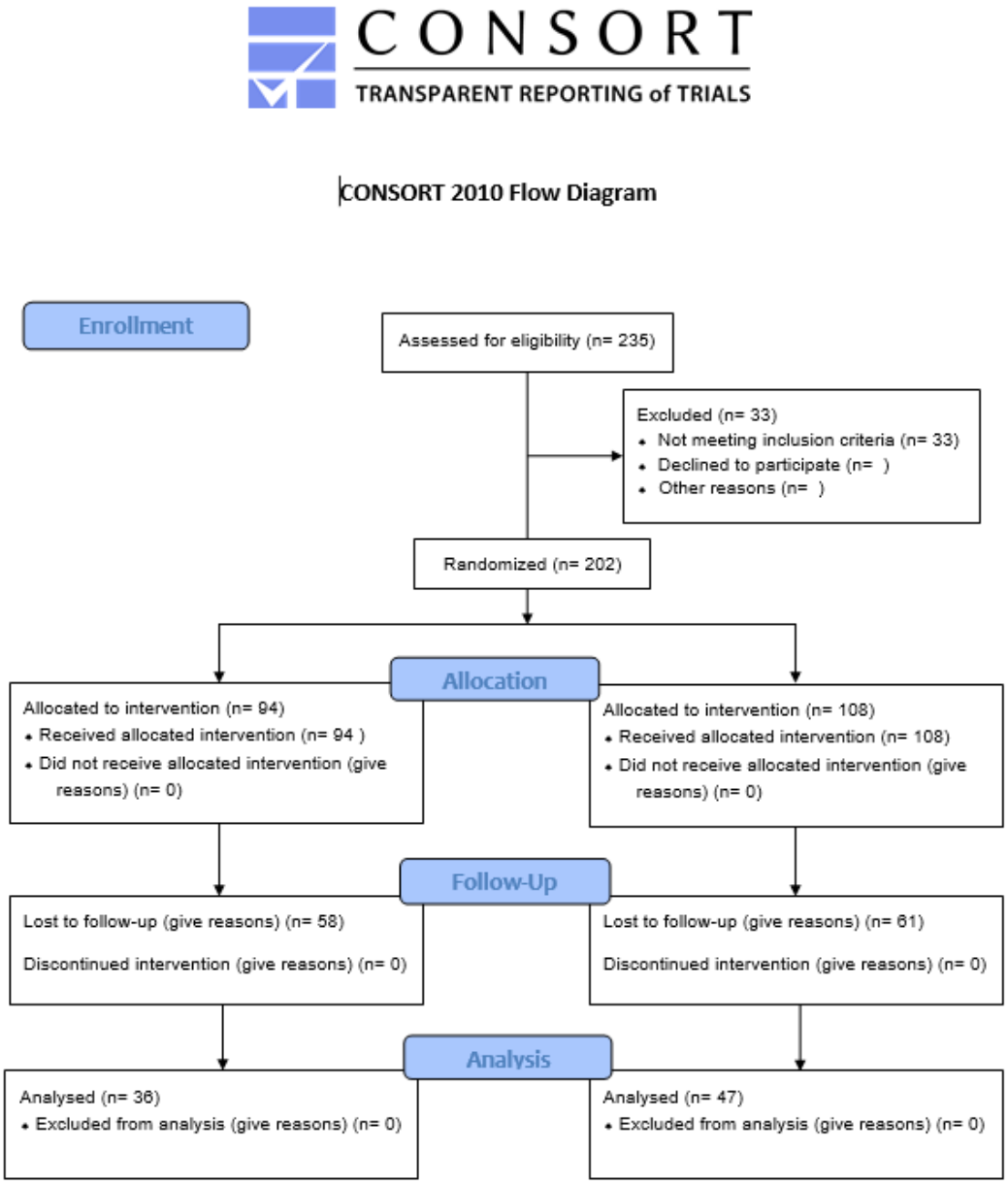


Figure 24. CONSORT 2010 Flow Diagram for the iMPROVE study.

For the baseline measurements, we used the data collected in-person during the baseline meeting, while self-reported data inserted in the online assessment tool by the participants themselves were used for the end of the first and second months of the intervention. Regarding the data at the end of month 3, we used the in-person measurements for the participants attending the in-person meeting and the online, self-reported measurements for the participants who did not attend the in-person session.

As shown in Table 20, in the overall sample, weight and BMI were statistically significantly lower at the end of month 3 compared to baseline (83kg vs 87kg, p<0.001 and 29.32kg/m^2 vs 31.35kg/m^2, p<0.001, respectively). Table 21 presents the within-month analyses which showed a similar, statistically significant decrease in weight and BMI from baseline up to the end of month 1 (87kg vs 84kg, p<0.001 and 31.35 kg/m^2 vs 30.14 kg/m^2, p<0.001, respectively), as well as from the end of month 1 up to the end of month 2 (84kg vs 82kg, p<0.001 and 30.14 kg/m^2 vs 29.71kg/m^2, p<0.001, respectively). No statistically significant change was observed from the end of month 2 up to the end of month 3.

Table 20. Weight, BMI and WHR measurements at the main points of the study.

Variable	Time	Total		Men		Women		
		N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	*P**
Weight	Baseline	202	87 (26)	59	103 (30)	143	83 (17)	**<0.001**
	Month 3	84	83 (23)	25	98 (27.5)	59	77 (16)	**<0.001**
	*p***		**<0.001**		**<0.001**		**<0.001**	
BMI	Baseline	202	31.35 (6.9)	59	31.2 (7.3)	143	31.4 (6.9)	0.920
	Month 3	83	29.32 (6.03)	25	29.63 (7.46)	58	28.93 (5.82)	0.333
	*p***		**<0.001**		**<0.001**		**<0.001**	
WHR	Baseline	183	0.86 (0.13)**	53	0.93 (0.08)**	130	0.83 (0.09)**	**<0.001**
	Month 3	73	0.86 (0.15)**	21	0.93 (0.07)**	52	0.83 (0.12)**	**<0.001**
	*p***		**0.003**		**0.009**		0.047	

p**: p-value showing differences within the two sexes, using the Mann-Whitney test.
p**: p-value showing overall change from baseline using the Wilcoxon signed-rank test.
** variable follows the normal distribution, so mean ± SD are shown.

Table 21. Weight, BMI, and WHR measurements during the three months of the study.

Variable	Time	Total		Men		Women		
		N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	*P**
Weight	Baseline	202	87 (26)	59	103 (30)	143	83 (17)	**<0.001**
	Month 1	118	84 (25)	35	101 (21)	83	79 (15)	**<0.001**
	*p***		**<0.001***		**<0.001**		**<0.001**	
	Month 2	89	82(25)	27	100 (25)	62	76.50 (13)	**<0.001**
	*p***		**0.001***		**0.009**		**0.023**	
	Month 3	84	83 (23)	25	98 (27.5)	59	77 (16)	**<0.001**
	*p***		0.819*		0.925		0.849	
BMI	Baseline	202	31.35 (6.9)	25	29.63 (7.46)	58	28.93 (5.82)	0.920
	Month 1	118	30.14 (6.08)	35	31.13 (7.51)	83	29.73 (5.87)	0.528
	*p***		**<0.001**		**<0.001**		**<0.001**	

	Month 2	89	29.71 (6.12)	27	31.01 (7.42)	62	29.33 (5.33)	0.215
	*p***		**0.001**		**0.010**		**0.044**	
	Month 3	83	29.31 (6.03)	25	29.63 (7.46)	58	28.93 (5.82)	0.333
	*p***		0.867		0.955		0.670	
WHR	Baseline	183	0.86 (0.13)**	53	0.93 (0.08)**	130	0.83 (0.09)**	**<0.001**
	Month 1	111	0.87 (0.15)	33	0.95 (0.06)**	78	0.84 (0.13)	**<0.001**
	*p***		0.349		0.636		0.394	
	Month 2	81	0.88 (0.15)	26	0.97 (0.07)	55	0.85 (0.13)	**<0.001**
	*p***		0.427		0.353		0.153	
	Month 3	73	0.86 (0.15)**	21	0.93 (0.07)**	52	0.83 (0.12)**	**<0.001**
	*p***		0.05		0.038		0.320	

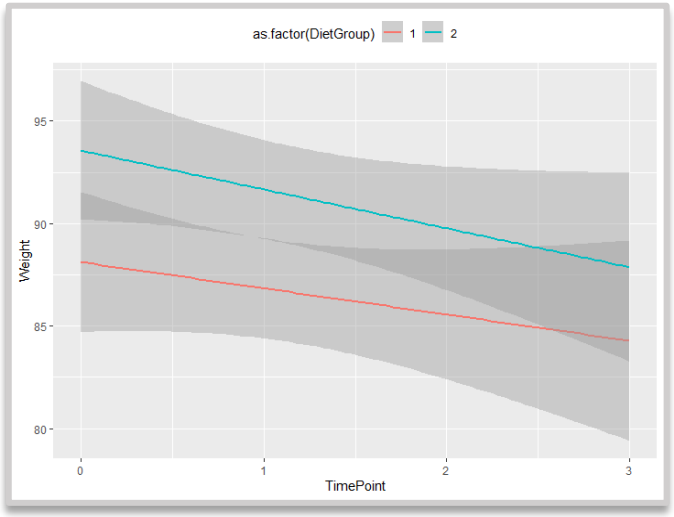
p**: p-value showing differences within the two sexes, using the Mann-Whitney test.
p:*** p-value showing overall change from the previous month using the Wilcoxon signed-rank test.
**: variable follows the normal distribution, so mean ± SD are shown.

Regarding the within-diet group changes, both weight and BMI were significantly reduced in both groups (Figure 25) from baseline to month 3 ($p<0.001$ for all), whereas WHR showed a statistically significant increase in the high-carbohydrate group only (0.85cm vs 0.88cm, $p=0.033$) (Table 22). Weight was also statistically significantly reduced from baseline to month 1 and month 1 to month 2 for participants in both groups ($p<0.05$ for all) (Table 23). Additionally, BMI levels decreased for both groups from baseline to the end of month 1 ($p<0.001$ for both), whereas participants in the high protein groups also displayed a statistically significant reduction from the end of month 1 up to the end of month 2 (31.18kg/m^2 vs 29.71kg/m^2, $p=0.006$).

Table 22. Weight, BMI and WHR measurements at the main points of the study, per diet group.

Variable	Time	N1 (High Carb)	Median ± IQR	N2 (High Prot)	Median ± IQR	*p**
Weight	Baseline	94	83.50 (26)	108	88.50 (25)	**0.014**
	Month 3	36	79 (25)	48	84.5 (20.5)	0.178
	*p***		**<0.001**		**<0.001**	
BMI	Baseline	94	30.5 (6.9)	108	32.3 (7.8)	0.920
	Month 3	36	29.21 (6.83)	47	29.32 (6.26)	0.333
	*p***		**<0.001**		**<0.001**	
WHR	Baseline	82	0.85 (0.12)	101	0.86 (0.12)	0.665
	Month 3	31	0.88 (0.099)***	42	0.85 (0.084)***	0.129
	*p***		**0.033**		0.063	

p**: p-value showing differences within the two diet groups, using the Mann-Whitney test.
p***: p-value showing change from baseline for each group, using the Wilcoxon signed-rank test.
***: variable follows the normal distribution, so mean ± SD are shown.



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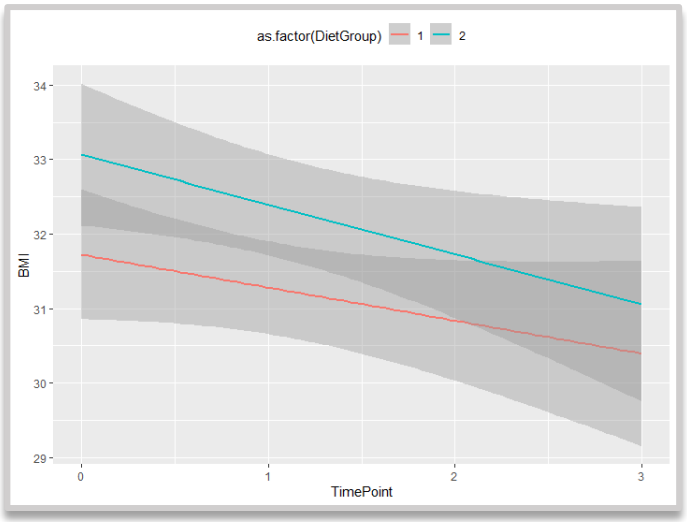


B.

Figure 25 Trajectories of weight (25.A) and BMI (25.B) change from baseline up to the end of month 3 of the intervention, per diet group (1=high carbohydrate and 2=high protein).

Table 23. Changes in weight, BMI and WHR per diet group during the three months of the study per diet group.

Variable	Time	N1(High Carb)	Median ± IQR	N2 (High Prot)	Median ± IQR	*p* *
Weight	Baseline	94	83.50 (26)	108	88.50 (25)	**0.014**
	Month 1	56	81.50 (21)	62	86 (26)	0.173
	p-value**		**<0.001**		**<0.001**	
	Month 2	42	80 (20)	47	86 (25)	0.149
	p-value**		**0.047**		**0.006**	
	Month 3	36	79 (25)	48	84.5 (20.5)	0.178
	p-value**		0.478		0.843	
BMI	Baseline	94	30.5 (6.9)	108	32.3 (7.8)	0.920
	Month 1	56	29.58 (6.34)	62	31.18 (6.09)	0.249
	p-value**		**<0.001**		**<0.001**	
	Month 2	42	29.84 (5.43)	47	29.71 (7.02)	0.421
	p-value**		0.088		**0.006**	
	Month 3	36	29.21 (6.83)	47	29.32 (6.26)	0.333
	p-value**		0.458		0.610	
WHR	Baseline	82	0.85 (0.12)	101	0.86 (0.12)	0.665
	Month 1	54	0.88 (0.15)	57	0.86 (0.09)***	0.246
	p-value**		0.339		0.726	
	Month 2	39	0.89 (0.15)	42	0.86 (0.16)***	0.354
	p-value**		0.839		0.230	
	Month 3	31	0.88 (0.099)***	42	0.85 (0.084)***	0.129
	p-value**		0.049		0.393	

**p*: p-value showing differences within the two diet groups, using the Mann-Whitney test.
*p***: p-value showing change from the previous month for each diet group, using the Wilcoxon signed-rank test.
***: variable follows the normal distribution, so mean ± SD are shown.

Imputation of Missing Values for Weight Measurements

In the effort to enhance the holistic assessment of the effect of the intervention on the primary outcomes, we proceeded to applying an imputation methodology for filling out the missing values observed for weight at the end of the three months (n=202 at baseline vs n=84 at the end of month 3 for weight). The imputation step was performed for the logarithm of weight, at the end of the first, second and third months, simultaneously, using a multivariate normal regression model.

For the process of the imputation, the role of multiple variables on the primary outcomes was examined, including age, sex, physical activity, baseline anthropometric measurements (body fat percentage, WC and visceral fat), baseline levels of biochemical indices such as total cholesterol, glucose, TG, HDL-C, as well as socio-economic characteristics like family status and education level. We used data from the former to assess the living status of the participants as living alone if they had reported to be single, separated, divorced, or a widower/widow or as not living alone if they had reported to be in married or in a steady relationship. We further categorized their education level on having received no education, having concluded the 1st, 2nd of 3rd

grade of education or other characteristics reported. Table 24 shows the comparisons between each group of the examined categorical values and the observed change in weight at the end of the 3 months. A statistically significant difference was revealed in the weight change observed among the participants in the two categories of living status, where individuals who were reported to be living alone noted lower change compared to the ones living with someone else (change=-1kg vs change=-3kg, p=0.003).

Furthermore, we conducted correlation analyses, in order to assess the potential effect of the remaining numerical variables on baseline weight and the observed weight change at 3 months (Table 25). Among the numerical variables, body fat percentage (p=0.007), WC, visceral fat, glucose, TG and HD-C levels were correlated to baseline weight (p<0.001), but not the change of weight at the end of the three months.

Table 24. Comparison of baseline weight and change in weight at 3 months across the categories of possible categorical predictors.

	Baseline weight, kg			Change in weight at 3 months, kg		
	N	Descriptives	*p*	N	Descriptives	*P*
Sex		Median (Q1, Q3)			Mean (SD)	
Male	59	103 (90, 120)	<0.001*	25	-4.22 (3.96)	0.250**
Female	143	83 (75, 92)		59	-2.47 (3.29)	
Physical activity		Median (Q1, Q3)			Median (Q1, Q3)	
Sedentary	64	88 (79, 105)		22	-1 (-4, 1)	
Mediocre	104	86 (77.5, 100)	0.369***	49	-3 (-6, 0)	0.197***
Active/intense	31	88 (81, 104)		12	-6.75 (-4.5, 0)	
Live alone		Median (Q1, Q3)			Mean (SD)	
Yes	59	86 (74, 104)	0.231*	21	-1.05 (3.01)	0.003**
No	142	88 (78, 104)		62	-3.70 (3.52)	
Education level		Median (Q1, Q3)			Median (Q1, Q3)	
No education	8	94.5 (84, 97.5)		6	-5.75 (-6, -3.5)	
1st grade	2	95 (86, 104)		1	3.5 (3.5, 3.5)	
2nd grade	54	87 (78, 110)	0.664***	22	-2.5 (-4.5, -1)	0.382***
3rd grade	125	86 (77, 103)		50	-3 (-6, 1)	
Other	12	86.5 (80, 90)		4	-3.5 (-6.5, 0)	

*: p-value showing differences within the two groups, using the Mann-Whitney test.
**: p-value showing differences within the two groups, using Student's t-test.
***: p-value showing

Table 25. Correlation of baseline weight and change in weight at 3 months with possible numerical predictors

	Correlation with baseline weight			Correlation with change in weight at 3 months		
	N	Spearman's Rho	*p*	N	Spearman's Rho	*P*
Age	202	-0.04	0.563	84	-0.09	0.413
Body Fat %	202	0.19	0.007	84	0.16	0.149
WC[2]	183	0.75	<0.001	77	-0.18	0.114
Visceral fat[3]	202	0.79	<0.001	84	-0.22	0.049
Total cholesterol[4]	193	-0.07	0.320	83	0.11	0.314
Glucose[5]	193	0.25	<0.001	83	-0.10	0.388
TG[6]	193	0.31	<0.001	83	-0.01	0.906
HDL-C[7]	193	-0.37	<0.001	83	0.14	0.213

[1]: correlated with WC, visceral fat, and HDL.
[2]: correlated with % fat, visceral fat, glucose, TG and HDL.
[3]: correlated with % fat, WC, glucose, TG and HDL.
[4]: correlated with TG and HDL.
[5]: correlated with WC, visceral fat, TG and HDL.
[6]: correlated with WC, visceral fat, total cholesterol, glucose, and HDL.
[7]: correlated with % fat, WC, visceral fat, total cholesterol, glucose, TG, and HDL.

For the determination of the optimal variables to be included in the process, we examined 4 different scenarios of examined variables, namely: scenario 1 including weight at baseline, sex, age and diet group; scenario 2 including weight at baseline, sex, age, diet group and living status; scenario three consisting of weight at baseline, sex, age, diet group, living status and education years; and the base case scenario including weight at baseline, sex, age, diet group, living status and body fat percentage at baseline.

Considering all participants, all four schemes displayed a statistically significant reduction in weight, with the base case scenario presenting a mean **-2.68kg** (p<0.0001) reduction (Table 26). An additional linear regression model fitted to investigate the difference of mean change in body weight at the 3 months between the two diet groups showed (Table 27) statistically significant changes from baseline but no significance difference between the two groups.

Table 26. Mean change in weight for overall population and the mean difference between diet groups at 3 months (in kg).

N=202				Change in weight at 3 months (kg)		Crude difference between diet groups*	
		M	N	Mean (95% CI)	*p*	b (95% CI)	*p*
CCS		0	84	-2.99 (-3.77, -2.22)	<0.0001	0.23 (-1.34, 1.80)	0.773
	Covariates used for imputation						
Sc.1	Weight Baseline, Sex, Age, Diet Group	100	202	-2.58 (-3.59, -1.57)	<0.0001	0.36 (-1.54, 2.26)	0.704
Sc.2	Weight Baseline, Sex, Age, Diet Group, Live alone	100	202	-2.64 (-3.56, -1.72)	<0.0001	0.36 (-1.39, 2.11)	0.682
Sc.3	Weight Baseline, Sex, Age, Diet Group, Live alone, education years	100	185	-2.54 (-3.56, -1.53)	<0.0001	0.31 (-1.72, 2.34)	0.762
Base case	Weight Baseline, Sex, Age, Diet Group, Live alone, Fat (%) baseline	100	202	-2.68 (-3.55, -1.80)	<0.0001	0.39 (-1.29, 2.06)	0.647

CCS: complete case scenario**; M:** # of imputations

***** Linear regression model of weight difference at 3 months. Coefficients (weight difference) presented for Diet Group (High protein vs. High Carbohydrate)

An additional linear regression model was fitted to investigate the difference of mean change in body weight at 3 months between the two diet groups. The corresponding mean change in weight observed at the end of the 3 months did not differ between the two groups and was measured at 0.05kg and 0.03 for the high carbohydrate and protein group respectively. Although statistically insignificant, the mean difference between the two diet groups was -0.02kg in favor of the high protein group (p=0.481).

Table 27. Mean change in weight by diet group at 3 months (in kg).

N=202	Change in weight at 3 months (kg)	High protein			High Carbohydrate		
		N	Mean (95% CI)	*p*	N	b (95% CI)	*p*
CCS		48	-2.90 (-4.03, -1.76)	<0.0001	36	-3.13 (-4.18, -2.07)	<0.0001
	Covariates used for imputation						
Sc.1	Weight Baseline, Sex, Age, Diet Group	108	-2.41 (-3.81, -1.02)	0.001	94	-2.77 (-4.19, -1.36)	<0.0001
Sc.2	Weight Baseline, Sex, Age, Diet Group, Live alone	108	-2.47 (-3.75, -1.19)	<0.0001	94	-2.83 (-4.12, -1.55)	<0.0001
Sc.3	Weight Baseline, Sex, Age, Diet Group, Live alone, education years	98	-2.40 (-3.82, -0.98)	<0.0001	87	-2.71 (-4.20, -1.22)	<0.0001
Base case	Weight Baseline, Sex, Age, Diet Group, Live alone, Fat (%) baseline	108	-2.50 (-3.65, -1.34)	<0.0001	94	-2.88 (-4.18, -1.59)	<0.0001

CCS: complete case scenario**.**

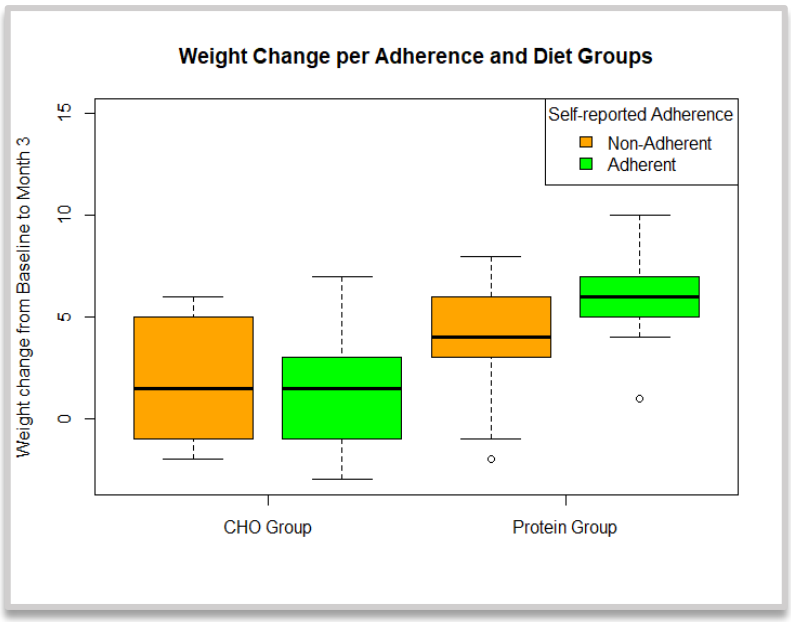
Assessment of Adherence to the Proposed Diets

Following the identification of a statistically significant reduction in body weight across the overall sample using both the observed and the imputed phenotypic data, we proceeded to additionally examining the effect of the participants' adherence to the two proposed diets. Data deriving from the monthly 24-hour dietary recalls conducted throughout the intervention period, revealed the participants' increased adherence to the consumption of the proposed calories but demonstrated their difficulty in completely adhering by 100% to the consumption of the increased proposed carbohydrate and protein intake. As such, for the purposes of exploring the effect of adherence to the proposed diet and primary outcome changes we used the data deriving from the self-reported adhrence score provided by the participants on a monthly basis via the online platform. The mean adherence score was calculated after adding all three rerported scores and diving them by a value of 3. We further proceeded to separating the individuals into two categories of "low adherence" or "non-adherent" and "high adherence" or "adherent" based on the sample median. Table 28 shows that for the overall sample, changes in both body weight and BMI differed statistically signifcantly between non-adherent and adherent participants, with the latter showing an increase in the 3-month trends of weight and BMI reduction. After examining potential differences within the two diet groups, adhrence did not appear to significantly affect the changes in the high carbohydrate group (p=0.067 and 0.079, respectively), while adherent participants in the high protein group displayed increased reduction in both weight and BMI, compared to the group's non-adherent volunteers (p<0.001 for both) (Figure 26).

Table 28. Differences between weight and BMI change at 3 months per adherence and diet groups.

Variable	Total		High Carbohydrate		High Protein	
	Z*	*p*	Z*	*p*	Z*	*P*
Weight change at 3 months	-4.280	**<0.001**	-1.830	0.067	-4.076	**<0.001**
BMI change at 3 months	-4.224	**<0.001**	-1.775	0.079	-4.094	**<0.001**

*Z-statistic from Mann Whitney test



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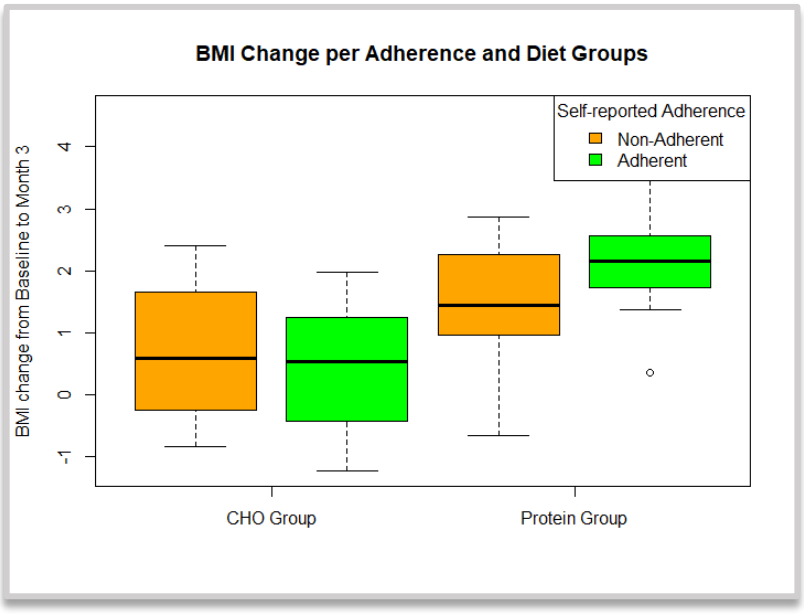


B

Figure 26. Clustered boxplot depicting the changes in A. weight and B. BMI from baseline up to the end of month 3 of the intervention, shown per adherence group (Adherent vs Non-adherent participants).

3.1.3. Study Results on Secondary Outcomes

Changes observed in Collected Data for Indices of Body Composition

Moving on to the study secondary outcomes, at the end of the three months of intervention, the overall sample showed statistically significant reductions in total and upper body fat (p<0.001, p=0.002, respectively), total and upper body fat-free mass (p<0.001 for both), total body water (p<0.001) and visceral fat (p<0.001) (Table 29). Within-sex comparisons revealed that the changes observed were mainly driven by women who constituted the vast majority of the sample, and who were the ones to note the observed reductions at the end of the 3 months.

Table 29. Body composition measurements at the main points of the study.

Variable	Time	Total		Men		Women		
		N	Median IQR	N	Median (IQR)	N	Median (IQR)	*P**
Body Fat %	Baseline	202	38.45 (10.8)	59	29.1 (8.9)	143	41.7 (8)	**<0.001**
	Month 3	64	38 (9)	17	30 (10)	47	40(7)	**<0.001**
	*p***		0.063		0.234		0.140	
Body Fat (kg)	Baseline	202	32.95 (13.3)	59	28.9 (14.2)	143	35.1 (12.6)	**0.001**
	Month 3	63	31 (12)	16	34.63 (15.59)**	47	31 (9)	0.962
	*p***		**<0.001**		0.079		**0.001**	
Fat-free Mass (kg)	Baseline	202	52 (18)	59	71 (15)	143	49 (7)	**<0.001**
	Month 3	64	49 (16)	17	73.47 (11.89)**	47	47 (6)	**<0.001**
	*p***		**<0.001**		**0.002**		**<0.001**	
Total Body Water (kg)	Baseline	202	38 (13)	59	52 (11)	143	36 (5)	**<0.001**
	Month 3	64	36 (11)	17	53.71 (8.75)**	47	34 (5)	**<0.001**
	*p***		**<0.001**		**0.002**		**<0.001**	
Visceral Fat	Baseline	202	10 (6)	59	14 (7)	143	9 (4)	**<0.001**
	Month 3	64	10 (6)	17	16.53 (6.52)**	47	9 (3)	**<0.001**
	*p***		**<0.001**		**0.038**		**0.001**	
Upper Body Fat %	Baseline	201	36.7 (6.98)**	59	32.34 (6.18)	142	38.51 (8)	**<0.001**
	Month 3	64	36.38 (6.65)**	17	33.24 (7.29)**	47	37.51 (6.1)**	**0.032**
	*p***		0.120		**0.039**		0.487	
Upper Body Fat (kg)	Baseline	201	18 (7)	59	18 (7)	142	17 (7)	0.288
	Month 3	64	16 (8)	17	20 (10)	47	16 (5)	**0.030**
	*p***		**0.002**		**0.011**		**0.039**	
Upper Body Ffm (kg)	Baseline	201	29 (9)	59	38 (7)	142	27 (3)	**<0.001**
	Month 3	64	27 (7)	17	39.29 (5.23)**	47	26 (3)	**<0.001**
	*p***		**<0.001**		**0.011**		**0.001**	

p**: p-value showing differences within the two sexes, using the Mann-Whitney test.
p**:** p-value showing overall change from baseline using the Wilcoxon signed-rank test.
** variable follows the normal distribution, so mean ± SD are shown.

Within-diet group analyses (Table 30) did not present any statistically significant differences for the majority of the 3-month changes in body composition measurements across the two groups. However, participants in the high-protein

group showed a significant reduction in kilograms of upper body fat (18kg at baseline vs 16kg at the end of the 3months, p=0.007), compared to the ones in the high-carbohydrate group.

Table 30. Changes in body composition measurements per diet group, during the three months of the study.

Variable	Time	N1 (High Carb)	Median ± IQR	N2 (High Prot)	Median ± IQR	*p**
Fat %	Baseline	94	38.35 (7.13)**	108	37.9 (13.3)	0.628
	Month 3	27	37.56 (6.05)**	37	37.84 (7.76)**	0.492
	*p***		0.269		0.135	
Fat kg	Baseline	94	31.3 (11.9)	108	33.95 (15.1)	0.381
	Month 3	26	31 (11)	37	32 (12)	0.364
	*p***		**0.008**		**0.014**	
Ffm kg	Baseline	94	50.5 (14)	108	53 (21)	**0.019**
	Month 3	27	49 (18)	37	49 (18)	0.509
	*p***		**<0.001**		**0.001**	
TBW kg	Baseline	94	37 (10)	108	39 (15)	**0.016**
	Month 3	27	36 (12)	37	36 (13)	0.479
	*p***		**<0.001**		**<0.001**	
Visceral Fat	Baseline	94	10 (4)	108	11 (5)	**0.041**
	Month 3	27	10 (6)	37	10 (6)	0.881
	*p***		**0.009**		**0.002**	
Upper Body Fat %	Baseline	94	36.59 (6.45)**	108	36.8 (7.45)**	0.778
	Month 3	27	35.63 (5.7)**	37	36.92 (7.3)**	0.247
	*p***		0.374		0.185	
Upper Body Fat kg	Baseline	94	16 (7)	107	18 (7)	**0.029**
	Month 3	27	15 (8)	37	16 (7)	0.190
	*p***		0.099		**0.007**	
Upper Body Ffm kg	Baseline	94	28 (7)	107	29 (10)	**0.028**
	Month 3	27	28 (8)	37	27 (8)	0.561
	*p***		**0.006**		**0.002**	

*__p__: p-value showing differences within the two diet groups, using the Mann-Whitney test.
__p**__: p-value showing change from the previous month for each diet group, using the Wilcoxon signed-rank test.
***: variable follows the normal distribution, so mean ± SD are shown.

Changes observed in Collected Data for Lifestyle Indices

Concerning the lifestyle measurements, the overall sample noted a statistically significant reduction in AIS score (5 vs 3.5, p=0.033), mainly driven by women (5 vs 3, p=0.134), denoting an improvement in sleeping qualities (Tables 31-32). Although statistically insignificant, the physical component of the SF-12 questionnaire presented a slight increase in the overall sample (52 vs 52.7, p=0.941). The SF mental component showed an insignificant increased from baseline up to the three months (49 vs 51.17,p=0.004, Figure 27), but noted a statistically significant increase from baseline up to the end of month 1 (49 vs 50.56, p=0.015).

Table 31. AIS, SF-PCS-12 and SF-MCS-12 measurements at the main points of the study.

Variable	Time	Total		Men		Women		
		N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	*P**
AIS	Baseline	140	5 (7)	42	3.5 (7.3)	98	5 (7)	0.246
	Month 3	62	3.5 (5)	19	4.21 (3.05)**	43	3 (5)	**0.033**
	*p***		**0.033**		0.098		0.134	
SF-PCS-12	Baseline	145	52 (12)	45	54 (9)	100	50(11)	**0.005**
	Month 3	80	52.7 (11.17)	22	55.91 (4.76)	58	50.40 (13.84)	**0.003**
	*p***		0.941		0.433		0.513	
SF-MCS-12	Baseline	145	49 (16)	45	47.04 (8.13)**	100	46.83 (10.31)	0.806
	Month 3	80	51.17 (15.32)	22	48.97 (12.51)	58	51.39 (15.23)	0.829
	*p***		0.444		0.911		0.324	

***p**: p-value showing differences within the two sexes, using the Mann-Whitney test.
***p*:** p-value showing overall change from baseline using the Wilcoxon signed-rank test.
** variable follows the normal distribution, so mean ± SD are shown.

Table 32. AIS, SF-PCS-12 and SF-MCS-12 measurements during the three months of the study.

Variable	Time	Total		Men		Women		
		N	Median ± IQR	N	Median ± IQR	N	Median ± IQR	*P**
AIS	Baseline	140	5 (7)	42	3.5 (7.3)	98	5 (7)	0.246
	Month 1	99	5 (6)	30	4 (6)	69	5 (6)	0.660
	*p***		0.660		0.970		0.642	
	Month 2	70	5 (7)	24	4.5 (6)	46	5 (8)	0.586
	*p***		0.586		0.555		0.758	
	Month 3	62	3.5 (5)	19	4.21 (3.05)**	43	3 (5)	0.299
	*p***		0.299		0.083		0.651	
SF-PCS-12	Baseline	145	52 (12)	45	54 (9)	100	50(11)	**0.005**
	Month 1	125	52.11 (9.59)	38	53.18 (7.22)	87	50.68 (10.46)	0.699
	*p***		0.699		0.530		0.868	
	Month 2	94	51.51 (10.51)	29	53.18 (6.34)	65	50.75 (11.01)	0.296
	*p***		0.296		0.627		0.365	

	Month 3	80	52.7 (11.17)	22	55.91 (4.76)	58	50.40 (13.84)	0.327
	*p***		0.327		0.163		0.712	
SF-MCS-12	Baseline	145	49 (16)	45	47.04 (8.13)**	100	46.83 (10.31)	0.806
	Month 1	125	50.56 (11.22)	38	52.48 (10.87)	87	49.19 (13.56)	**0.015**
	*p***		**0.015**		0.082		0.079	
	Month 2	94	50.75 (10.28)	29	50.64 (6.46)**	65	50.58 (10.72)	0.638
	*p***		0.638		0.563		0.802	
	Month 3	80	51.17 (15.32)	22	48.97 (12.51)	58	51.39 (15.23)	0.327
	*p***		0.327		0.124		0.798	

p**: p-value showing differences within the two sexes, using the Mann-Whitney test.
p*:* p-value showing overall change from the previous month using the Wilcoxon signed-rank test.
**: variable follows the normal distribution, so mean ± SD are shown.

Within the two diet groups (Tables 33-34), lifestyle characteristics did not present major changes from baseline up to month three. The statistically significant increase of the SF-MCS-12 in the overall sample appears to be driven by the participants in the high carbohydrate group, who showed a statistically important change from a mean value of 46.35 at baseline to a median of 50.56 at the end of month 1 (p=0.022).

Table 33. AIS, SF-PCS-12 and SF-MCS-12 measurements per diet group at the main points of the study.

Variable	Time	N1 (High Carb)	Median ± IQR	N2 (High Prot)	Median ± IQR	*p**
AIS	Baseline	67	5 (7)	73	4 (6.5)	0.730
	Month 3	27	4.81 (3.86)***	35	3 (5)	0.637
	*p***		0.272		0.062	
SF-PCS-12	Baseline	62	50 (14)	83	52 (8)	0.272
	Month 3	36	52 (11.67)	44	53.04 (10.58)	0.336
	*p***		0.469		0.547	
SF-MCS-12	Baseline	62	46.35 (9.74)***	83	50 (13)	0.507
	Month 3	36	53 (17.71)	44	50.39 (12.46)	0.578
	*p***		0.741		0.567	

p**: p-value showing differences within the two sexes, using the Mann-Whitney test.
p*:* p-value showing overall change from baseline using the Wilcoxon signed-rank test.
** variable follows the normal distribution, so mean ± SD are shown.

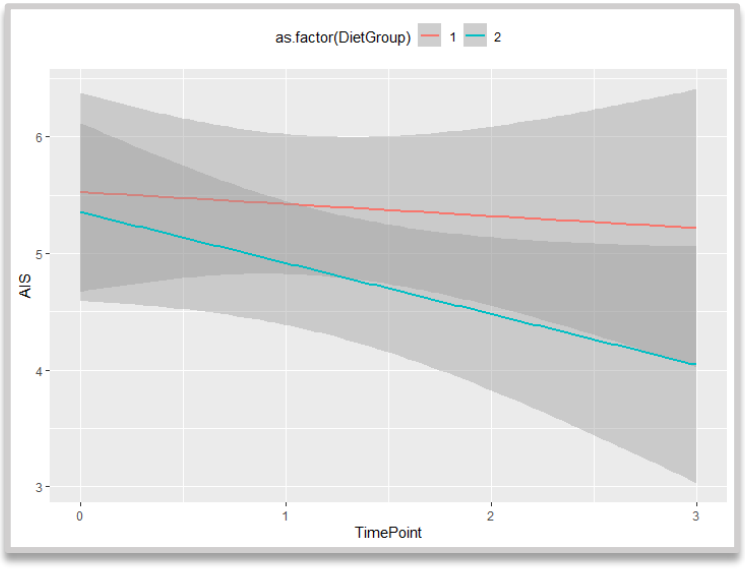
Table 34. Changes in AIS, SF-PCS-12 and SF-MCS-12 per diet group during the three months of the study.

Variable	Time	N1 (High Carb)	Median ± IQR	N2 (High Prot)	Median ± IQR	*p**
AIS	Baseline	67	5 (7)	73	4 (6.5)	0.730
	Month 1	46	5 (6)	53	5 (5)	0.516
	p-value**		0.693		0.843	
	Month 2	33	6 (7)	37	4 (6)	0.210
	p-value**		0.419		0.889	
	Month 3	27	4.81 (3.86)***	35	3 (5)	0.637

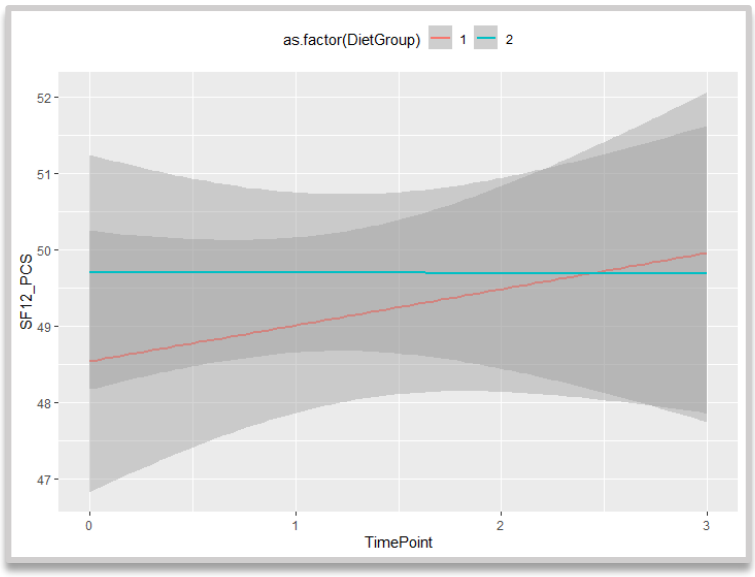
	p-value**		0.193		0.979	
SF-PCS-12	Baseline	62	50 (14)	83	52 (8)	0.272
	Month 1	58	52.10 (10.9)	67	52.11 (9.01)	0.995
	p-value**		0.637		0.399	
	Month 2	42	51.94 (13.23)	52	51.1 (9.13)	0.918
	p-value**		0.723		0.247	
	Month 3	36	52 (11.67)	44	53.04 (10.58)	0.336
	p-value**		0.869		0.090	
SF-MCS-12	Baseline	62	46.35 (9.74)***	83	50 (13)	0.507
	Month 1	58	50.56 (10.89)	67	50.58 (13.13)	0.622
	p-value**		**0.022**		0.243	
	Month 2	42	50.75 (13.11)	52	50.78 (9.22)	0.115
	p-value**		0.407		0.159	
	Month 3	36	53 (17.71)	44	50.39 (12.46)	0.578
	p-value**		0.544		0.080	

*__p__: p-value showing differences within the two diet groups, using the Mann-Whitney test.
__p**__: p-value showing change from the previous month for each diet group, using the Wilcoxon signed-rank test.
***: variable follows the normal distribution, so mean ± SD are shown.

A.



B.



C.

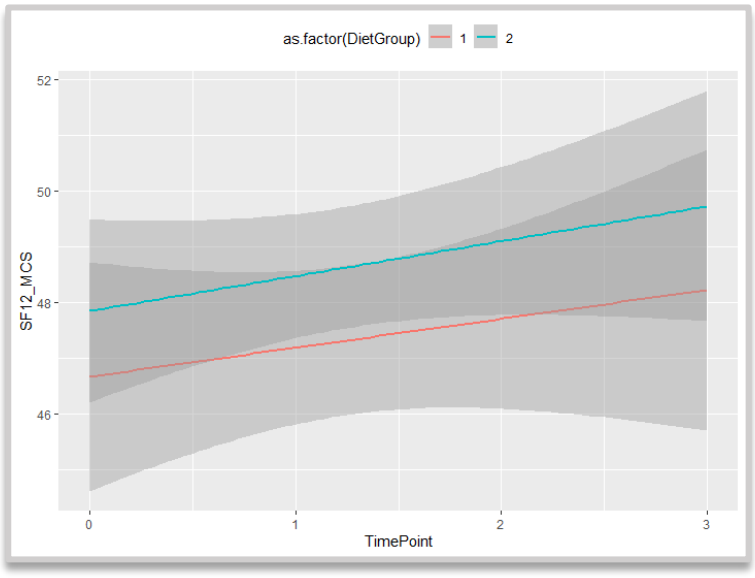


Figure 27. Trajectories of A. AIS, B. SF-PCS-12 and C. SF-MCS-12 change from baseline up to the end of month 3 of the intervention, per diet group (1=high carbohydrate and 2=high protein).

3.1.4. Genetic Predisposition and Anthropometric/Lifestyle Parameters

3.1.4.1. Baseline Associations

Proceeding to exploring the effect of genetic predisposition on the population's baseline characteristics, we set out by conducting linear regressions for the baseline anthropometric and lifestyle indices displayed in Table 35. Interestingly, both GRSs were statistically significantly associated with lower levels of AIS, denoting that an elevated genetic risk for increased BMI does not necessarily imply increased risk for disrupted sleep habits.

Table 35. Associations between the cohort's baseline characteristics and the uGRS and wGRS.

	Model 1			Model 2		
	Estimate	***SE***	***p***	***Estimate***	***SE***	***p***
logWeight						
uGRS	-0.0004	0.001	0.711	-0.0003	0.001	0.749
wGRS	-0.005	0.037	0.886	-0.003	0.037	0.934
logBMI						
uGRS	-0.001	0.001	0.328	-8.605e-04	9.847e-04	0.383
wGRS	-0.026	0.032	0.419	-2.199e-02	3.258e-02	0.501
logWHR						
uGRS	0.001	0.001	0.183	-0.009	0.006	0.126
wGRS	0.023	0.019	0.233	0.001	0.001	0.128
logFat_kg						
uGRS	-0.001	0.002	0.513	-0.001	0.002	0.547
wGRS	-0.020	0.065	0.759	-0.017	0.065	0.793
logAIS						
uGRS	-0.017	0.005	**0.001**	-0.017	0.005	**0.002**
wGRS	-0.546	0.175	**0.002**	-0.537	0.178	**0.003**
LogCESD-R-10						
uGRS	-0.005	0.004	0.264	-0.005	0.004	0.236
wGRS	-0.073	0.144	0.612	-9.071e-02	1.469e-01	0.537
LogSF-PCS-12						
uGRS	9.574e-05	1.486e-03	0.949	-0.001	0.001	0.724
wGRS	-0.003	0.049	0.951	-0.025	0.048	0.597
LogSF-MCS-12						
uGRS	0.002	0.001	0.185	0.002	0.002	0.203
wGRS	0.046	0.059	0.439	0.052	0.058	0.369

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, PAL, smoking

Furthermore, we used the 10 BMI-related and the 10 fat-related SNPs to separately examine associations as shown in Table 36. Associations with the forme3r did not present any statistically significant trends. Interestingly, carriers of the T allele of the rs6265 steadily presented nominal associations with the body fat indices, namely logbody fat in kg (Model 1: β=0.037, p=0.027; Model 2: β=0.035, p=0.032), logVisceral fat Model 1: β=0.039, p=0.016; Model 2: β=0.038, p=0.019), and logUpper Body fat in kg (Model 1: β=0.038, p=0.032; Model 2: β=0.036, p=0.041).

Table 36. Associations between the cohort's baseline characteristics and the 10 candidate, fat-related variants.

	Model 1			Model 2		
	Beta	*Stat**	*p*	*Beta*	*Stat**	*p*
logFat_kg						
rs574367_T	-0.029	-1.506	0.134	-0.027	-1.407	0.161
rs2605100_A	-0.006	-0.409	0.683	-0.003	-0.157	0.875
rs4846567_T	0.010	0.583	0.561	0.015	0.854	0.394
rs10195252_T	-0.006	-0.374	0.709	-0.009	-0.594	0.554
rs206936_G	0.022	0.969	0.333	0.042	1.813	0.071
rs4994_G	-0.004	-0.127	0.899	0.001	0.023	0.982
rs11191548_C	-0.001	-0.023	0.982	0.009	0.339	0.735
rs6265_T	**0.037**	**2.234**	**0.027**	**0.035**	**2.163**	**0.032**
rs1443512_A	-0.026	-1.435	0.153	-0.032	-1.767	0.079
rs12970134_A	-0.018	-1.009	0.314	-0.023	-1.265	0.207
logVisceral Fat						
rs574367_T	-0.023	-1.183	0.238	-0.021	-1.105	0.270
rs2605100_A	-0.002	-0.099	0.921	0.001	0.090	0.928
rs4846567_T	0.006	0.333	0.739	0.009	0.534	0.594
rs10195252_T	-0.004	-0.286	0.775	-0.007	-0.486	0.628
rs206936_G	0.014	0.657	0.512	0.029	1.254	0.211
rs4994_G	-0.012	-0.378	0.701	-0.008	-0.262	0.794
rs11191548_C	0.002	0.069	0.945	0.008	0.326	0.745
rs6265_T	**0.039**	**2.437**	**0.016**	**0.038**	**2.367**	**0.019**
rs1443512_A	-0.019	-1.113	0.267	-0.025	-1.413	0.159
rs12970134_A	-0.014	-0.808	0.419	-0.018	-1.042	0.299
logUpper Body Fat kg						
rs574367_T	-0.026	-1.262	0.209	-0.024	-1.18	0.239
rs2605100_A	-0.005	-0.299	0.765	-0.001	-0.055	0.956
rs4846567_T	0.007	0.399	0.690	0.013	0.674	0.501
rs10195252_T	-0.010	-0.6044	0.520	-0.014	-0.866	0.387
rs206936_G	0.015	0.635	0.526	0.039	1.593	0.113
rs4994_G	-0.005	-0.146	0.884	0.001	0.019	0.985
rs11191548_C	0.006	0.224	0.823	0.019	0.663	0.508
rs6265_T	**0.038**	**2.161**	**0.032**	**0.036**	**2.062**	**0.041**
rs1443512_A	-0.034	-1.808	0.072	-0.040	-2.099	**0.037**
rs12970134_A	-0.019	-1.009	0.314	-0.024	-1.269	0.206

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, PAL, smoking

Interactions between GRSs and Dietary Patterns at Baseline

Additionally, in an effort to assess the effect of gene-diet interactions on the examined indices at baseline, we proceeded to conducting multivariate linear regressions including the effect of the interaction between: a) the constructed GRSs and the extracted dietary patterns (statistically significant results shown at Tables 37-38); and b) the selected BMI candidate variants and the dietary patterns (Table 39). Interestingly, we found that the interaction between the GRSs and adherence to the "Mixed" Pattern was associated with increased levels of logSF-PCS-12 (uGRS Model 1: β=0.004, p=0.003; Model 2: β=0.004, p=0.028, wGRS Model 1: β=0.148, p=0.001; Model 2: β=0.132, p=0.009).

Table 37. Interactions between the cohort's baseline characteristics, dietary patterns and the uGRS.

	Model 1			Model 2		
LogSF-PCS-12						
uGRS* Mixed Pattern	0.004	0.001	**0.003**	0.004	0.002	**0.028**
uGRS*Med-proxy Pattern	4.824e-04	1.622e-03	0.767	6.089e-05	1.565 e-03	0.969
uGRS* Eating-out Pattern	-1.650e-03	2.323e-03	0.479	-0.001	0.002	0.693
uGRS* Traditional, vegetarian-alike Pattern	1.976e-03	1.496e-03	0.189	0.0002	0.002	0.870
uGRS* High in unsaturated fats and fruit juice consumption Pattern	-0.016	0.059	0.781	-0.002	0.002	0.309
LogSF-MCS-12						
uGRS* Mixed Pattern	-0.001	0.002	0.637	-0.001	0.002	0.795
uGRS*Med-proxy Pattern	-0.003	0.002	0.174	-0.003	0.002	0.105
uGRS* Eating-out Pattern	-0.002	0.003	0.532	-0.0004	0.003	0.882
uGRS* Traditional, vegetarian-alike Pattern	0.001	0.002	0.454	0.001	0.002	0.524
uGRS* High in unsaturated fats and fruit juice consumption Pattern	-0.0001	0.002	0.931	-0.001	0.002	0.661

Model 1: Adjusted for age, sex, uGRS and each dietary pattern**; Model 2:** Adjusting for age, sex, PAL, smoking, uGRS and each dietary pattern

Table 38. Interactions between the cohort's baseline characteristics, dietary patterns and the wGRS.

	Model 1			Model 2		
	Estimate	*SE*	*p*	*Estimate*	*SE*	*p*
LogSF-PCS-12						
wGRS* Mixed Pattern	**0.148**	**0.042**	**0.001**	0.132	0.049	**0.009**
wGRS*Med-proxy Pattern	0.034	0.052	0.519	0.013	0.050	0.792
wGRS* Eating-out Pattern	-0.107	0.067	0.114	-0.082	0.065	0.215
wGRS* Traditional, vegetarian-alike Pattern	7.061e-02	5.223e -02	0.179	-0.016	0.059	0.781
wGRS* High in unsaturated fats and fruit juice consumption Pattern	0.009	0.053	0.858	-0.079	0.055	0.151
LogSF-MCS-12						
wGRS* Mixed Pattern	-0.006	0.055	0.908	-0.001	0.064	0.992
wGRS*Med-proxy Pattern	-0.081	0.062	0.191	-0.101	0.06	0.096
wGRS* Eating-out Pattern	-0.081	0.081	0.323	-0.044	0.080	0.583

wGRS* Traditional, vegetarian-alike Pattern	0.0002	0.064	0.998	-0.011	0.071	0.878
wGRS* High in unsaturated fats and fruit juice consumption Pattern	-0.024	0.063	0.706	-0.052	0.066	0.437

Model 1: Adjusted for age, sex, wGRS and each dietary pattern**; Model 2:** Adjusting for age, sex, PAL, smoking, wGRS and each dietary pattern

Interactions between Candidate Variants and Dietary Patterns at Baseline

Subsequently, a similar set of regressions was conducted to test the interactions between the dietary patterns and the BMI-candidate variants. Table 40 shows the statistically significant associations observed. Overall, we observe that carriers of BMI-raising alleles tended to present higher levels of anthropometric indices and worse lifestyle indices when adhering to patterns with increased sugar or fat content, such as the "Mixed" and "High in unsaturated fats and fruit juice consumption" ones, and lower such levels when adhering to the more balanced patterns like the Traditional, vegetarian-alike one.

In the case of weight, we observed that carriers of BMI-raising alleles in the variants rs1421085 (C), rs1121980 (A), rs17817449 (G), rs3751812 (T), rs9939609 (A) who adhere to the "Traditional, vegetarian-alike" pattern tended to display nominally significant associations with lower levels of weight (nominal associations of $p<0.05$ and $p<0.01$). On the contrary, carriers of the BMI-positively associated T allele of the rs3751812 variant who adhered to the "High in unsaturated fats and fruit juice consumption" pattern showed increased levels of weight, after adjusting for age and sex (Model 1: $\beta=0.022$, $p=0.048$). Similar trends were observed for BMI, where rs3751812-T and rs9939609-A allele carriers noted lower levels of BMI when adhering to the "Traditional, vegetarian-alike" pattern $p<0.05$ and $p<0.01$, respectively) , while rs3751812-T allele carriers consuming the "High in unsaturated fats and fruit juice consumption" pattern presented increased BMI. Associations with baseline total body fat in kg also presented akin findings with rs3751812_T and rs9939609_A being associated with lower levels when adhering to the Traditional patterns, whereas the former being associated with higher levels when adhering to the High in unsaturated fats pattern.

Regarding the lifestyle measurements, attrition to the latter was associated with increased CESD-R-10 score in carriers of the BMI-raising T allele of the rs925946 variant. Interestingly, the physical component of the SF-12 questionnaire presented most of the nominally significant interactions, namely: i) the aggravating effect of the rs1421085 (C), rs1121980 (A), rs17817449 (G), rs9939609 (A), rs17782313 (C) variants in SF-PCS-12 scores when adherence to the "Mixed" dietary pattern was present; ii) a negative association between the rs6548238 T allele and increased BMI values when adhering to the "Mixed" pattern; iii) a positive relation between BMI-raising rs3751812- T allele and the score when the interaction with the "Mixed" Pattern was present); iv) the aggravating effect of the rs1421085 (C), rs1121980 (A), rs17817449 (G) and rs9939609 (C) variants in SF-PCS-12, even when adherence to the "Med-Proxy" pattern was present.

Table 39. Statistically significant interactions between the cohort's baseline characteristics, dietary patterns and the 10 candidate, BMI-related variants.

	Model 1			Model 2		
	Beta	*Stat**	*p*	*Beta*	*Stat**	*p*
logWeight						

rs1421085_C* Traditional, vegetarian-alike Pattern	-0.016	-1.945	0.054	**-0.017**	**-2.015**	**0.046**
rs1121980_A* Traditional, vegetarian-alike Pattern	-0.017	-1.954	0.053	**-0.017**	**-1.99**	**0.049**
rs17817449_G* Traditional, vegetarian-alike Pattern	**-0.028**	**-2.393**	**0.018**	**-0.029**	**-2.487**	**0.014**
rs3751812_T* Traditional, vegetarian-alike Pattern	**-0.026**	**-2.307**	**0.023**	**-0.026**	**-2.292**	**0.023**
rs9939609_A* Traditional, vegetarian-alike Pattern	**-0.003**	**-2.692**	**0.008**	**-0.030**	**-2.663**	**0.009**
rs3751812_T* High in unsaturated fats and fruit juice consumption Pattern	**0.022**	**1.997**	**0.048**	0.021	1.862	0.065
logBMI						
rs3751812_T* Traditional, vegetarian-alike Pattern	**-0.026**	**-2.307**	**0.023**	**-0.023**	**-2.189**	**0.030**
rs9939609_A* Traditional, vegetarian-alike Pattern	**-0.030**	**-2.692**	**0.008**	-0.020	-1.932	0.056
rs3751812_T* High in unsaturated fats and fruit juice consumption Pattern	**0.022**	**1.997**	**0.048**	**0.021**	**2.06**	**0.041**
logFat_kg						
rs3751812_T* Traditional, vegetarian-alike Pattern	**-0.047**	**-2.256**	**0.026**	**-0.047**	**-2.256**	**0.026**
rs9939609_A* Traditional, vegetarian-alike Pattern	**-0.043**	**-2.104**	**0.037**	**-0.043**	**-2.075**	**0.039**
rs3751812_T* High in unsaturated fats and fruit juice consumption Pattern	**0.046**	**2.249**	**0.026**	**0.044**	**2.135**	**0.035**
LogCESD-R-10						
rs925946_T* High in unsaturated fats and fruit juice consumption Pattern	**0.104**	**2.163**	**0.032**	**0.097**	**2.006**	**0.047**
LogSF-PCS-12						
rs6548238_T* Mixed Pattern	**-0.030**	**-2.11**	**0.037**	**-0.031**	**-2.151**	**0.034**
rs1421085_C* Mixed Pattern	**-0.026**	**-3.235**	**0.002**	**-0.027**	**-3.474**	**0.001**
rs1121980_A* Mixed Pattern	**-0.024**	**-3.056**	**0.003**	**-0.026**	**-3.269**	**0.001**
rs17817449_G* Mixed Pattern	**-0.029**	**-3.572**	**0.001**	**-0.030**	**-3.821**	**0.0002**
rs3751812_T* Mixed Pattern	**0.020**	**2.062**	**0.042**	**0.021**	**2.17**	**0.032**
rs9939609_A* Mixed Pattern	**-0.026**	**-3.227**	**0.002**	**-0.027**	**-3.455**	**0.001**
rs17782313_C* Mixed Pattern	**-0.030**	**-2.238**	**0.027**	**-0.028**	**-2.122**	**0.036**
rs1421085_C* Med-Proxy Pattern	**-0.018**	**-2.133**	**0.035**	-0.017	-1.912	0.059
rs1121980_A* Med-Proxy Pattern	**-0.018**	**-2.029**	**0.045**	-0.016	-1.787	0.077
rs17817449_G* Med-Proxy Pattern	**-0.022**	**-2.261**	**0.026**	**-0.019**	**-2.073**	**0.041**
rs9939609_A* Med-Proxy Pattern	**-0.024**	**-2.456**	**0.016**	**-0.022**	**-2.231**	**0.028**

Model 1: Adjusted for age, sex, SNP and each dietary pattern**; Model 2:** Adjusting for age, sex, PAL, smoking, SNP and each dietary pattern

3.1.4.2. Associations with changes post-intervention

Effect of GRSs and Candidate Variants on Imputed Weight Loss

Proceeding to exploring the effect of genetic predisposition in the changes observed after the intervention period, we set out by using the imputed data for weight loss at the end of the 3-month period to examine potential associations between the scores and the observed changes in the index. As shown in Table 40, neither the uGRS not the wGRS were statistically significantly associated with weight change post-intervention. Within-diet group analyses also showed no statistically significant relations for weight loss in participants following either the high carbohydrate or the high protein diet.

Table 40. Multivariate linear regressions between the constructed GRSs and imputed weight loss post-intervention in the overall sample.

	Model 1			Model 2			Model 3		
	*Coef**	*SE*	*p*	*Coef**	*SE*	*p*	*Coef**	*SE*	*p*
Weight change									
uGRS	-0.024	0.084	0.772	-0.023	0.084	0.784	-0.022	0.083	0.789
wGRS	-0.034	2.851	0.905	-0.455	2.854	0.874	-0.0425	2.850	0.882

***Coef**: Coefficient

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, PAL, smoking; **Model 3:** Adjusting for age, sex, PAL, smoking and diet group

In a similar manner to the above, we subsequently used the 10 aforementioned, BMI-related SNPs to separately examine associations with post-intervention weight change, using the imputed weight data. As displayed in Table 41, the SNPs did not display statistically significant associations for the observed weight change post-intervention. Within-diet group analyses also showed no statistically significant relations for weight loss in participants following either the high carbohydrate or the high protein diet.

Table 41. Multivariate linear regressions between the 10 examined SNPs and imputed weight loss post-intervention in the overall sample.

	Model 1			Model 2			Model 3		
	*Coef**	*SE*	*p*	*Coef**	*SE*	*p*	*Coef**	*SE*	*p*
Weight change									
rs6548238_C									
Heterozygote	0.284	2.901	0.922	1.158	3.001	0.700	1.078	3.005	0.720
Homozygote	1.716	2.758	0.535	1.158	3.001	0.700	2.468	2.886	0.391
rs1801282_G									
Heterozygote	-2.658	4.007	0.509	-2.780	3.996	0.489	-2.727	3.999	0.498
Homozygote	-3.874	3.706	0.299	-4.048	3.709	0.668	-3.962	3.718	0.290
rs2241766_G									
Heterozygote	-0.163	1.122	0.885	-0.168	1.121	0.881	-0.135	1.122	0.904
Homozygote	-0.486	1.275	0.707	-0.431	1.288	0.739	-0.409	1.285	0.751
rs925946_T									
Heterozygote	0.850	1.068	0.427	0.889	1.061	0.404	0.902	1.059	0.396
Homozygote	-0.878	2.193	0.690	-1.129	2.194	0.608	-1.177	2.194	0.593
rs17817449_G									
Heterozygote	-0.077	1.326	0.953	-0.307	1.382	0.824	-0.294	1.381	0.832
Homozygote	0.298	1.262	0.813	0.131	1.312	0.921	1.135	1.311	0.918
rs3751812_T									

Heterozygote	-0.722	1.463	0.623	-0.551	1.441	0.703	-0.388	1.445	0.789
Homozygote	-0.544	1.343	0.686	-0.327	1.341	0.808	-0.145	1.362	0.916
rs17782313_C									
Heterozygote	-1.809	3.404	0.597	-2.353	3.522	0.506	-2.456	3.535	0.490
Homozygote	-0.591	3.325	0.859	-1.157	3.459	0.739	-1.233	3.469	0.723
rs9939609_A									
Heterozygote	0.089	1.394	0.949	-0.105	1.442	0.942	-0.086	1.442	0.952
Homozygote	0.237	1.267	0.852	0.481	1.324	0.971	0.068	1.325	0.959
rs1421085_C									
Heterozygote	-0.309	1.310	0.814	-0.291	1.326	0.827	-0.318	1.325	0.811
Homozygote	0.498	1.147	0.665	0.485	1.174	0.680	0.466	1.173	0.692
rs1121980_A									
Heterozygote	-0.246	1.298	0.850	-0.219	1.311	0.867	-0.238	1.309	0.856
Homozygote	0.502	1.148	0.299	0.479	1.174	0.684	0.458	1.173	0.697

***Coef**: Coefficient

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, PAL, smoking; Model 3: Adjusting for age, sex, PAL, smoking and diet group.

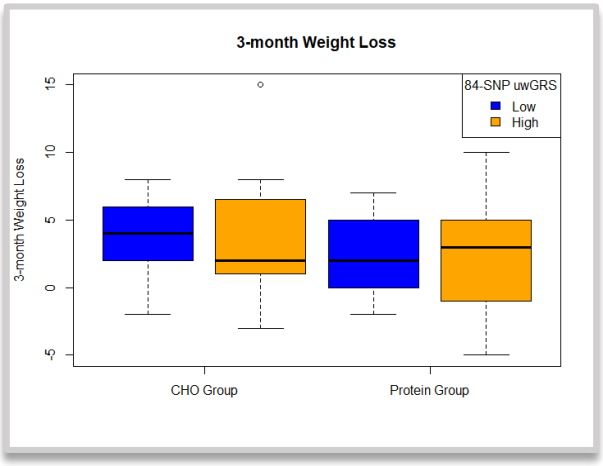
Effect of GRSs and Candidate Variants on Observed Changes

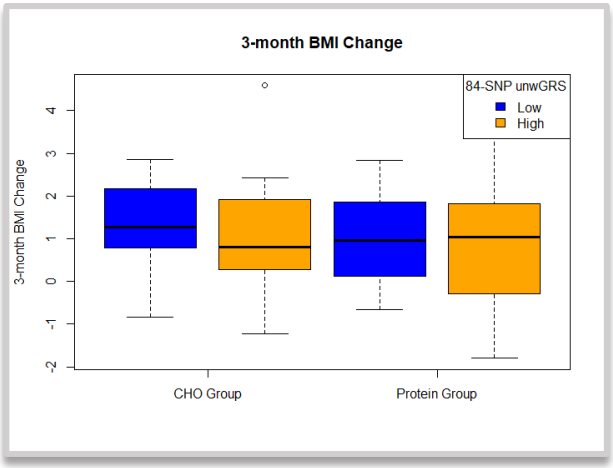
Using the observed values for weight and BMI change post-intervention, we moved on to splitting the overall sample in categories of either "High" or "Low" uGRs and wGRS, based on the observed scores' sample medians. Although differences between the two groups were not statistically significant for both the uGRS and the wGRS, a steady decrease was observed in both the weight and BMI change from baseline in the high GRS groups (Table 42). Within-diet group analyses also showed no statistically significant relations for weight loss in participants following either the high carbohydrate or the high protein diet (p=0.273 and p=0.639 for the uGRS and p=0.777 and p=0.207 for the wGRS, respectively). Similarly to above, although statistically insignificant, a steady decrease in changes was observed in groups with high GRSs for both diet categories (Figure 28).

Table 42. Mean changes in weight and BMI post-intervention, per GRS groups in the overall sample.

Variable	Low GRS		High GRS		
	N	Mean (SD)	N	Mean (SD)	*P**
Weight Change					
uGRS	34	3.53 (3.69)	42	2.57 (3.47)	0.289
wGRS	31	3.61 (3.77)	44	2.55 (3.46)	0.245
BMI Change					
uGRS	34	1.17 (1.24)	41	0.94 (1.23)	0.444
wGRS	31	1.19 (1.26)	43	0.93 (1.23)	0.390

A.





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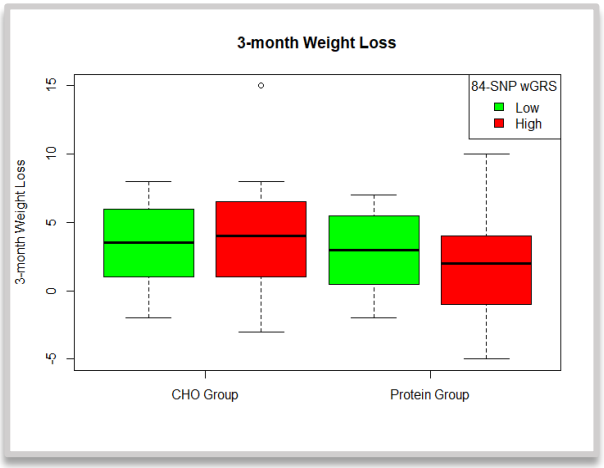

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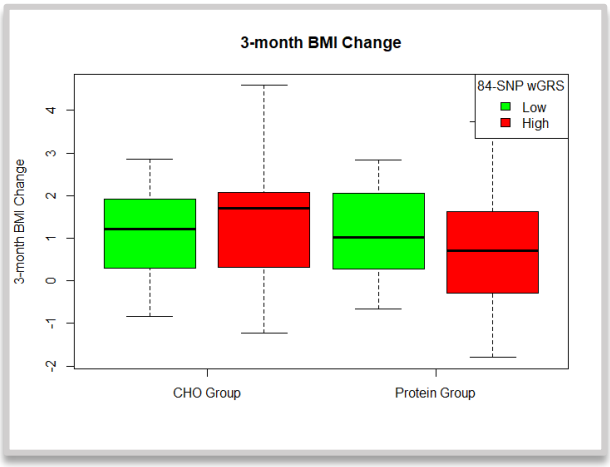


D.

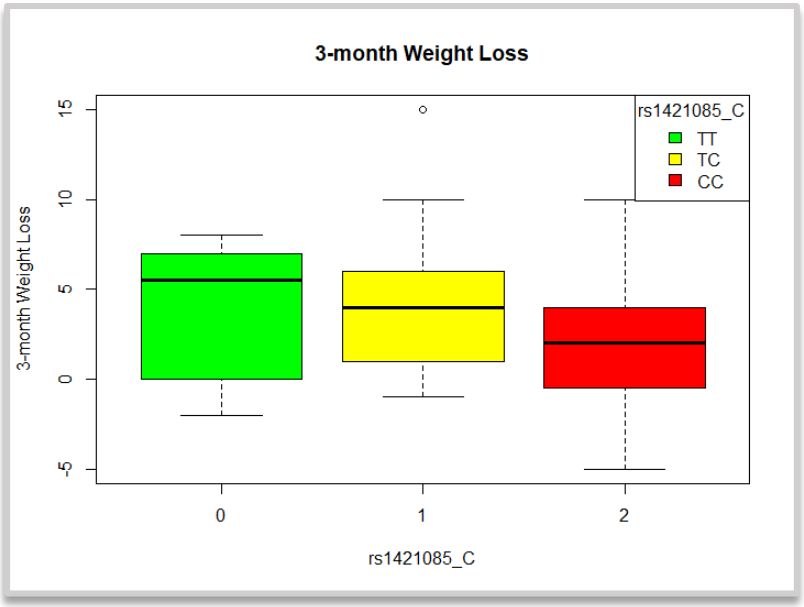
Figure 28. Clustered boxplots depicting A. 3-month weight loss per uGRS and diet group, B. 3-month BMI change per uGRS and diet group, C. 3-month weight loss per wGRS and diet group and D. 3-month BMI change per wGRS and diet group.

Additionally, we proceeded to examining potential differences in post-intervention weight and BMI within the separate groups of genotypes for the 10 candidate, BMI-related variants (Table 43). Kruskal-Wallis tests revealed statistically significant differences in the observed weight change across the groups of the

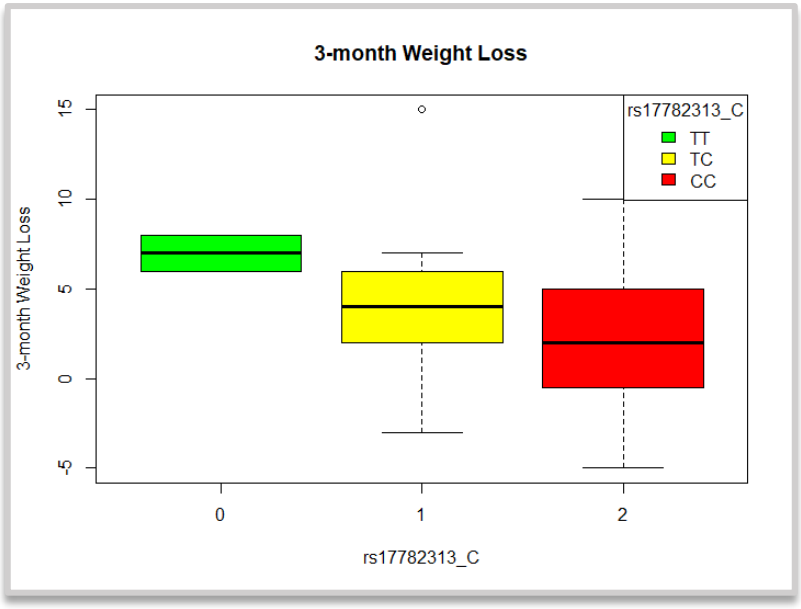
rs1421085 and the rs17782313 variants. Carriers of the BMI-raising C allele of the former displayed statistically significantly lower change in weight post-intervention (p<0.036, Figure 29). Similarly, carriers of the BMI-positively associated C allele of the rs17782313 SNP also showed statistically significantly lower weight changes after the 3-month period (p=0.043, Figure 29).

Table 43. Mean changes in weight and BMI post-intervention, per groups of the 10 candidate variants in the overall sample.

Variable	Homozygotes for the non-effect allele		Heterozygotes		Homozygotes for the non-effect allele		
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	*P**
Weight Change							
rs6548238_C	2	5.5 (2.12)	10	4.7 (4.64)	71	2.9 (3.35)	0.161
rs1801282_G	2	0.5 (2.12)	4	1 (2.94)	77	2.99 (3.60)	0.310
rs2241766_G	14	1.86 (3.68)	47	2.96 (3.79)	22	3.18 (2.99)	0.575
rs925946_T	66	2.61 (3.73)	14	3.36 (2.79)	3	5.33 (2.08)	0.237
rs1421085_C	10	4.2 (3.82)	21	4.19 (3.89)	52	2.02 (3.17)	**0.036**
rs1121980_A	10	4.2 (3.82)	23	3.96 (3.80)	50	2.04 (3.23)	0.056
rs17817449_G	10	4.2 (3.82)	23	3.70 (4.16)	50	2.16 (3.09)	0.091
rs3751812_T	8	2.5 (3.30)	23	2.91 (3.30)	52	2.85 (3.76)	0.876
rs9939609_A	10	4.2 (3.82)	21	3.29 (4.39)	52	2.38 (3.09)	0.246
rs17782313_C	2	7 (1.41)	17	4.17 (4.00)	64	2.34 (3.33)	**0.043**
BMI Change							
rs6548238_C	2	1.50 (0.69)	10	1.66 (1.50)	70	0.86 (1.18)	0.411
rs1801282_G	2	0.27 (0.98)	4	0.26 (1.09)	76	1.04 (1.24)	0.327
rs2241766_G	14	0.70 (1.33)	46	1.03 (1.31)	22	1.07 (1.03)	0.381
rs925946_T	65	0.91 (1.30)	14	1.22 (0.99)	3	1.66 (0.44)	0.238
rs1421085_C	10	1.36 (1.34)	21	1.42 (1.27)	51	0.73 (1.15)	0.119
rs1121980_A	10	1.36 (1.34)	23	1.35 (1.24)	49	0.74 (1.17)	0.125
rs17817449_G	10	1.35 (1.35)	23	1.25 (1.37)	49	0.79 (1.13)	0.163
rs3751812_T	8	0.86 (1.17)	22	1.07 (1.20)	52	0.97 (1.28)	0.744
rs9939609_A	10	1.35 (1.35)	21	1.09 (1.43)	51	0.87 (1.13)	0.212
rs17782313_C	2	2.32 (0.05)	17	1.44 (1.32)	63	0.82 (1.18)	0.063



A.



B.

Figure 29. Clustered boxplots depicting3-month weight loss A. per genotype groups of the rs1421085 SNP and B. per genotype groups of the rs17782313 SNP.

Additionally, we conducted multiple linear regressions using the observed data to examine further associations between the tested variants and changes in the observed indices (Table 44). Presence of the T allele of the rs6548238 variant was nominally associated with increased change in total body fat in kg, after adjustment for both models of confounding factors (Model 1: β=2.329, p=0.016; Model 2: β=2.639, p=0.006). Presence of the aggravating G and A alleles of the rs17817449 and rs9939609 SNPs were also nominally associated with lower changes in the SF-PCS-12 across both adjusting Models (p<0.01 for all associations). Interestingly, presence of the BMI-raising alleles for the rs1421085 (C), rs1121980 (A), rs17817449 (G), rs9939609 (A) SNPs were nominally associated with increased changes in SF-MCS-12 post-intervention. Finally, out of the selected fat-related variants, presence of the G allele of the rs4995 SNP was found nominally related with lower change in upper body fat in kg (Model 2: β=-1.715, p=0.033). No statistically significant associations were observed between the changes post-intervention in fat-related indices and the 10 candidate, fat-related variants.

Table 44. Associations between the changes observed post-intervention and the 10 candidate, BMI-related SNPs.

	Model 1			Model 2		
	Beta	*Stat**	*p*	*Beta*	*Stat**	*p*
BMI Change						
rs6548238_T	0.530	1.599	0.114	0.607	1.828	0.072
rs1801282_G	-0.516	-1.397	0.166	-0.582	-1.538	0.128
rs2241766_G	-0.143	-0.675	0.502	-0.153	-0.724	0.471
rs925946_T	0.301	1.101	0.2744	0.318	1.15	0.254
rs1421085_C	0.382	1.95	0.055	0.306	1.437	0.155
rs1121980_A	0.359	1.833	0.071	0.279	1.325	0.189
rs17817449_G	0.301	1.531	0.129	0.209	0.957	0.342
rs3751812_T	0.013	0.062	0.950	0.055	0.259	0.796

rs9939609_A	0.199	0.997	0.323	0.091	0.411	0.683
rs17782313_C	**0.650**	**2.338**	**0.022**	0.576	1.905	0.061
WHR Change						
rs6548238_T	0.046	0.489	0.627	0.015	1.045	0.300
rs1801282_G	0.11	0.863	0.394	0.016	0.836	0.407
rs2241766_G	0.063	0.837	0.408	-0.004	-0.469	0.640
rs925946_T	0.015	0.177	0.861	**0.028**	**2.244**	**0.029**
rs1421085_C	-0.013	-0.178	0.859	0.011	1.043	0.301
rs1121980_A	-0.013	-0.178	0.859	0.006	0.625	0.534
rs17817449_G	0.012	0.176	0.862	0.019	1.925	0.059
rs3751812_T	0.111	1.276	0.211	0.002	0.153	0.879
rs9939609_A	-0.013	-0.178	0.859	0.015	1.441	0.155
rs17782313_C	-0.043	-0.433	0.668	0.021	1.497	0.139
Fat_kg Change						
rs6548238_T	**2.329**	**2.497**	**0.016**	**2.639**	**2.873**	**0.006**
rs1801282_G	-1.658	-1.839	0.071	-1.724	-1.872	0.067
rs2241766_G	-0.235	-0.408	0.685	-0.296	-0.515	0.609
rs925946_T	0.457	0.616	0.541	0.495	0.667	0.507
rs1421085_C	1.019	1.903	0.061	0.811	1.354	0.181
rs1121980_A	0.988	1.848	0.069	0.773	1.302	0.198
rs17817449_G	0.837	1.562	0.124	0.517	0.843	0.403
rs3751812_T	0.3675	0.660	0.512	0.561	1.005	0.319
rs9939609_A	0.715	1.32	0.192	0.409	0.668	0.507
rs17782313_C	1.393	1.877	0.066	1.035	1.27	0.209
AIS Change						
rs6548238_T	0.435	0.434	0.667	0.284	0.281	0.780
rs1801282_G	0.233	0.137	0.892	1.106	0.597	0.554
rs2241766_G	0.726	1.3	0.201	0.629	1.091	0.282
rs925946_T	0.532	0.774	0.444	0.580	0.837	0.408
rs1421085_C	-0.473	-1.027	0.311	-0.838	-1.703	0.097
rs1121980_A	-0.473	-1.027	0.311	-0.828	-1.703	0.097
rs17817449_G	-0.223	-0.474	0.638	-0.659	-1.245	0.221
rs3751812_T	-0.614	-1.182	0.244	-0.471	-0.868	0.391
rs9939609_A	-0.279	-0.575	0.569	-0.808	-1.485	0.146
rs17782313_C	0.692	1.019	0.314	1.046	1.422	0.164
SF-PCS-12 Change						
rs6548238_T	-2.699	-1.134	0.261	-2.407	-0.989	0.327
rs1801282_G	0.167	0.055	0.956	-0.156	-0.050	0.960
rs2241766_G	-0.039	-0.027	0.979	-0.027	-0.018	0.985
rs925946_T	-1.377	-0.767	0.446	-1.475	-0.804	0.425
rs1421085_C	-2.356	-1.676	0.099	-2.521	-1.63	0.109
rs1121980_A	-2.115	-1.507	0.137	-2.194	-1.436	0.157
rs17817449_G	**-3.758**	**-2.681**	**0.009**	**-4.594**	**-2.91**	**0.005**
rs3751812_T	-1.882	-1.205	0.233	-1.834	-1.157	0.252
rs9939609_A	**-3.202**	**-2.247**	**0.028**	**-4.093**	**-2.474**	**0.016**
rs17782313_C	-0.151	-0.069	0.945	-1.38	-0.587	0.559
SF-MCS-12 Change						
rs6548238_T	5.984	1.916	0.060	5.614	1.737	0.088
rs1801282_G	-1.161	-0.288	0.775	-0.209	-0.049	0.961
rs2241766_G	1.03	0.521	0.605	1.091	0.544	0.589
rs925946_T	1.508	0.627	0.533	1.284	0.516	0.608
rs1421085_C	**4.304**	**2.337**	**0.023**	**4.556**	**2.223**	**0.030**
rs1121980_A	**4.108**	**2.235**	**0.029**	**4.266**	**2.109**	**0.039**
rs17817449_G	**5.7**	**3.093**	**0.003**	**6.602**	**3.127**	**0.003**
rs3751812_T	0.351	0.166	0.869	0.106	0.049	0.961
rs9939609_A	**4.921**	**2.617**	**0.011**	**5.781**	**2.6**	**0.012**
rs17782313_C	4.506	1.585	0.118	**6.535**	**2.135**	**0.037**

Model 1: Adjusted for age, sex**; Model 2:** Adjusting for age, sex, PAL, smoking

Interactions between GRSs and Candidate Variants and Diet Groups on Observed Changes

A final set of examinations was conducted to test for the potential effect of the interaction between the suggested diet group and genetic background on the samples' observed changes (Tables 45). Overall, no significant associations were observed for interactions with the calculated GRSs or the BMI-related variants in changes of anthropometric and lifestyle indices post-intervention.

Table 45. Associations between the changes observed post-intervention and the interactions between diet group and the uGRS and wGRS.

	Model 1			Model 2		
	Beta	*Stat**	*p*	*Beta*	*Stat**	*p*
Weight Change						
uGRS*Diet Group	-6.378	5.445	0.245	-0.125	0.159	0.438
wGRS*Diet Group	-0.136	0.159	0.398	-5.916	5.509	0.287
BMI Change						
uGRS*Diet Group	-0.046	0.057	0.427	-0.041	0.057	0.476
wGRS*Diet Group	-2.096	1.939	0.284	-1.880	1.960	0.341
WHR Change						
uGRS*Diet Group	-0.0004	0.003	0.873	-0.0004	0.003	0.875
wGRS*Diet Group	-0.027	0.091	0.771	-0.025	0.095	0.794
Fat kg Change						
uGRS*Diet Group	-0.106	0.161	0.514	-0.073	0.163	0.660
wGRS*Diet Group	-4.395	5.632	0.439	-3.306	5.767	0.569
AIS Change						
uGRS*Diet Group	0.032	0.192	0.870	-0.078	0.190	0.685
wGRS*Diet Group	0.594	7.589	0.939	-7.252	8.495	0.399
SF-PCS-12 Change						
uGRS*Diet Group	-0.193	0.390	0.623	-0.265	0.386	0.496
wGRS*Diet Group	-2.298	13.364	0.864	-7.603	13.431	0.574
SF-MCS-12 Change						
uGRS*Diet Group	0.855	0.599	0.159	0.878	0.609	0.156
wGRS*Diet Group	18.578	20.749	0.370	20.61	21.525	0.343

Model 1: Adjusted for age, sex, uGRS or wGRS and diet group**; Model 2:** Adjusting for age, sex, PAL, smoking, uGRS or wGRS and diet group

Interestingly, the interaction between diet group and the T allele of the fat-related rs6265 variant was steadily associated with increased changes for all examined fat indices (Table 46), namely change in total body fat in kg (Model 1: β=3.282, p=0.009; Model 2: β=3.398, p=0.007), change in visceral fat (Model 1: β= 0.947 , p=0.043) and change in upper body fat in kg (Model 1: β=2.468, p= 0.002; Model 2: β=2.564, p= 0.001). One more nominal association was revealed between the rs1443512- T allele and lower change in visceral fat, after adjusting for age and sex (Model 1: β= -1.043, p= 0.048).

Table 46. Associations between the changes observed post-intervention in fat-related indices and the interactions between diet group the 10 candidate, fat-related SNPs.

	Model 1			Model 2		
	Beta	*Stat**	*p*	*Beta*	*Stat**	*p*
Fat_kg Change						
rs574367_T*Diet Group	-1.304	-0.940	0.351	-1.087	-0.782	0.438
rs2605100_A*Diet Group	-0.978	-0.718	0.476	-1.979	-1.373	0.176
rs4846567_T*Diet Group	-1.894	-1.531	0.131	-1.726	-1.343	0.185
rs10195252_T*Diet Group	0.113	0.085	0.933	0.425	0.322	0.749
rs206936_G*Diet Group	-2.466	-1.198	0.236	-2.723	-1.336	0.187
rs4994_G*Diet Group	-0.713	-0.209	0.835	-0.485	-0.141	0.889
rs11191548_C*Diet Group	0.101	0.047	0.963	0.103	0.048	0.962
rs6265_T*Diet Group	**3.282**	**2.705**	**0.009**	**3.387**	**2.826**	**0.007**
rs1443512_A*Diet Group	-2.676	-1.928	0.059	-2.242	-1.567	0.123
rs12970134_A*Diet Group	-0.404	-0.256	0.799	-0.586	-0.365	0.717
Visceral Fat Change						
rs574367_T*Diet Group	-0.297	-0.576	0.567	-0.293	-0.559	0.579
rs2605100_A*Diet Group	-0.473	-0.932	0.356	-0.644	-1.156	0.253
rs4846567_T*Diet Group	-0.881	-1.936	0.058	-0.823	-1.702	0.095
rs10195252_T*Diet Group	-0.017	-0.035	0.972	0.052	0.103	0.919
rs206936_G*Diet Group	-0.955	-1.239	0.220	-0.974	-1.251	0.216
rs4994_G*Diet Group	-1.636	-1.3	0.199	-1.465	-1.129	0.264
rs11191548_C*Diet Group	0.163	0.206	0.838	0.119	0.147	0.884
rs6265_T*Diet Group	**0.947**	**2.071**	**0.043**	1.011	2.205	0.032
rs1443512_A*Diet Group	**-1.043**	**-2.016**	**0.048**	-0.925	-1.703	0.094
rs12970134_A*Diet Group	0.087	0.149	0.882	0.136	0.227	0.821
Upper Body Fat kg Change						
rs574367_T*Diet Group	-1.15	-1.305	0.197	-1.084	-1.218	0.228
rs2605100_A*Diet Group	-0.593	-0.682	0.498	-1.091	-1.163	0.249
rs4846567_T*Diet Group	-0.789	-0.987	0.328	-0.631	-0.753	0.455
rs10195252_T*Diet Group	0.418	0.488	0.628	0.593	0.694	0.491
rs206936_G*Diet Group	-1.327	-0.993	0.325	-1.431	-1.071	0.289
rs4994_G*Diet Group	2.505	1.17	0.247	3.004	1.388	0.171
rs11191548_C*Diet Group	-0.311	-0.222	0.825	-0.384	-0.264	0.793
rs6265_T*Diet Group	**2.468**	**3.262**	**0.002**	**2.564**	**3.375**	**0.001**
rs1443512_A*Diet Group	-1.217	-1.336	0.187	-0.914	-0.969	0.337
rs12970134_A*Diet Group	0.003	0.003	0.998	-0.042	-0.041	0.968

Model 1: Adjusted for age, sex, SNP and Diet Group**; Model 2:** Adjusting for age, sex, PAL, smoking, SNP and Diet Group

3.1.5. Discussion

Baseline Characteristics and Dietary Patterns

The following information in 3.1.5. constitute information published under the publication Nutrients 2021, 13, 3495. https://doi.org/10.3390/nu13103495 and can be found in Appendix C.

The present analyses display the design and the baseline population characteristics and dietary habits of the iMPROVE study. Overall, our baseline sample of 202 volunteers displayed satisfactory levels of lifestyle quality, with the majority of participants not reporting depression symptoms or heavily disrupted sleep quality. Five dietary patterns were identified, including: (a) a "Mixed" pattern; (b) a pattern including food groups similar to those of the Mediterranean diet, entitled "Med-proxy" pattern; (c) the "Eating-out" pattern consisting of food combinations usually found in restaurants or fast-food environments (i.e., pies); (d) the "Traditional, vegetarian-alike" pattern, characterized by plant-based, Greek, traditional recipes; and (e) the "High in unsaturated fats and fruit juice consumption" pattern, including foods groups with high unsaturated fats and magnesium content (i.e., small fish and nuts) and highlighting representative habits of healthy snacking across Greek adults (i.e., olives, nuts and fruit juice). Interestingly, while the "Mixed" pattern included a vast majority of processed foods with added sugars and high fat content (i.e., chocolate, croissants, tray sweets, soft drinks, chips, seed oil, margarine, and butter), it was also characterized by light products and chicken and potatoes' consumption. This can be potentially attributed to the representative consumption of specific food groups by overweight and obese Greeks, who tend to adhere to short-term, self-imposed attempts to follow a more balanced diet. The latter do not result in successful weight management and/or weight loss efforts, but are exactly characterized by increased consumption of light products and simple food combinations, such as chicken and potatoes. The "Med-proxy" and the "Traditional, vegetarian-alike" patterns are representative of the dietary habits of the Greek population, evidently influenced by the Mediterranean diet and its increased content in fruit, vegetables, and legumes. Apparently, due to its high sugar and fat content, the "Mixed" pattern was associated with higher levels of anthropometric and biochemical characteristics. On the other hand, the plant-based, traditional recipes presented negative associations with body fat and positive relations with increased levels of HDL cholesterol. We further evaluated the within-group tertile categorization of adherence to each pattern, showing that higher tertiles were related to stronger associations for specific patterns, such as the positive relationship between adherence to the "Mixed" pattern and logBMI levels and the negative relationship between the increased adherence to the same pattern and logHDL values. The concept of obese adults and the effect of dietary intake in the formation of their cardiometabolic profile display great interest, with current literature to be reporting similar findings to the ones outlined in the present study. Interestingly, the majority of studies aiming at identifying dietary patterns in overweight/obese populations, usually provide results for dietary habits adhering to the Western diet (including food groups with increased

content of processed foods and/or foods in high fat and sugar content) or to a more balanced dietary pattern including fruit and vegetables, relating to higher and lower values of BMI, respectively [343]. Such patterns may include food combinations each time representative of the region of living, while maintaining a strong influence of the dietary habits and combinations usually found in the Western and/or the Mediterranean diet. A 2021 study by Saghafi-Asi et al. investigating the relationship between dietary patterns and biochemical biomarkers of 151 healthy obese Iranian adults, also underlined a positive association between a "Western" dietary pattern with high fat and sugar content and BMI and body fat levels [344]. Additionally, a different study in Romanian obese adults also underlined the identification of a "high meat/high fat", a "Western," and a "Prudent" pattern [345]. Similar findings were reported in a cohort of 410 Polish participants of a case-control study, where adherence to a pattern influenced by the Western one was related with higher levels of fat tissue and waist circumference, in contrast with the adherence to a "Healthy" pattern [345]. In their 2019 longitudinal study, Neri-Sanchez et al. also underlined the positive association between adherence to a "Risky" dietary pattern, including high fat and high sugar content, with the presence of central obesity in Mexican adults [346]. A different pattern consisting of poultry, vegetables, red meat, and red meat products, among others, was also associated with obesity in male, Chinese adults, in a 2021 study of 1739 adults by Wang et al. [348]. Additionally, a different cross-sectional study of our group identified similar associations between dietary patterns and biochemical biomarkers in the adults of the POMAK population. More specifically, the dietary pattern including increased consumption of products high in sugars was related to low levels of HDL cholesterol [349]. Similar trends were also noted when investigating the dietary patterns of adolescent populations, where a dietary pattern with high protein and animal fat content was associated with elevated levels of logBMI and logTriglycerides, in French teenagers [311]. Furthermore, the development of the novel Lifestyle Index using the data deriving from the study sample, allowed for further investigation of the quality of life characteristics on the anthropometric and biochemical indices. Consisting of five variables, including two of the present dietary patterns extracted, the Index displayed negative associations with logBMI and body fat levels, as well as levels of the log-transformed variables of fasting glucose, SGOT, and SGPT. Thus, LI confirmed that higher quality of dietary intake and higher levels of physical activity reduced depression symptoms and improved self-reported conception of health status and may display a protective effect on body composition, as well as a favorable influence on improved glycemic profile. Overall, development of lifestyle indices as a means of quantifying and evaluating the potential influence of specific lifestyle aspects on body weight is mounting, as analyzed in the beginning of the paper, lifestyle indices can also incorporate dietary information via calculation of diet quality indices. A 2017 systematic review of 34 studies by Asghari et al., sought to investigate the effect of diet quality indices in obesity-related traits, showing that Healthy Eating Index (HEI) displayed an inverse association with obesity. The same review also concluded that different dietary scores, in general, did not efficiently assess diet quality, with most significant findings being presented in populations of

the United States [350]. Furthermore, different research groups have investigated the effect of lifestyle characteristics, such as sedentary behavior and screen-time, in adolescent populations [328, 351, 352]. In adults, current research refers to potential associations between constructed lifestyle indices and specific diseases or disease-related outcomes, namely cardiovascular disease [354], cancer [353], and type 2 diabetes [355]. Lenz et al., showed that creation of a Lifestyle Index for evaluation of life quality in adults at risk for cardiovascular disease can be a useful tool [356]. Furthermore, in a similar effort to evaluate the lifestyle aspects and weight characteristics, Roda et el in 2016, also investigated the potential effect of sleep qualities, screen time, and dietary intake, among others, highlighting a strong positive association between sedentary behavior and overweight [357].

Baseline Characteristics, MD and PA

Furthermore, regarding the samples' baseline characteristics, our findings showed that the combination of increased adherence to the MD and elevated physical activity was not only associated with lower levels of the core anthropometric indices (i.e. BMI, WHR, total body fat and visceral fat), but also a better profile of lifestyle characteristics as evidenced by its inverse association with depression characteristics and its positive relation with the mental component of the SF-12 short questionnaire. Evidently, apart from the well-known effects of MD in reducing cardiometabolic risk and elevating overall quality of life [113], literature firmly supports its additional role in the construction of a better mental health profile. In their 2020 literature review, Ventriglio et al highlighted the beneficial influence of MD adherence in the reduction of anxiety symptoms and the overall improvement of outcomes related to disorders of psychiatric nature [358]. Similar findings to the ones presented hereby were also reported by the SUN project, where adherence to the MD was associated with favorable levels for both the physical and the mental component of SF-36, in almost 10000 adult participants [359]. A different study by Yin et al also demonstrated a lower risk for presenting depression symptoms in adult women with high adherence to MD compared to the ones demonstrating lower adherence [360]. Similarly, the beneficial character of increased PA in overall physical and mental health status has been long demonstrated, irrespective of the presence of overweight or obesity. Using data for approximately 870.000 adults, Xu et al demonstrated the favorable effects of various types of PA on mental health characteristics for people with normal weight, as well as obesity [361]. In the case of the latter, Pojednic et al also highlighted that even slight increases in physical activity were related with better health outcomes, even in the absence of targeting or achieving weight loss [362]. Therefore, naturally, the combination of increased adherence to MD and not leading a sedentary lifestyle has been associated with enhanced physical and mental health status, with Di Lorenzo et al even showing positive results in the anthropometric indices of a cohort of patients with psychiatric disorders [363]. It is therefore interesting to argue that a balanced diet combined with increased physical activity can positively influence parameters of mental health in populations with increased weight, rather than MD or increased PA alone. This

combination could be potentially even brought to the forefront of personalized recommendations for populations presenting overweight or obesity, as even the slightest increase in following the MD and exercising can massively impact mental health status, which could subsequently positively influence physical health by practically reducing cardiometabolic risk and by even positively shaping the individuals' relationship with food.

Intervention Outcomes

Regarding the outcomes of the proposed hypocaloric intervention, our findings showed that participants in the study presented a statistically significant weight loss at the end of the 3-month period, irrespective of the proposed dietary regimen (mean reduction of 2.68kg, $p<0.0001$ for all participants). As previously mentioned, research on the effect of different macronutrient content for weight loss has not yielded significant differences for proposed diets with increased carbohydrate versus increased protein content. In line with the results presented hereby, the aforementioned POUNDS lost trial reported similar outcomes for the 345 participants with available pre- and post-intervention data. The trial noted a similar drop-out rate as the one observed for the iMPROVE participants, with 42.55% of the participants completing the POUNDS lost trial (811 individuals with baseline data vs 345 individuals with data at baseline and end of the 6-month period) and 41.58% of the participants completing the iMPROVE trial (202 individuals with baseline data vs 84 individuals with data at baseline and end of the 3-month period). Compared to iMPROVE, POUNDS lost noted almost double weight loss in the double months of the intervention period, i.e. a mean weight loss of 6 kg at the end of the 6 months. In line with the iMPROVE trial results, the trial also noted no differences for weight loss within the four groups of the different proposed diets [168, 364] Similar findings were also presented by the DIETFITS trial, which yielded no statistically significant differences in the weight loss observed at the end of the 12-month intervention period between the healthy low-fat and the healthy low-carbohydrate groups [365].

Analogous results were also presented by Parr et al, who investigated the effect of adhering to a 4-month hypocaloric diet with three groups of different macronutrient content, i.e. a high protein, moderate carbohydrate content (30% fat, 30% protein and 40% carbohydrate); a high protein, high carbohydrate content (15% fat, 30% protein, 55% carbohydrate) or a control regimen (30% fat, 15% protein and 55% carbohydrate) The trial showed a mean 7.7kg weight loss in 89 adults with overweight or obesity after the 4-month intervention period, without differences in the changes observed within the examined diet groups [366]. Additionally, the older NUGENOB project also investigated the effect of a 10-week hypocaloric diet of either low fat content (i.e. 20- 25% fat, 15% protein, ~60 to 65% carbohydrate) or high-fat composition (i.e.~40 to 45% fat, 15% protein, ~40-45% carbohydrates) in the observed weight loss of 771 adults with obesity. The study showed a respective mean weight loss of 6.9kg and 6.7kg for each group, without noting statistically significant differences between the two [300,367]. Similar to the findings of the iMPROVE trial,

NUGENOB also showed no statistically significant interactions between the suggested diet groups and the weight loss outcome [300].

In the same trial, Handjieva-Darlenska et al attempted to further evaluate the rate of the observed weight loss throughout the intervention period. The authors found that a weight loss of less than 4kg mid-way through the 10-week trial could possibly be predictive of a lower overall weight loss at the end of the intervention period [300]. The authors attributed this to the potential effect of motivation in such intervention schemes, where personal incentive appears to be a main contributor in driving successful outcomes for each individual. In the present study, we find that changes in the primary outcomes (i.e. weight and BMI change) remain statistically significant up to the end of month 2 ($p<0.05$). This finding could potentially strengthen the notion that dietary interventions of such nature could be most effective when conducted in short time periods where weight loss appears to peak and which are able to maximize the enhanced effect of the increased motivation usually observed in participants at the beginning of trials targeting weight loss. In this spectrum, a 2020 systematic review by Ge et al., showed not only that low-carbohydrate and low-fat diets yielded similar results for weight loss at the standard intervention period of 6 months, but also that the observed weight loss diminished at a period of 12 months irrespective of the dietary regimen followed [259]. On the contrary, use of behavioral treatments has shown that weight loss was in fact enhanced later-on during the intervention period, with Butryn et al showing that participants following such approaches showed higher weight loss closer to the end of the intervention period [368]. This could be potentially attributed to the fact that participants in such programs master the proposed measures later on within the trial. Ahern et al., attempted to compare different kinds of interventions for weight loss, demonstrating that participants in a behavioral arm (Weight Watchers) noted higher weight loss at one year compared to those following a brief dietary intervention [369]. Therefore, more research is needed to identify potential combinations of dietary and behavioral interventions for identifying the optimal window to maximize weight loss in intervention trials, capable of leading to substantial, beneficial changes in body weight and body composition while ensuring long-term loss maintenance.

In the same spectrum, adherence to the proposed diets varied between the individuals, with their self-reporting adherence scores varying throughout the intervention period. As participants were closely monitored and communicated bi-weekly with their allocated nutrition experts, they frequently reported difficulty in fully adhering to the proposed meals due to a hectic daily lifestyle, the need to organize meals or a false subjective view of increased quantities due to the increased meal frequency (i.e. 5 or 6 proposed meals per day). We therefore chose to account for their subjective adherence to the proposed diets as a means to more accurately capture the role of adherence to their observed changes in weight. Current literature has investigated the role of the adherence to dietary interventions in the observed results, with Lemstra M et al demonstrating an overall adherence percentage of 60.5% in a meta-analysis of 27 studies in the field [370]. In their 2017 paper for identifying beneficial strategies to improve adherence, Gibson et Sainsbury highlighted the need

for the proposed interventions to account for the increased will to eat observed during such attempts to lose weight, as well as tailor proposed suggestions to the individuals' needs while also promoting self-monitoring techniques [371].

Proceeding to the intervention results on body composition, WHR did not present statistically significant changes at the end of the 3-month period. The slight increases in the WHR measurements at the ends of months one and two can be potentially due to the marginal error attributed to the fact that the participants performed said measurements by themselves. However, participants displayed significant changes In fat-related indices post-intervention, including reductions in total body fat, fat-free mass and visceral fat ($p<0.001$ for all). Women noted the majority of the significant reductions at the end of the three months, whereas no differentiations were observed for the two diet groups. POUNDS lost reported similar findings, with participants showing reductions for fat and lean mass, as well as abdominal, subcutaneous and visceral fat ($p<0.0001$ compared to baseline). In line with iMPROVE, women appeared to lose more visceral fat than men and no differences were observed for the 4 diet groups [372]. Changes in body composition are mostly reported in the literature when combining a dietary intervention with recommendations for increased PA. Similarly, Rojo-Tirado et al demonstrated that body fat percentage was reduced across 3 groups of different PA types (strength, endurance, combination of strength and endurance and control group) in 239 adults who followed a 6-month hypocaloric diet and exercise-based intervention [373]. We could therefore argue that more evident changes in WHR or other anthropometric indices could be achieved if providing additional recommendations for PA.

Moving on to the observed changes in the lifestyle indices, AIS presented a statistically significant decrease post-intervention by 1.5 units (5 at baseline vs 3.5 at the end of 3 months, $p=0.033$), without differences observed between the two diet groups. As previously mentioned, the bidirectional relationship between sleep and weight has been well established in current literature [374]. The present sample did not show elevated levels of AIS at baseline, denoting that the majority of participants did not demonstrate sleep-related disruptions and the latter would, thus, probably not constitute an obstructive or confounding factor in their attempt to lose weight. The observed reduction in AIS aligns with the beneficial effect of weight loss in the amelioration of sleep qualities, with most studies having reported results for its impact on obstructive sleep apnea. In 2021, de Melo et al also demonstrated a beneficial effect of adherence to a one-month, hypocaloric, high-protein diet in AOS parameters of patients with obesity [374]. Concomitantly, Georgoulis et al showed that a behavioral intervention including a 6-month, hypocaloric MD diet for weight loss, was associated with enhanced inflammatory levels in patients with Obstructive sleep apnea [375].

Regarding the remaining changes, a statistically significant improvement for SF-MCS-12 was observed by the end of the first month of the intervention (46.4 at baseline vs 50.56 at the end of month 1, $p=0.022$), but disappeared by the end of the three-month period. It is likely, that the short-term increase in the mental component could be associated with the increased participant motivation observed in the

beginning of the intervention and the presence of significantly yielded results in weight loss by the end of the first month. In their 2024 systematic review, Lasikiewicz et al showed that weight loss resulting from dietary or behavioral interventions was associated with ameliorations in participants' psychological characteristics, including health-related quality of life [376]. Moreover, Alhalel et al also noted that weight loss following a lifestyle intervention was related with improvement in the mental health status of 92 adult women with overweight or obesity [377].

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Associations between genetic makeup and baseline characteristics

In an effort to holistically assess the potential impact of the genetic factor, we chose to examine both the role of a 84-SNP unweighted and weight GRS, as well as separate candidate variants known for their relations with BMI and fat-related indices. The constructed GRSs did not present statistically significant interactions with the baseline anthropometric indices, potentially due to the limited size of the examined population. Regarding the examined variants, carriers of the T allele of the BDNF-located rs6265 SNP demonstrated lower levels for all examined fat-related indices, namely total body fat in kg (β=0.035, p=0.032 after adjusting for age, sex, PAL and smoking), visceral fat (β=0.038, p=0.019 after adjusting for age, sex, PAL and smoking) and upper body fat in kg (β=0.036, p=0.041 after adjusting for age, sex, PAL and smoking). Presence of the C allele of the variant has been well-associated with increased anthropometric measurements in current literature, namely WHR (β=4.00, $p=2.00e^{-7}$), HC ($p<0.05$), weight ($p<0.001$) and BMI levels ($p=1.88e^{-12}$), in a variety of populations with different ancestries [378-381]. As previously mentioned, the polymorphism is associated with impaired intracellular signaling and reduced BDNF secretion. Therefore, the present finding of the protective T allele being associated with lower levels of fat indices agrees with the current literature, given the association between the C allele and reduced BDNF levels leading to increased food intake and body composition measurements.

Regarding the observed interactions between the candidate variants and the cohort's baseline dietary patterns, carriers of BMI-raising alleles demonstrated several nominal interactions for multiple anthropometric and lifestyle characteristics. Interactions between BMI-raising alleles and dietary patterns rich in sugar or fat content appeared to aggravate the already existing predisposing effect, while adherence to patterns with more balanced meal combinations revealed a protective effect of diet in the variants' impact on anthropometric indices. More specifically, interactions between the "Traditional, vegetarian-alike" pattern and several risk variants for elevated BMI (i.e. rs1421085-C, rs1121980-A, rs17817449-G, rs3751812-T, rs9939609-A) were nominally linked to lower levels of body weight and BMI at baseline. In a similar manner, rs3751812-T and rs9939609-A alleles were associated with lower levels when adhering to the "Traditional, vegetarian-alike" pattern, whereas the former was associated with higher levels when adhering to the High in unsaturated fats pattern. On the contrary, presence of the BMI-positively associated T allele of the rs3751812 variant who adhered to the "High in unsaturated fats and fruit juice consumption" pattern showed increased levels of weight and BMI. In our case, although we did not observe significant associations for the "Med-proxy" pattern, positive associations in attenuating the genetic effect were shown for the

“Traditional, vegetarian-alike” pattern, which, in turn, comprises of meal combinations, traditional of the Greek daily life and usually presenting high content of fiber. Furthermore, the aggravating effect shown for the “High in unsaturated fats and fruit juice consumption” pattern could potentially emphasize the effect of rich fat and sugar sources, irrespective of fat type (i.e. unsaturated and not saturated, per se).

This is not the first time that diet appears to either modify or aggravate the predisposing effect of genetic makeup. in line with the results presented hereby, a 6-SNP GRS created by Hosseini-Esfahani et al created a 6-SNP GRS including the rs1421085, rs1121980, rs17817449, rs3751812, rs8050136 and rs9939973, to investigate associations between dietary fiber intake and obesity phenotypes. In line with the findings presented hereby for the first four SNPs used in this GRS and their interaction with the “Traditional” pattern, the latter was found to significantly interact with fiber intake in modifying BMI levels [282]. The same team also showed that adhering to the WD appeared to aggravate the genetic effect in obesity phenotypes of individuals with increased levels of the same GRS [282].

Previous attempts have also shown concomitant results when investigating such associations in cohorts of large populations with multiple thousands of participants. In a similar attempt to the present one, Ding et al investigated potential interactions between the GRS of the 97 SNPs for BMI identified by Locke et al [326] and three indices of diet quality, namely the AHEI, Alternative Mediterranean Diet score (AMED), and the DASH score in approximately 31000 individuals. The study showed a consistent attenuation of the genetic effect in the cases where the diet scores presented higher values [383]. A similar effect was shown by Wang et al in the case of almost 14000 health professionals, where a 20-year follow up showed attenuation of the effect of BMI-elevating variants in the individuals with higher AHEI scores [384]. To boot, examination of gene-diet interactions in a sample of 68317 European adults also yielded nominally significant associations between a favorable diet score and two BMI-related variants -rs10968576 and rs4771122- in the population’s BMI [385]. A different study investigated the effect of the rs17782313 SNP on depression characteristics of women with overweight and obesity. The study showed a significant interaction between presence of the aggravating C allele and increased depression scores in women adhering to an unhealthy dietary pattern, when compared to the ones following a healthy pattern [386].

Lastly, a 2022 systematic review by Tan et al also demonstrated the favorable effect of adhering to diets with principles similar to the ones of MD or DASH on decreasing the risk for increased weight in individuals with BMI-raising alleles in the FTO, MC4R, PPARG or APOA5 genes [387].

Proceeding to the interactions observed for the lifestyle measurements, attrition to the “High in unsaturated fats and fruit juice consumption” pattern was associated with increased CESD-R-10 score in carriers of the BMI-raising T allele of the rs925946 SNP. In 2012, Gyekis et al. examined the potential effect of several BMI-related polymorphisms on the incidence of major depression highlighting that the SNPs, alone, were not associated with the appearance of the disorder [388]. Literature has generally highlighted the importance of gene-diet interactions in the appearance or the modification of depression-related disorders and subsequent characteristics [389, 390]. Subsequently, the finding presented hereby could potentially highlight the

connective link in the modifying effect of an unbalanced diet in the final increase of depression-related symptoms.

Another interesting finding presented hereby concerns the gene-diet interactions observed for the physical component of the SF-12 questionnaire. More specifically, adherence to the "Mixed" pattern in carriers of the BMI-raising rs1421085-C, rs1121980-A, rs17817449-G, rs9939609-A, rs17782313-C, rs6548238-C alleles was associated with lower levels of SF-PCS-12; this strengthens the notion that the combination of obesity-predisposing genotypes and adherence to a WD-alike diet can aggravate quality of life, even when comparing to genetic makeup or diet separately. Interestingly, we also found that the rs1421085-C, rs1121980-A, rs17817449-G, rs9939609-A variants were associated with lower levels of SF-PCS-12 even when the individuals adhered to the "Med-proxy" pattern. Always taking into account the limited percentage of variance explained by the patterns, this finding could potentially reveal a greater impact of unhealthy dietary habits on aggravating the predisposition for lower quality of life, compared to the potential attenuating effect that a healthy diet could potentially present.

Associations between genetic makeup and intervention outcomes

When looking into the effect of genetic makeup on the observed changes in the cohort's characteristics post-intervention, we detected no statistically significant associations with the GRSs or the examined candidate variants, again, potentially due to the limited size of our population. However, although statistically insignificant, individuals with higher GRSs steadily presented lower rates of weight and BMI change when compared to the ones with lower genetic risk. Accordingly, changes in the examined indices did not statistically significantly differentiate between the two diet groups.

Using the observed data, within-genotype group tests revealed a statistically significant difference between the three groups of genotypes for the FTO rs1421085 and the MC4R rs17782313 variants and change in body weight (p-0.036 and p-0.043, respectively). As mentioned previously, the relationship between both variants and increased weight is well-documented in the literature, hence our choosing to investigate their potential impact on weight loss. Franzago et al previously investigated the impact of 5 target SNPs, among them the in-LD-with-rs1421085 FTO rs9939609 SNP and the MC4R rs17782313 variant, in the observed weight loss of patients with obesity following a nutritional intervention. Their study showed that carriers of the rs9939069-A allele presented lower BMI decrease from baseline up to the end of the 12-month of intervention [391].

Generally, to date, other attempts of dietary interventions targeting weight loss have yielded analogous results to the ones of the iMPROVE cohort, with most studies investigating and reporting the effect of candidate polymorphisms or GRSs of tens of selected variants in outcomes of interest. In its sample of 609 adults with overweight, the DIETFITS trial also showed no statistically significant associations between gene-diet interactions and weight loss at the end of the 12-month intervention period [365]. While investigating the potential effect of 42 candidate SNPs in 648 participants, the

previously mentioned NUGENOB project also noted only nominally significant associations for lower weight loss in participants with the effect alleles who followed the low-fat diet [392]. Concomitantly, the trans-NIH consortium for genetics of weight loss in response to lifestyle interventions showed that a higher 59-SNP GRS for WHR was only limitedly associated with lower WC or WHR reduction in Caucasian individuals, after 1 year in the Look AHEAD, DPP, DPS, DIETFITS and PREDIMED-Plus interventions [393].

Interestingly, BMI-raising alleles also presented associations with the components of the SF-12 questionnaire, denoting the additional presence of corresponding relations in our sample. The FTO rs17817449 and rs9939609 variants were associated with reduced changes in SF-PCS-12 post-intervention, potentially signifying that although the variants were not associated with the baseline levels of the component, they could still negatively affect the potentially positive change caused by the weight loss that accompanied the dietary intervention. On the contrary, in addition to those two polymorphisms, the FTO rs1421085,rs1121980 and the MC4R rs17782313 SNPs presented positive nominal associations for increased changes in the mental component of the questionnaire. This could potentially denote that although the intervention results might not have been clearly evident on improving the physical component, individuals with increased predisposition to obesity were mentally benefited by the observed changes post-intervention.

Regarding the examined interactions with the proposed dietary group, in the present study we found no statistically significant associations between the GRSs or the BMI-variants and the changes post-intervention. Interestingly, current literature focuses on the interaction of the rs17782313 MC4R variant (or other variants in LD with the rs17782313) and macronutrient intake on various dietary or cardiometabolic indices. In the POUNDS Lost study, an important relation was demonstrated between the MC4R rs17782313 SNP and dietary protein intake in the protein diet groups, regarding the increase in appetite and cravings, when using data from 735 participants at the end of the 2-year period [394]. In like manner, Adamska-Patruno et al examined the effect of four MC4R variants, including the rs17782313 one, in the cardiometabolic parameters of 819 individuals with obesity. The study showed that carriers of the C allele presented increased visceral fat, glucose and triglyceride levels when noting higher levels of protein intake [395]. A different study in 282 Iranian women showed a statistically significant association between carbohydrate intake and the presence of the C allele in the variant with higher BMI, WC and BMR [396]. An additional finding deriving from the POUNDS lost trial concerned the in-LD-with-rs1421085 FTO rs1558902 variant. The study showed that carriers of the BMI-raising allele presented higher reductions in weight and fat-related indices when adhering to the high-protein vs the low-protein diet [397]. We could, therefore, argue that, in our case, associations were not presented between the variants and macronutrient intake potentially due to the limited sample size.

Interestingly, the protective T allele of the rs6265 variant presented nominally significant interactions with diet group, denoting that T carriers in the high-protein group presented enhanced post-intervention changes observed for total and upper

body fat in kg, as well as visceral fat (β=3.387, p=0.007, β=2.564, p=0.001 and β=1.011, p=0.032, respectively adjusting for age, sex, PAL and smoking). As previously discussed, the role of the variant is central in BDNF production, with Deng et al having reported its ability to regulate BDNF protein phosphorylation and even bone mineral density [398]. This is not the first time that the variant is found to be interacting with macronutrient intake on modifying cardiometabolic determinants. Miksza et al found that adult carriers of the C allele presented higher levels of BMI, WC and glucose when noting a daily dietary protein intake great than 18% [399]. Furthermore, in a sample of 634 diabetic patients, Naeini et al showed that carriers who presented increased DQI or PI scores demonstrated higher rates in the reduction of total cholesterol or IL-18 [400]. Moreover, in a sample of 8840 Korean adults with and without T2D, Daily et Park also found consistent interaction between the variant and protein intake in modifying the risk for presenting T2D in older adults [401]. In terms of other gene-diet interactions for changes in body composition, it is worth mentioning that the POUNDS lost trial reported a similar interaction between carbohydrate consumption but the FGF21 rs8381147 variants in modifying total fat change in 715 participants at the end of the 2-year period. Carriers of the carbohydrate intake-decreasing allele showed lower changes in WC and total fat mass when adhering to the low carbohydrate diet [402]. Hence, our present findings lays interesting ground for further inquiring the interaction between: i) the MC4R variant and protein intake; and ii) future attempts for more variants in more anthropometric and cardiometabolic risk factors.

Interestingly, apart from the present findings displayed for the FTO variants, the rs17782313 and rs6265 SNPs demonstrating significant associations in the iMPROVE study have also been further specifically examined for their associations with increased anthropometrics. Farooq et al chose the two variants as most representative to investigate metabolomic pathways implicated in increased BMI levels. Interestingly, the study found several associations for both polymorphisms and a multitude of metabolites with emphasis on alterations in fat metabolism [403] . This research further solidifies the findings presented hereby, demonstrating a dominating effect of the two variants in the changes of the anthropometric indices examined post-intervention.

Overall, major advantages of the present study are: i) the conduct of the first-ever dietary intervention examining the role of macronutrient composition and potential gene-diet interaction on weight loss in Greek adults; and ii) the use of the online assessment tool, as a means enabling long-distance communication and monitoring, during the time of social distancing due to the novel coronavirus disease 19 (COVID-19) pandemic. On the other hand, limitations of the present study included: i) the limited sample size included due to the substantial impact of the COVID-19 pandemic on volunteer recruitment rates caused by social-distancing protocols implemented in recruitment sites and the limited expression of interest for participation in the study; ii) the long-distance maintenance of an increased adherence rate to the proposed diets, due to the extended time period between the in-person follow-up meetings; and iii) the proper use of the online assessment tool by older adults who had both limited access and knowledge on the use of state-of-the-art technological devices and

online tools. The present constitutes the first attempt of its kind in a Greek population of adults, yielding significant insights on the associations between genetic predisposition and anthropometric and lifestyle indices in this populations. The heterogeneity of the results should, thus, be viewed as a strength allowing to lay the ground for future work, in line with the procedures and findings of larger, similar initiatives such as the POUNDS lost, the DIETFITS and the DiOGenes trials.

3.2. The 2018 Gutenberg Chair Project: TEENAGE and STANISLAS Cohorts

The following constitute information published under the publications Nutrients 2021, 13, 198 https://doi.org/10.3390/nu13010198 and Nutrients 2023, 15, 1884. https://doi.org/10.3390/nu15081884 and can be found in Appendix D.

3.2.1. Baseline Characteristics and Dietary Patterns of the Studies' Populations

We used baseline anthropometric, biochemical and dietary data for all analyses on both adolescent cohorts. For the TEENAGE Study, we analyzed data from a total sample of 766 adolescents (45.56% boys, 54.43% girls), with a median age of 13.30 years (Table 47). The sample presented various statistically significant sex-related differences, with the boys presenting higher levels of weight (p=0.001), WHR (p<0.001), SBP (p=0.001), glucose and CRP (p-values<0.001), compared to girls. On the contrary, girls showed increased levels of the HOMA-IR, insulin and HDL-C indices. Moreover, the Greek teenagers reported a median energy intake of 1741.00 kcal/d (IQR = 760), significantly different between the two genders, with boys reporting a higher intake than girls (p<0.001).

Table 50. Baseline characteristics of the adolescent cohort of the TEENAGE Study (n=766).

	TEENAGE Study						
	All		Boys		Girls		
	n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	*p* *
Age (years)	766	13.30(1.31)	349	13.36 (1.38)	417	13.26 (1.25)	<0.001
Weight (kg)	766	55.00 (14.00)	349	56.00 (16.00)	417	54.00 (13.00)	0.001
BMI (kg/m²)	766	20.88 (4.38)	349	20.85 (4.45)	417	20.93 (4.37)	0.517
WHR	763	0.76 (0)	349	0.79 (0)	414	0.73 (0)	<0001
SBP (mmHg)	743	119.00 (16)	335	120.67 (11.93)**	408	118.00 (15)	0.001
DBP (mmHg)	743	70.00 (12)	335	71.00 (12)	408	70.00 (12)	0.825
Energy Intake (kcal/day)	766	1741.00 (760)	349	1939.00 (779)	417	1574.00 (609)	<0.001
Glucose (mg/dL),	611	80.00 (12)	283	81.00 (11)	328	79.00 (12)	<0.001
HOMA-IR	539	2.28 (2)	255	2.12 (2)	284	2.37 (2)	<0.001
Insulin (mg/dL)	539	11.00 (7)	255	10.00 (7)	284	12.00 (8)	<0.001
TC (mg/dL)	611	157.00 (33)	283	156.49 (25.18) **	328	157.50 (31)	0.210
HDL- C (mg/dL)	611	89.20 (27)	283	53.00 (16)	328	56.00 (17)	0.001
LDL- C (mg/dL)	611	54.00 (16)	283	90.57 (21.78) **	328	88.40 (26)	0.651
Triglycerides (mg/dL)	611	56.00 (24)	283	55.00 (25)	328	57.00 (24)	0.090
CRP (mg/dL)	540	0.30 (1)	254	0.45 (1)	286	0.20 (0)	<0.001

* All hypothesis testing took place via use of the Mann–Whitney test.

** Variable follows the normal distribution and is presented as mean± SD.

p= p-value, **BMI**: Body Mass Index, **WHR**: Waist-to-hip Ratio, **SBP**: Systolic Blood Pressure, **DBP**: Diastolic Blood Pressure, **HOMA-IR:** Homeostatic Model Assessment for Insulin Resistance**, LDL-C**: Low-density cholesterol, **HDL-C:** High-density cholesterol, **CRP:** C-reactive protein

Regarding the STANISLAS cohort, we analyzed a sample of 287 teenagers with available dietary assessment data. Our sample composed of 47.73% boys and 52.26% girls, with a median age of 13.08 years (Table 48). Overall, as in the case of the TEENAGE adolescents, boyo\s presented higher values of WHR, SBP (p< 0.001) and glucose (p=0.018), compared to girls. However, in this sample, girls showed higher levels of TC and LDL-C (p=0.002, p=0.030, respectively) than the boys. The French teenagers reported a median energy intake of 2056.03 kcal/d (IQR = 662.24), without presenting significant differences between sexes.

Table 48. Baseline characteristics of the adolescent cohort of the STANISLAS Study.

	STANISLAS Family Study						
	All		Boys		Girls		
	n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	*p* *
Age (years)	287	13.08 (2.92)	137	13.08 (2.92)	150	13.08 (2.85)	0.416
Weight (kg)	263	46.59 (18.10)	129	47.20 (21.90)	134	46.05 (14.84)	0.136
BMI (kg/m²)	263	18.44 (3.61)	129	18.30 (3.20)	134	18.52 (4.18)	0.853
WHR	221	0.77 (0.04) **	110	0.81 (0.03) **	111	0.75 (0.06)	<0.001
SBP (mmHg)	263	112.00 (14.50)	129	115.60 (11.53) **	134	110.46 (8.76) **	<0.001
DBP (mmHg)	263	57.00 (15.50)	129	56.69 (16.00) **	134	57.02 (10.23) **	0.829
Energy Intake (kcal/d)	287	2056.03 (662.24)	137	2070.99 (495.20)**	150	2094.92 (681.16)	0.469
Glucose (mg/dL)	263	88.28 (6.12) **	129	89.18 (6.48) **	134	87.38 (5.76) **	0.018 ***
TC (mg/dL)	263	179.15 (40.93)	129	173.36 (30.89) **	134	183.01 (36.29)	0.002
HDL-C (mg/dL)	263	54.05 (20.08)	129	54.44 (15.44) **	134	56.37 (16.99)	0.222
LDL-C (mg/dL)	263	116.99 (33.98)	129	113.13 (28.19) **	134	120.85 (32.05)	0.030
TG(mg/dL)	263	51.33 (33.63)	129	52.21 (38.05)	134	46.56 (30.09)	0.930
CRP (mg/L)	243	0.30 (0.53)	118	0.32 (0.54)	125	0.26 (0.55)	0.765

* Hypothesis testing took place via use of the Mann–Whitney test wherever at least one variable did not follow the normal distribution.

** Variable follows the normal distribution and is presented as mean ± SD.

*** Hypothesis testing took place via the Student's Independent Samples *t*-test

p= p-value, **BMI**: Body Mass Index, **WHR**: Waist-to-hip Ratio, **SBP**: Systolic Blood Pressure, **DBP**: Diastolic Blood Pressure, **HDL-C:** High-density cholesterol, **LDL-C**: Low-density cholesterol, **CRP**: C-reactive protein

Baseline Dietary Patterns for the Two Cohorts

For the TEENAGE study, PCA was conducted on 15 food groups deriving from the 24-hour dietary recalls. KMO test was calculated at 0.545 for the TEENAGE teenagers, indicating mediocre to sufficient data adequacy. PCA for the TEENAGE cohort resulted in the identification of 5 dietary patterns, accounting for 49.35% of the sample's total variance. All food group factor loadings are presented in Table 49. Based on the inclusion of corresponding food groups, the five dietary patterns identified were categorized as follows:

i. a "western breakfast" dietary pattern, consisting of cheese, dairy and processed meat (explaining 15.61% of the total variance);
ii. a "legumes and good fat" pattern, with high consumption of legumes, olives, olive oil and nuts(explaining 10.32% of the total variance);
iii. a "homemade meal" pattern, with high consumption of red meat and potatoes and associated with lower fish consumption explaining (8.33% of the total variance);
iv. a "chicken and sugars" pattern, with high consumption of chicken and sweets and associated with lower the consumption of fruits and juices (explaining 7.60% of the total variance explained); and
v. an "eggs and fibers" pattern, with high consumption of non-refined cereals, vegetables and eggs and associated with lower consumption of refined cereals (explaining 7.47% of the total variance).

Table 49. Principal Components Analysis' factor loadings for the 15 food groups in the TEENAGE study (n = 766).

	Component				
Food Groups*	**1**	**2**	**3**	**4**	**5**
Cheese	0.897	-	-	-	-
Dairy	0.863	-	-	-	-
Processed Meat	0.635	-	-	-	-
Legumes	-	0.739	-	-	-
Olives, Olive Oil, Nuts	-	0.668	-	-	-
Red Meat	-	-	0.712	−0.429	-
Potatoes	-	-	0.661	-	-
Fish	-	−0.358	−0.480	-	-
Chicken	-	-	-	0.649	-
Sweets	-	-	-	0.518	-
Fruit and Juices	-	-	-	−0.368	-
Non-refined cereals	-	-	-	-	0.674
Vegetables	-	-	-	-	0.342
Eggs	-	-	-	-	0.303
Refined Cereals	0.512	-	-	-	−0.595
Total Variance Explained (%)	15.61	10.32	8.33	7.60	7.47

***Only loadings with an absolute values> 0.3 are presented in the table**

Following the extraction of the dietary patterns, we proceeded to conducting multiple linear regressions adjusting for all aforementioned models of confounding

factors. As shown in Table 50, the “chicken and sugars” pattern was statistically significantly associated with lower values of logCRP in all models (Model 1: β = −0.051, p-value = 0.006, Model 2: β = −0.057, p-value = 0.004, Model 3: β = −0.050, p-value = 0.008, Model 4: β = −0.051, p-value = 0.008). The same pattern was nominally associated with elevated logGlucose (Model 1 β = 0.015, p-value = 0.017), but lower logInsulin after adjusting for Model 1 (β = −0.020, p-value = 0.030), Model 3 (β = 0.018, p-value = 0.049) and Model 4 (β = 0.018, p-value = 0.041). Further nominal associations were further observed for: i) the “legumes and good fat” pattern and lower values of logBMI (Model 1: β = −0.006, p-value = 0.017) and logInsulin (β = −0.020, p-value = 0.030); and ii) the “homemade meal” pattern and lower values of logBMI (Model 1: β = −0.005, p-value = 0.042).

Table 50. Linear Regression Analyses on the association between the dietary patterns, anthropometric indices and biomarkers of glycemic and lipidemic control in the TEENAGE study.

	Model 1			Model 2			Model 3			Model 4		
	β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*
LogBMI												
Western Breakfast	−0.004	0.003	0.150	−0.003	0.003	0.308	-	-	-	-	-	-
Legumes and Good Fat	−0.006	0.003	0.017	−0.004	0.003	0.194	-	-	-	-	-	-
Homemade Meal	−0.005	0.003	0.042	−0.003	0.003	0.242	-	-	-	-	-	-
Chicken and Sugars	−0.005	0.003	0.069	−0.004	0.003	0.128	-	-	-	-	-	-
Eggs and Fibers	0.004	0.003	0.111	0.004	0.003	0.115	-	-	-	-	-	-
LogWHR												
Western Breakfast	0.013	0.012	0.270	0.016	0.13	0.247	0.017	0.013	0.198	0.017	0.014	0.250
Legumes and Good Fat	−0.006	0.011	0.622	−0.008	0.013	0.527	−0.007	0.013	0.608	−0.007	0.013	0.597
Homemade Meal	−0.009	0.011	0.445	−0.008	0.013	0.517	−0.007	0.013	0.599	−0.008	0.013	0.562
Chicken and Sugars	−0.003	0.011	0.760	−0.005	0.013	0.696	−0.003	0.013	0.828	−0.003	0.013	0.800
Eggs and Fibers	−0.011	0.011	0.320	−0.001	0.013	0.339	−0.015	0.013	0.268	−0.015	0.013	0.267
LogSBP												
Western Breakfast	−0.003	0.002	0.085	−0.002	0.002	0.174	−0.002	0.002	0.295	−0.001	0.002	0.646
Legumes and Good Fat	0.000	0.002	0.838	0.001	0.002	0.729	0.001	0.002	0.499	0.001	0.002	0.472
Homemade Meal	0.000	0.002	0.937	0.000	0.002	0.819	0.001	0.002	0.579	0.001	0.002	0.481
Chicken and Sugars	0.002	0.002	0.169	0.002	0.002	0.246	0.003	0.002	0.090	0.003	0.002	0.071
Eggs and Fibers	2.294×10^{-5}	0.002	0.988	−0.001	0.002	0.680	−0.001	0.002	0.409	−0.001	0.002	0.411
LogDBP												
Western Breakfast	−0.003	0.002	0.224	−0.003	0.002	0.256	−0.002	0.002	0.361	0.000	0.003	0.894
Legumes and Good Fat	−0.002	0.002	0.482	−0.001	0.002	0.786	0.000	0.002	0.948	-3.047×10^{-5}	0.002	0.990
Homemade Meal	0.001	0.002	0.551	0.003	0.002	0.155	0.004	0.002	0.097	0.004	0.002	0.063
Chicken and Sugars	0.001	0.002	0.609	0.001	0.002	0.528	0.002	0.002	0.333	0.003	0.002	0.271
Eggs and Fibers	0.001	0.002	0.802	0.000	0.002	0.878	0.000	0.002	0.914	0.000	0.002	0.919
LogGlucose												
Western Breakfast	−0.003	0.007	0.655	−0.003	0.007	0.632	−0.003	0.007	0.631	−0.004	0.008	0.615
Legumes and Good Fat	0.010	0.006	0.120	0.011	0.007	0.111	0.011	0.007	0.110	0.011	0.007	0.111
Homemade Meal	−0.002	0.006	0.740	−0.004	0.007	0.531	−0.004	0.007	0.531	−0.004	0.007	0.532
Chicken and Sugars	0.015	0.006	0.017	0.013	0.007	0.051	0.013	0.007	0.051	0.013	0.007	0.051

Eggs and Fibers	0.003	0.006	0.588	0.003	0.007	0.659	0.003	0.007	0.659	0.003	0.007	0.660
LogInsulin												
Western Breakfast	−0.015	0.010	0.119	−0.015	0.010	0.139	−0.009	0.010	0.356	−0.007	0.010	0.521
Legumes and Good Fat	−0.020	0.009	0.030	−0.019	0.010	0.066	−0.017	0.009	0.066	−0.017	0.009	0.064
Homemade Meal	0.011	0.010	0.247	0.011	0.010	0.250	0.013	0.009	0.167	0.014	0.009	0.142
Chicken and Sugars	0.012	0.009	0.191	0.013	0.010	0.173	0.018	0.009	0.049	0.018	0.009	0.041
Eggs and Fibers	−0.015	0.009	0.113	−0.011	0.010	0.281	−0.014	0.010	0.133	−0.014	0.010	0.132
LogHOMA-IR												
Western Breakfast	−0.016	0.011	0.158	−0.016	0.012	0.180	−0.035	0.011	0.422	−0.004	0.012	0.728
Legumes and Good Fat	−0.020	0.010	0.054	−0.020	0.011	0.074	−0.019	0.011	0.075	−0.019	0.011	0.072
Homemade Meal	0.014	0.011	0.205	0.013	0.011	0.231	0.015	0.010	0.157	0.016	0.010	0.124
Chicken and Sugars	0.010	0.010	0.349	0.010	0.011	0.345	0.015	0.010	0.139	0.016	0.010	0.114
Eggs and Fibers	−0.018	0.010	0.089	−0.017	0.012	0.157	−0.020	0.011	0.067	−0.020	0.011	0.066
LogTotalCholesterol												
Western Breakfast	−0.005	0.003	0.066	−0.006	0.003	0.060	−0.006	0.003	0.054	−0.003	0.003	0.422
Legumes and Good Fat	0.001	0.003	0.721	0.001	0.003	0.863	0.000	0.003	0.883	0.000	0.003	0.908
Homemade Meal	0.002	0.003	0.402	0.002	0.003	0.538	0.002	0.003	0.549	0.003	0.003	0.353
Chicken and Sugars	0.000	0.003	0.917	2.502×10^{-5}	0.003	0.993	-5.600×10^{-5}	0.003	0.985	0.000	0.003	0.868
Eggs and Fibers	0.003	0.003	0.269	0.002	0.003	0.521	0.002	0.003	0.511	0.002	0.003	0.511
LogHDL- C												
Western Breakfast	−0.002	0.004	0.553	−0.002	0.004	0.692	−0.004	0.004	0.313	−0.002	0.005	0.643
Legumes and Good Fat	0.006	0.004	0.160	0.005	0.004	0.210	0.004	0.004	0.343	0.004	0.004	0.351
Homemade Meal	0.001	0.004	0.832	0.001	0.004	0.900	0.000	0.004	0.919	0.000	0.004	0.958
Chicken and Sugars	0.009	0.004	0.022	0.007	0.004	0.080	0.006	0.004	0.153	0.006	0.004	0.128
Eggs and Fibers	−0.001	0.004	0.885	−0.002	0.004	0.600	−0.001	0.004	0.761	−0.001	0.004	0.759
LogLDL- C												
Western Breakfast	−0.008	0.005	0.099	−0.009	0.005	0.053	−0.009	0.005	0.073	−0.004	0.005	0.460
Legumes and Good Fat	−0.001	0.004	0.761	−0.003	0.005	0.547	−0.002	0.005	0.610	−0.003	0.005	0.586
Homemade Meal	0.003	0.004	0.566	0.001	0.005	0.800	0.001	0.005	0.753	0.003	0.005	0.537
Chicken and Sugars	−0.005	0.004	0.246	−0.005	0.005	0.278	−0.004	0.005	0.324	−0.004	0.004	0.411
Eggs and Fibers	0.005	0.004	0.233	0.004	0.005	0.389	0.004	0.005	0.423	0.004	0.005	0.423
LogTriglycerides												
Western Breakfast	−0.003	0.006	0.632	0.002	0.007	0.747	0.001	0.006	0.831	0.004	0.007	0.573
Legumes and Good Fat	0.006	0.006	0.307	0.008	0.006	0.208	0.010	0.006	0.101	0.010	0.006	0.103
Homemade Meal	−0.005	0.006	0.441	−0.004	0.006	0.550	−0.002	0.006	0.686	−0.002	0.006	0.745

Chicken and Sugars	−0.006	0.006	0.329	−0.004	0.006	0.491	−0.002	0.006	0.728	−0.002	0.006	0.764
Eggs and Fibers	−0.002	0.006	0.776	−0.005	0.007	0.418	−0.007	0.006	0.288	−0.007	0.006	0.287
LogCRP												
Western Breakfast	0.002	0.020	0.939	0.006	0.021	0.775	0.018	0.020	0.383	0.021	0.022	0.349
Legumes and Good Fat	0.006	0.019	0.759	0.019	0.021	0.369	0.022	0.020	0.275	0.022	0.020	0.276
Homemade Meal	0.015	0.020	0.444	0.005	0.021	0.795	0.007	0.019	0.714	0.007	0.020	0.714
Chicken and Sugars	−0.051	0.019	0.006	−0.057	0.020	0.004	−0.050	0.019	0.008	−0.051	0.019	0.008
Eggs and Fibers	0.016	0.019	0.418	0.029	0.021	0.175	0.023	0.020	0.266	0.023	0.020	0.266

Model 1: Adjusted for age and sex**; Model 2: Adjusted for age, sex,** physical activity; **Model 3:** Adjusted for age, sex, physical activity**,** BMI**; Model 4:** Adjusted for age, sex, physical activity, BMI, energy intake.

p= p-value

Concerning the analyses of STANISLAS cohort, PCA was conducted based on 15 food groups deriving from the available dietary data of the 3-day food consumption diary. KMO test was set at 0.576, again indicating mediocre to sufficient data quality. Five dietary patterns were subsequently extracted, accounting for 46.69% of the sample's total variance (Table 51), namely:

i. a "western breakfast" dietary pattern, with high consumption of cheese, breads and flours, processed meat and vegetables (explaining 10.58% of the total variance);
ii. a "prudent snacking" pattern, with high consumption of eggs and vegetable fats and lower consumption of salty snacks (explaining 10.44% of the total variance);
iii. a "high protein and animal fat" pattern, with high consumption of red meat, animal fat and milk and yogurt (explaining 9.26% of the total variance);
iv. a "fish and seafood" pattern, with high consumption of fish and seafood and lower consumption of poultry (explaining 8.19% of the total variance); and
v. a "sugary snacks" pattern, with high consumption of soft drinks, sugars, sweets and cereal bars (explaining 8.19% of the total variance).

Table 51. Principal Components Analysis' factor loadings for the 15 food groups in the STANISLAS Family study (n = 287).

	Component				
Food Groups	**1**	**2**	**3**	**4**	**5**
Cheese	0.664	-	-	-	-
Breads and Flours	0.605	-	-	-	-
Processed Meat	0.523	-	-	-	-
Vegetables	0.483	-	-	-	-
Eggs	-	0.630	-	-	-
Salty Snacks	-	−0.580	-	-	-
Vegetable Fat	-	0.576	-	-	-
Red Meat	-	-	0.703	-	-
Animal Fat	-	-	0.610	-	-
Milk and Yogurt	-	-	0.473	−0.338	-
Fish	-	-	-	0.666	-
Seafood	-	-	-	0.628	-
Poultry	-	-	-	−0.380	-
Soft Drinks	-	-	-	-	0.777
Sugars, Sweets and Cereal Bars	-	-	-	-	0.746
Total Variance Explained (%)	10.58	10.44	9.26	8.19	8.19

***Only loadings with an absolute values >0.3 are presented in the table.**

In the case of STANISLAS teenagers, extraction of the dietary patterns was followed by conduct of linear mixed models adjusted for all the models of aforementioned confounding factors. Statistically significant associations were observed for the "high protein and animal fat" pattern and higher values of logBMI (Model 1: est = 0.011, p-value = 0.002, Model 2: est = 0.009, p-value = 0.020), as well as higher values of logTriglycerides (Model 1: est = 0.054, p-value < 0.001; Model 2: est = 0.049, p-value = 0.001; Model 3: est = 0.045, p-value = 0.002, Model 4:est = 0.041, p-value = 0.009). Nominal associations included: i) the "western breakfast" pattern and lower logCRP (Model 4: est = −0.076, p-value = 0.024); ii) the "high protein and

animal fat" pattern and lower values of logDBP (Model 3: est = −0.010, p-value = 0.045, Model 4: est = −0.012, p-value=0.028, respectively); iii) the "fish and seafood" pattern and lower logDBP values (Model 1: est = 0.009, p-value = 0.039), in Model 1; and iv) the "sugary snacks" pattern and lower values of logHDL-C (Model 3: est = −0.014, p-value = 0.049) (Table 52).

Table 52. Linear Mixed Model Analyses on the association between the dietary patterns and measured indices in the STANISLAS Family study.

	Model 1			Model 2			Model 3			Model 4		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***
LogBMI												
Western Breakfast	0.000	0.003	0.878	0.000	0.005	0.459	-	-	-	-	-	-
Prudent Snacking	0.000	0.003	0.950	0.001	0.003	0.738	-	-	-	-	-	-
High Protein and Animal Fat	0.011	0.003	0.002	0.009	0.003	0.018	-	-	-	-	-	-
Fish and Seafood	−0.002	0.003	0.430	-0.001	0.003	0.700	-	-	-	-	-	-
Sugary Snacks	-0.001	0.003	0.701	-0.002	0.003	0.437	-	-	-	-	-	-
LogWHR												
Western Breakfast	−0.000	0.001	0.800	−0.000	0.001	0.539	−0.000	0.001	0.540	−0.000	0.001	0.840
Prudent Snacking	3.965729×10^{-5}	0.001	0.976	0.000	0.001	0.809	0.000	0.001	0.797	0.000	0.001	0.722
High protein and animal Fat	0.000	0.001	0.723	0.000	0.001	0.616	0.000	0.001	0.757	0.001	0.001	0.486
Fish and Seafood	0.001	0.001	0.134	0.002	0.001	0.146	0.002	0.001	0.126	0.002	0.001	0.130
Sugary Snacks	−0.001	0.001	0.392	−0.001	0.001	0.363	−0.001	0.001	0.409	−0.000	0.001	0.691
LogSBP												
Western Breakfast	-2.28874×10^{-5}	0.002	0.991	0.000	0.002	0.892	0.000	0.002	0.837	−0.000	0.002	0.792
Prudent Snacking	0.003	0.002	0.114	0.003	0.002	0.181	0.002	0.002	0.189	0.002	0.002	0.215
High protein and Animal Fat	0.000	0.002	0.733	0.000	0.002	0.822	−0.000	0.002	0.802	−0.001	0.002	0.504
Fish and Seafood	−0.000	0.002	0.751	−0.000	0.002	0.766	−0.000	0.002	0.801	−0.000	0.002	0.794
Sugary Snacks	0.000	0.002	0.640	0.000	0.002	0.787	0.000	0.002	0.673	−0.000	0.002	0.894
LogDBP												
Western Breakfast	−0.000	0.004	0.948	0.003	0.004	0.510	0.003	0.004	0.483	0.003	0.005	0.464
Prudent Snacking	0.002	0.004	0.593	0.001	0.004	0.833	0.000	0.004	0.841	0.000	0.004	0.845
High Protein and Animal Fat	−0.008	0.004	0.089	−0.008	0.005	0.099	−0.010	0.005	0.045	−0.012	0.005	0.028
Fish and Seafood	0.009	0.004	0.039	0.008	0.004	0.077	0.008	0.004	0.069	0.008	0.004	0.070
Sugary Snacks	−0.000	0.004	0.936	−0.002	0.005	0.651	−0.001	0.005	0.718	−0.002	0.006	0.632
LogGlucose[1]												
Western Breakfast	0.000	0.001	0.604	0.001	0.002	0.448	0.001	0.002	0.462	0.000	0.002	0.868
Prudent Snacking	−0.000	0.001	0.917	−0.000	0.002	0.793	−0.000	0.002	0.805	−0.000	0.002	0.727
High Protein and Animal Fat	−0.001	0.002	0.428	−0.001	0.002	0.632	−0.000	0.002	0.708	−0.002	0.002	0.365
Fish and Seafood	−0.002	0.001	0.202	−0.001	0.001	0.331	−0.001	0.001	0.323	−0.001	0.001	0.323
Sugary Snacks	0.001	0.001	0.568	0.000	0.002	0.906	0.000	0.002	0.928	−0.001	0.002	0.502
LogTotalCholesterol[1]												

Western Breakfast	−0.001	0.004	0.728	−0.002	0.004	0.644	−0.002	0.004	0.66	−0.002	0.005	0.703
Prudent Snacking	0.002	0.004	0.599	0.004	0.004	0.347	0.004	0.004	0.369	0.004	0.004	0.358
High Protein and Animal Fat	−0.003	0.005	0.490	−0.006	0.005	0.236	−0.007	0.005	0.157	−0.008	0.005	0.151
Fish and Seafood	0.005	0.004	0.224	0.006	0.004	0.173	0.006	0.004	0.171	0.006	0.004	0.172
Sugary Snacks	−0.001	0.004	0.712	6.926×10^{-7}	0.005	1.000	0.000	0.005	0.940	0.001	0.006	0.833
LogHDL- C[1]												
Western Breakfast	0.006	0.006	0.303	0.005	0.007	0.426	0.005	0.007	0.443	0.011	0.007	0.139
Prudent Snacking	−0.005	0.006	0.419	−0.004	0.007	0.547	−0.003	0.007	0.584	−0.003	0.007	0.657
High Protein and Animal Fat	−0.003	0.007	0.621	−0.002	0.008	0.762	0.000	0.008	0.983	0.004	0.008	0.622
Fish and Seafood	0.004	0.006	0.462	0.002	0.006	0.710	0.002	0.006	0.728	0.002	0.006	0.746
Sugary Snacks	−0.007	0.006	0.237	−0.014	0.007	0.065	−0.014	0.007	0.049	−0.013	0.008	0.114
LogLDL- C[1]												
Western Breakfast	−0.006	0.006	0.333	−0.007	0.006	0.275	−0.007	0.006	0.292	−0.060	0.053	0.254
Prudent Snacking	0.004	0.006	0.493	0.007	0.006	0.293	0.006	0.006	0.332	0.041	0.047	0.391
High Protein and Animal Fat	−0.005	0.007	0.472	−0.010	0.007	0.168	−0.013	0.007	0.073	−0.112	0.057	0.050
Fish and Seafood	0.004	0.006	0.475	0.007	0.006	0.292	0.006	0.006	0.288	0.035	0.045	0.435
Sugary Snacks	−0.001	0.006	0.810	0.005	0.007	0.492	0.005	0.007	0.410	0.042	0.059	0.473
LogTriglycerides[1]												
Western Breakfast	0.011	0.012	0.338	0.009	0.013	0.467	0.010	0.013	0.444	−0.001	0.014	0.911
Prudent Snacking	0.003	0.012	0.237	0.000	0.013	0.990	-6.768×10^{-5}	0.013	0.996	−0.001	0.013	0.893
High Protein and Animal Fat	0.054	0.013	<0.001	0.049	0.014	0.001	0.045	0.014	0.002	0.041	0.015	0.009
Fish and Seafood	0.014	0.012	0.252	0.019	0.012	0.133	0.020	0.012	0.114	0.021	0.012	0.093
Sugary Snacks	0.009	0.012	0.428	0.010	0.013	0.462	0.011	0.013	0.399	−0.002	0.016	0.855
LogCRP												
Western Breakfast	−0.045	0.029	0.125	−0.053	0.031	0.085	−0.050	0.030	0.096	−0.076	0.033	0.024
Prudent Snacking	0.031	0.028	0.274	0.037	0.030	0.217	0.037	0.029	0.201	0.036	0.029	0.222
High Protein and Animal Fat	0.009	0.031	0.757	−0.005	0.033	0.873	−0.019	0.032	0.558	−0.033	0.034	0.334
Fish and Seafood	0.018	0.029	0.516	0.009	0.030	0.745	0.010	0.029	0.733	0.008	0.030	0.774
Sugary Snacks	0.010	0.031	0.743	0.011	0.032	0.729	0.016	0.032	0.603	0.004	0.036	0.905

[1] Original data values in mmol/l were used for creation of the logGlucose, logTotalCholesterol, logHDL- C, logLDL- C, LogTriglycerides variables

Model 1: Adjusted for age and sex; **Model 2**: Adjusted for age, sex, physical exercise; **Model 3**: Adjusted for age, sex, physical activity, BMI; **Model 4**: Adjusted for age, sex, physical activity, BMI, energy intake

p= p-value

3.2.2. VEGF-A variants and cardiometabolic and dietary parameters in the TEENAGE

For the purposes of investigating the potential impact of the 11 VEGF-A-related SNPS (Table 53) on the TEENAGE adolescents' cardiometabolic indices, we proceeded to conducting multiple linear regressions for each of the target variants' risk alleles. The rs4416670 and rs7043199 and the variants presented statistically significant associations, where presence of the C allele of the former was related with lower logSBP (Model 1: β = -0.007, *p* = 0.002, Model 2: β = -0.007, *p* =0.002, Model 3: β = -0.07, *p* = 0.0035) (Table 54). On the contrary, presence of the A allele of the rs7043199 variant was related with increased levels of both logSBP, (Model 2: β= 0.009, *p* = 0.004) and logDBP (Model 3: β= 0.0138, *p* = 0.0046).

Table 53. List of the VEGF-A-related Single Nucleotide Polymorphisms (SNPs) (n=11) investigated for cardiometabolic associations in the TEENAGE cohort.

		Consortial Summary Statistics						TEENAGE Cohort	
SNP	**Gene**	**Chr**	**Position**	**Alleles**	**MAF**	**Effect Allele**	**Direction of effect for VEGF**	**EAF**	**Ref**
rs114694170	MEF2C,MEF2C-AS1	5	5:88884379	T/C	0.02 (C)	T	Negative (beta=-0.15)	0.96	[6]
rs6921438	SCIRT, LOC100132354	6	6:43957870	G/A/C	0.44 (A)	A	Negative (beta-0.72)	0.39	[6-7]
rs1740073	LINC02537, SCIRT, C6orf223	6	6:43979661	T/A/C	0.20 (T)	T	Positive (beta=0.09)	0.35	[6]
rs4416670	SCIRT	6	6:43982716	T/A/C	0.47 (C)	C	Negative (beta-0.13)	0.44	[7]
rs6993770	ZFPM2-AS1,ZFPM2	8	8:105569300	A/T	0.36 (T)	T	Negative (beta=0.17)	0.31	[6-7]
rs7043199	VLDLR-AS1	9	9:2621145	T/A	0.11 (A)	A	Negative (beta=-0.10)	0.19	[6]
rs10738760	VLDLR, KCNV2	9	9:2691186	A/G	0.41 (G)	G	Negative (beta=-0.28)	0.46	[7]
rs2375981	VLDLR, KCNV2	9	9:2692583	C/A/G/T	0.41 (G)	C	Positive (beta=0.21)	0.44	[6]
rs74506613 / proxy rs10761741 used	JMJD1C	10	10:63306426	G/T	0.37 (T)	T	Positive (beta=0.08)	0.47	[6]
rs4782371	ZFPM1	16	16:88502423	T/A/C/G	0.41(G)	T	Negative (beta= -0.07)	0.36	[6]
rs2639990	ZADH2	18	18:75203596	T/C	0.10(C)	T	Positive (beta=0.11)	0.10	[6]

SNP: Single Nucleotide Polymorphism**, Chr:** Chromosome, **bp:** base pairs, **MAF:** Minor Allele Frequency (as shown in GWAS Catalog**), Ref:** Reference

Table 54. Associations between the 11 VEGF-A-related SNPs and cardiometabolic indices in the TEENAGE cohort.

	Model 1		Model 2		Model 3	
	beta	p-value	beta	p-value	beta	p-value
logBMI						
rs114694170	0.01009	0.3424	0.01317	0.2385	0.01239	0.2707
rs6921438	-0.00631	0.1131	-0.0053	0.2038	-0.00475	0.2564
rs1740073	0.005531	0.1785	0.003664	0.3826	0.002784	0.5088
rs4416670	-0.00698	0.06125	-0.00389	0.3099	-0.00363	0.3452
rs6993770	-0.00649	0.1252	-0.00866	0.04606	-0.00858	0.0483
rs7043199	-0.01265	0.01352	-0.01202	0.02304	-0.01185	0.02551
rs10738760	0.003147	0.4208	0.002341	0.5588	0.00203	0.6125
rs2375981	0.003426	0.3883	0.002837	0.4846	0.002472	0.5432
rs10761741	0.003055	0.4467	0.003455	0.3978	0.003062	0.4544
rs4782371	0.00442	0.2833	0.003158	0.4576	0.002953	0.4892
rs2639990	-0.00297	0.6463	-0.00232	0.7241	-0.0021	0.7516
logTriglycerides						
rs114694170	0.008907	0.7274	0.02828	0.2978	0.029	0.292
rs6921438	0.001028	0.9184	0.01319	0.2007	0.01328	0.2003
rs1740073	0.006261	0.5473	0.002573	0.8058	0.00253	0.8107
rs4416670	1.83E-05	0.9984	0.00513	0.5827	0.004898	0.6018
rs6993770	0.006058	0.5595	-0.00307	0.7726	-0.00332	0.7567
rs7043199	-0.01681	0.1822	-0.01787	0.1588	-0.01938	0.1304
rs10738760	-0.02382	0.01482	-0.0201	0.04157	-0.0201	0.04306
rs2375981	-0.01995	0.04558	-0.01675	0.09515	-0.01696	0.09375
rs10761741	0.004158	0.6738	-0.00254	0.7989	-0.00198	0.844
rs4782371	-0.00071	0.9448	0.00189	0.8571	0.001944	0.8546
rs2639990	-0.01428	0.3776	-0.01309	0.4196	-0.0138	0.4033
logCholesterol						
rs114694170	-0.00314	0.7859	-0.00783	0.5438	-0.00896	0.4916
rs6921438	-0.00051	0.9111	0.000254	0.9586	-9.61E-05	0.9844
rs1740073	0.000767	0.8706	0.000225	0.9639	-0.00033	0.947

rs4416670	0.001849	0.6564	0.004052	0.3602	0.004303	0.3322
rs6993770	0.0042	0.3709	0.002885	0.567	0.002729	0.5901
rs7043199	-0.00066	0.908	-9.11E-05	0.9879	-0.00107	0.8596
rs10738760	-0.00256	0.5642	-0.00355	0.4489	-0.00351	0.4558
rs2375981	-0.00357	0.4299	-0.00446	0.3497	-0.00424	0.3768
rs10761741	-0.00642	0.1503	-0.00856	0.0695	-0.0087	0.06685
rs4782371	0.003328	0.4736	0.001601	0.7478	0.002173	0.6649
rs2639990	-0.00337	0.645	-0.00521	0.4986	-0.00315	0.6864
logSBP						
rs114694170	0.004856	0.4602	0.01095	0.1322	0.01002	0.1704
rs6921438	-0.00528	0.03273	-0.00571	0.03214	-0.00614	0.02126
rs1740073	0.006211	0.01456	0.007036	0.008435	0.007113	0.007929
rs4416670	**-0.00707**	**0.002172**	**-0.00744**	**0.002407**	**-0.00716**	**0.003524**
rs6993770	-0.005	0.05437	-0.00489	0.07711	-0.005	0.07093
rs7043199	0.007357	0.02104	**0.009594**	**0.004338**	0.009446	0.005093
rs10738760	-0.00105	0.6643	-0.00018	0.9445	-0.0002	0.9368
rs2375981	-0.00048	0.8464	0.000475	0.8549	0.000676	0.7948
rs10761741	0.004394	0.07559	0.003574	0.1711	0.003634	0.1643
rs4782371	-0.0017	0.5082	-0.00148	0.5885	-0.00099	0.7192
rs2639990	-0.00027	0.9467	-0.00181	0.6667	-0.00112	0.7913
logDBP						
rs114694170	-0.00538	0.5747	-0.00023	0.9829	-0.00073	0.945
rs6921438	-0.00617	0.08685	-0.00804	0.03627	-0.00845	0.0283
rs1740073	0.005599	0.1311	0.006755	0.07975	0.006983	0.07167
rs4416670	-0.00556	0.09872	-0.00686	0.05272	-0.00661	0.06318
rs6993770	-0.00621	0.101	-0.0043	0.281	-0.00443	0.2685
rs7043199	0.01191	0.01033	0.01359	0.005051	**0.0138**	**0.004611**
rs10738760	6.32E-06	0.9986	0.001639	0.6575	0.001642	0.6579
rs2375981	-0.00022	0.9508	0.001781	0.6339	0.002048	0.5851
rs10761741	0.005385	0.135	0.006435	0.08701	0.006501	0.0848
rs4782371	0.000505	0.8928	0.002055	0.6027	0.002789	0.4824

rs2639990	0.004213	0.4671	0.003025	0.6163	0.003598	0.5553
logPP						
rs114694170	0.02169	0.1799	0.03011	0.0877	0.02892	0.1044
rs6921438	-0.00429	0.4814	-0.00136	0.8342	-0.00166	0.7989
rs1740073	0.008354	0.1826	0.008206	0.2063	0.007979	0.223
rs4416670	-0.01232	0.03026	-0.01075	0.07144	-0.0104	0.08316
rs6993770	-0.0003	0.9623	-0.00313	0.6417	-0.0031	0.6466
rs7043199	-0.00119	0.8798	0.002393	0.77	0.001466	0.859
rs10738760	-0.0021	0.7244	-0.00156	0.8026	-0.00142	0.8201
rs2375981	-0.00033	0.9559	-0.00017	0.9786	9.90E-05	0.9875
rs10761741	0.005041	0.4081	0.000931	0.8832	0.000839	0.8954
rs4782371	-0.00663	0.2943	-0.00846	0.2027	-0.00844	0.2076
rs2639990	-0.00571	0.5596	-0.00865	0.3943	-0.00733	0.4765
logGlucose						
rs114694170	0.01915	0.4259	0.01844	0.488	0.01499	0.5762
rs6921438	-0.00684	0.4689	-0.01078	0.2855	-0.01227	0.2245
rs1740073	0.00942	0.3361	0.007099	0.4879	0.006708	0.5143
rs4416670	0.000832	0.9235	0.000346	0.9698	0.000223	0.9806
rs6993770	-0.01043	0.2856	-0.00569	0.5839	-0.00679	0.5148
rs7043199	0.008424	0.4782	0.008428	0.4973	0.006293	0.6144
rs10738760	0.006866	0.457	0.003822	0.6927	0.002642	0.7852
rs2375981	0.007188	0.445	0.004344	0.6588	0.003512	0.722
rs10761741	0.003465	0.7095	0.004664	0.6322	0.006317	0.5187
rs4782371	-0.01497	0.1213	-0.00968	0.3456	-0.00954	0.3557
rs2639990	-0.00127	0.9336	-0.0042	0.7913	-0.00359	0.8233
logLDL						
rs114694170	-0.0082	0.6443	-0.02002	0.3046	-0.02187	0.2661
rs6921438	-0.00502	0.4711	-0.00418	0.573	-0.00419	0.5718
rs1740073	0.000988	0.8914	-0.00091	0.9035	-0.0022	0.7704
rs4416670	0.001987	0.7558	0.006226	0.3529	0.006893	0.3039
rs6993770	-0.00281	0.6968	-0.00581	0.4461	-0.00551	0.4718

rs7043199	0.006725	0.4431	0.006013	0.5094	0.005337	0.5605
rs10738760	-0.01029	0.1306	-0.01186	0.09438	-0.01145	0.1071
rs2375981	-0.01274	0.06626	-0.01425	0.04787	-0.01372	0.05769
rs10761741	-0.00519	0.4493	-0.00794	0.2667	-0.0091	0.2047
rs4782371	0.01135	0.1115	0.007783	0.3015	0.008257	0.2758
rs2639990	-0.00388	0.7136	-0.00713	0.517	-0.00744	0.5042
logHDL						
rs114694170	0.001151	0.9449	-0.00031	0.9867	-0.00111	0.9524
rs6921438	0.002231	0.7332	0.00056	0.9363	-0.00014	0.9837
rs1740073	0.002099	0.7572	0.005597	0.4303	0.00606	0.3951
rs4416670	0.002402	0.6887	0.000127	0.984	-0.00021	0.9737
rs6993770	0.01151	0.08893	0.0148	0.03953	0.01427	0.04781
rs7043199	-0.00711	0.3875	-0.00429	0.6186	-0.00585	0.4992
rs10738760	0.01409	0.02729	0.01249	0.06206	0.01223	0.06815
rs2375981	0.01261	0.05275	0.01139	0.09454	0.01129	0.09822
rs10761741	-0.01029	0.1098	-0.01098	0.1037	-0.00975	0.15
rs4782371	-0.00762	0.2552	-0.0072	0.3117	-0.0068	0.3417
rs2639990	-0.00388	0.7136	-0.00713	0.517	-0.00744	0.5042
logCRP						
rs114694170	-0.0379	0.6541	-0.04237	0.6554	-0.03521	0.711
rs6921438	-0.0418	0.1947	-0.04414	0.2017	-0.04039	0.241
rs1740073	-0.00433	0.8972	-0.0181	0.606	-0.02466	0.482
rs4416670	-0.0194	0.511	-0.01528	0.6242	-0.0162	0.6012
rs6993770	-0.01718	0.6107	-0.00339	0.9251	-0.0048	0.8941
rs7043199	0.02666	0.5029	0.003378	0.9353	0.000455	0.9913
rs10738760	0.02319	0.4658	0.02242	0.5016	0.02371	0.4762
rs2375981	0.02867	0.3747	0.02603	0.441	0.02572	0.4462
rs10761741	0.0237	0.4588	0.01415	0.6735	0.01207	0.7179
rs4782371	-0.04092	0.2165	-0.03658	0.3002	-0.03689	0.2958
rs2639990	-0.05523	0.2803	-0.05647	0.2884	-0.05193	0.3325

Model 1: Adjusted for age and sex**, Model 2:** Adjusted for age,_sex and exercise**, Model 3:** Adjusted for age,_sex, exercise and dietary patterns**, p:** p-value

After examining the individual associations between the target variants and the indices in reference (Table 54), we proceeded to the creation of the 9-SNP uGRS and the subsequent conduct of multiple linear regressions for all the cardiometabolic indices (Table 55). Increased uGRS was associated with increased levels of logBMI (Model 1: β = 0.0044, *p*-value =0.003, Model 2: β = 0.0043, *p*-value = 0.005, Model 3: β = 0.004, *p*-value = 0.009) and logSBP (Model 1: β= 0.002, *p*-value = 0.03, Model 2: β= 0.019, *p*-value = 0.047, Model 3: β= 0.002, *p*-value = 0.037), but decreased values of logHDL (Model 1: β= -0.005, *p*-value = 0.032) (Table 58). We further used the sample median to split the uGRS in two categories of "low" and "high" risk and examine potential differences between the two groups of genetic risk. Indeed, the observed associations were additionally noted in the within-group analyses, with logBMI displaying statistically significant differences with individuals in the higher category presenting greater logBMI values (p <0.05) (Figure 30). Similarly, individuals in the higher percentile of uGRS presented statistically significantly higher values of logSBP compared to the ones in the lower group (p < 0.05), while subjects with higher versus lower uGRS displayed statistically significantly lower levels of logHDL (p < 0.05) (Figure 30).

Table 55. Associations between the 9-SNP uGRS and selected cardiometabolic indices in the TEENAGE cohort.

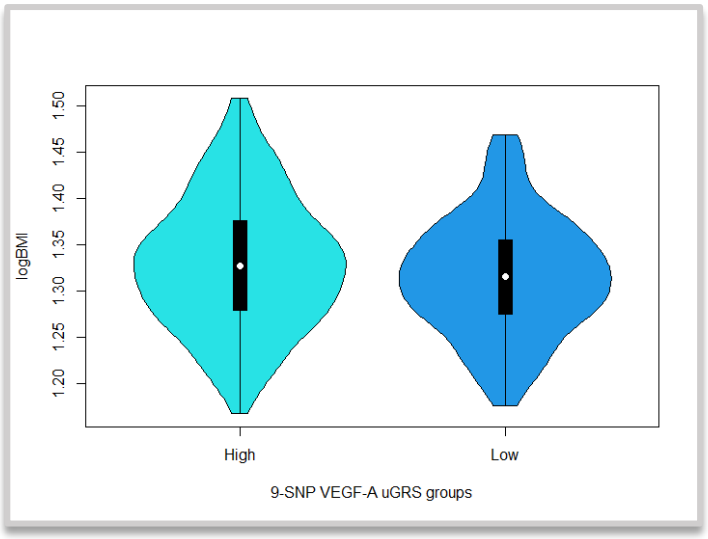
	Model 1			Model 2			Model 3		
	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p
logBMI									
9-SNP uGRS for VEGF-A	**0.004445**	**0.001494**	**0.00305**	**0.004349**	**0.001553**	**0.005277**	**0.0040937**	**0.0015678**	**0.009281**
logTriglycerides									
9-SNP uGRS for VEGF-A	0.005892	0.003854	0.127	0.004260	0.003915	0.2771	0.004650	0.003994	0.2450
logCholesterol									
9-SNP uGRS for VEGF-A	-0.0001979	0.0017479	0.90992	-0.000716	0.001859	0.70024	-0.0007685	0.0018917	0.68474
logSBP									
9-SNP uGRS for VEGF-A	**0.002006**	**0.000924**	**0.0303**	**0.0019840**	**0.0009974**	**0.047203**	**0.0020983**	**0.0010045**	**0.037205**
logDBP									
9-SNP uGRS for VEGF-A	0.001891	0.001351	0.161963	0.002211	0.001441	0.12569	0.002365	0.001455	0.10458
LogPP									
9-SNP uGRS for VEGF-A	0.002425	0.002268	0.2854	0.001599	0.002413	0.50779	0.0015523	0.0024439	0.52558
LogGlucose									
9-SNP uGRS for VEGF-A	0.0009057	0.0036448	0.804	0.001952	0.003840	0.611	0.0028415	0.0038989	0.4665
logLDL									
9-SNP uGRS for VEGF-A	0.003038	0.002688	0.2589	0.002300	0.002818	0.4148	0.001733	0.002863	0.5454

LogHDL									
9-SNP uGRS for VEGF-A	**-0.005336**	**0.002493**	**0.03279**	-0.004999	0.002631	0.05812	-0.004455	0.002673	0.09630
LogCRP									
9-SNP uGRS for VEGF-A	0.001437	0.012397	0.90778	-0.0001663	0.0131008	0.98988	-0.001631	0.013250	0.90207

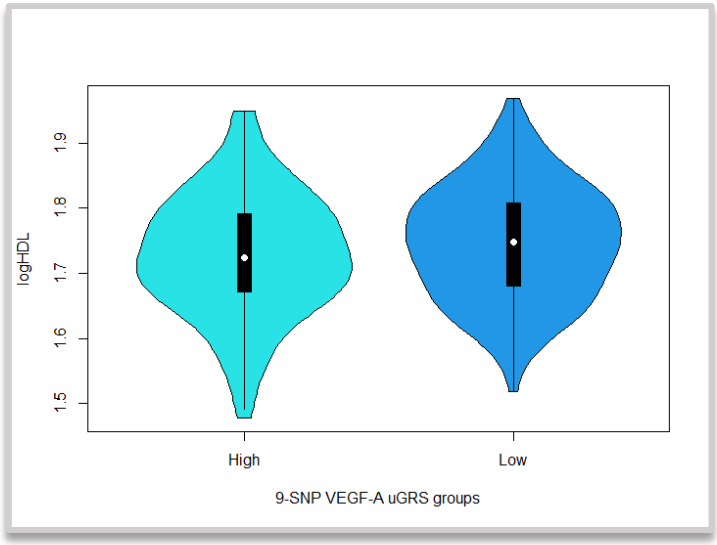
Model 1: Adjusted for age and sex, **Model 2:** Adjusted for age, sex and exercise, **Model 3:** Adjusted for age, sex, exercise and dietary patterns.

p: p-value

A.



B.



C.

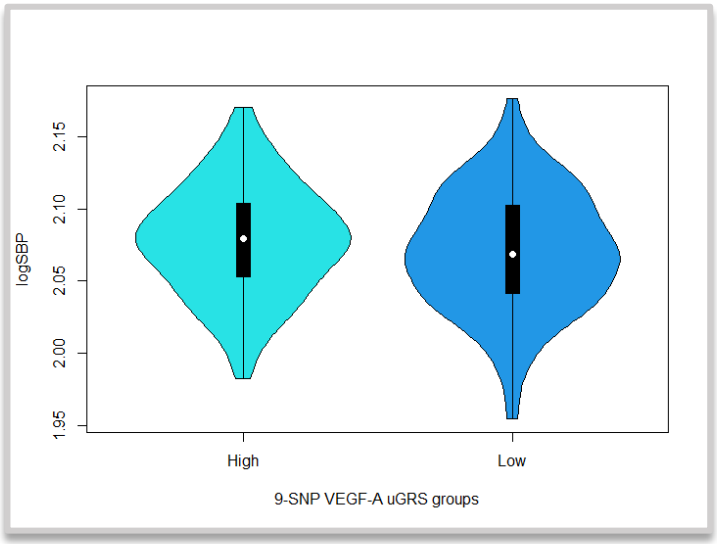


Figure 30: Violin plots depicting the distribution of A. logBMI, B. logSBP and C. logHDL between the two groups of the 9-SNP VEGF-A unweighted GRS (low versus high), separated by the sample median (*p*-values < 0.05).

Interactions between the uGRS and Dietary Patterns

In order to test for potential gene-diet interactions, we proceeded to conducting multiple linear regressions investigating the impact of the interaction of the calculated uGRS and the extracted dietary patterns on the various cardiometabolic indices (Table 56). A statistically significant association was revealed concerning the interaction between the uGRS and the "Western Breakfast" pattern was associated with higher levels of logDBP (Model 1: β = 0.0060, *p*-value = 4.28e-05, Model 2: β = 0.00568, *p*-value = 0.000239). A different nominally statistically significant association was found for the interaction between the uGRS and consumption of the "Eggs and Fibers" pattern and increased levels of logGlucose (Model 2: β = 0.00883, *p*-value = 0.0132).

Table 56. Associations between the 9-SNP uGRS for VEGF-A and dietary patterns in the TEENAGE cohort.

	Model 1			Model 2		
	Estimate	SE	p	Estimate	SE	p
logBMI						
uGRS*Western Breakfast	0.0006259	0.0016544	0.70532	0.0009623	0.0016699	0.564684
uGRS*Legumes and Good Fat	0.0004362	0.0014115	0.75742	-0.0002951	0.0015027	0.844375
uGRS*Homemade Meal	-0.001836	0.001302	0.15906	-0.001894	0.001326	0.153652
uGRS*Chicken and Sugars	-0.001955	0.001442	0.17566	-0.001508	0.001577	0.339236
uGRS*Eggs and Fibers	-0.000687	0.001204	0.56840	0.0004325	0.0014616	0.767393
logTriglycerides						
uGRS*Western Breakfast	-0.003976	0.004121	0.335	-0.003394	0.004147	0.4135
uGRS*Legumes and Good Fat	-0.003084	0.003643	0.398	-0.002993	0.003701	0.4192
uGRS*Homemade Meal	-0.0003673	0.0031521	0.907	-0.0004249	0.0031042	0.8912
uGRS*Chicken and Sugars	-0.000562	0.003527	0.873	0.000446	0.003723	0.9047
uGRS*Eggs and Fibers	0.0004714	0.0029163	0.872	-8.952e-07	3.645e-03	0.9998
logCholesterol						
uGRS*Western Breakfast	-0.0003120	0.0018673	0.86737	-0.0003595	0.0019652	0.85495
uGRS*Legumes and Good Fat	4.399e-04	1.654e-03	0.79038	0.0006190	0.0017604	0.72529
uGRS*Homemade Meal	0.0022544	0.0014247	0.11421	0.0024594	0.0014679	0.09455
uGRS*Chicken and Sugars	0.0005882	0.0015997	0.71324	0.0011419	0.0017668	0.51840
uGRS*Eggs and Fibers	-0.0024429	0.0013171	0.064231	-0.0035654	0.0017221	0.0390
logSBP						
uGRS*Western Breakfast	0.0019835	0.0010171	0.05164	0.0021791	0.0010716	0.042500
uGRS*Legumes and Good Fat	0.0009800	0.0008694	0.2601	0.001112	0.000966	0.250296
uGRS*Homemade Meal	-0.0004048	0.0008249	0.6238	-0.0006534	0.0008508	0.442827
uGRS*Chicken and Sugars	0.0003776	0.0008987	0.6745	0.0003459	0.0010081	0.731659
uGRS*Eggs and Fibers	-0.0011855	0.0007341	0.1068	-0.0018073	0.0009354	0.053889
logDBP						
uGRS*Western Breakfast	**0.0060753**	**0.0014736**	**4.28e-05**	**0.005687**	**0.001537**	**0.000239**

uGRS*Legumes and Good Fat	0.0009039	0.0012713	0.477344	0.001483	0.001396	0.28856
uGRS*Homemade Meal	-0.0008981	0.0012064	0.45691	-0.001097	0.001229	0.37234
uGRS*Chicken and Sugars	1.822e-05	1.316e-03	0.988960	0.001229	0.001457	0.39932
uGRS*Eggs and Fibers	0.0001876	0.0010752	0.86156	-0.0009524	0.0013559	0.48273
logPP						
uGRS*Western Breakfast	-0.004375	0.002501	0.08081	-0.003179	0.002602	0.22237
uGRS*Legumes and Good Fat	0.0006745	0.0021355	0.75221	0.0001765	0.0023393	0.93989
uGRS*Homemade Meal	0.0001585	0.0020281	0.93772	-5.986e-05	2.067e-03	0.97691
uGRS*Chicken and Sugars	0.0006736	0.0022094	0.76055	-0.001662	0.002442	0.49637
uGRS*Eggs and Fibers	-0.003235	0.001801	0.07296	-0.002587	0.002269	0.2548
logGlucose						
uGRS*Western Breakfast	-0.0002371	0.0038992	0.952	-0.0006882	0.0040671	0.866
uGRS*Legumes and Good Fat	-0.004075	0.003441	0.237	-0.002575	0.003628	0.478
uGRS*Homemade Meal	-0.0035228	0.0029773	0.237	-0.003946	0.003039	0.195
uGRS*Chicken and Sugars	0.003550	0.003317	0.285	0.003922	0.003634	0.281
uGRS*Eggs and Fibers	5.869e-03	2.745e-03	0.0330	**0.008830**	**0.003550**	**0.0132**
logLDL						
uGRS*Western Breakfast	-0.0003845	0.0028733	0.8936	-0.0008217	0.0029791	0.7828
uGRS*Legumes and Good Fat	0.001102	0.002545	0.6652	0.001857	0.002669	0.4870
uGRS*Homemade Meal	0.002229	0.002194	0.3103	0.002617	0.002230	0.2412
uGRS*Chicken and Sugars	0.0024563	0.0024563	0.9468	0.0008795	0.0026757	0.7425
uGRS*Eggs and Fibers	-0.004027	0.002024	0.0472	-0.005950	0.002606	0.0229
logHDL						
uGRS*Western Breakfast	0.0007058	0.0026675	0.79145	0.001002	0.002789	0.71958
uGRS*Legumes and Good Fat	0.0004628	0.0023529	0.84413	-7.341e-05	2.485e-03	0.97644
uGRS*Homemade Meal	0.003719	0.002032	0.06787	0.003693	0.002080	0.07649
uGRS*Chicken and Sugars	0.001880	0.002275	0.40903	0.002321	0.002496	0.3529
uGRS*Eggs and Fibers	-0.0003372	0.0018861	0.85819	-0.0007087	0.0024472	0.77227
logCRP						
uGRS*Western Breakfast	-0.009797	0.013082	0.45430	-0.0072781	0.0136345	0.59379
uGRS*Legumes and Good Fat	0.002883	0.011393	0.80035	-0.0031947	0.0119986	0.79019
uGRS*Homemade Meal	0.010795	0.009823	0.27239	0.011024	0.010010	0.27144
uGRS*Chicken and Sugars	0.004140	0.010979	0.70632	-0.0006592	0.0120081	0.95625
uGRS*Eggs and Fibers	-0.006220	0.008995	0.48963	0.0010038	0.011644	0.93135

Model 1: Adjusted for age, sex, uGRS and each dietary pattern**, Model 2:** Adjusted for age, sex and exercise. uGRS and each dietary pattern

3.2.3. Discussion

Analyses using TEENAGE and STANISLAS Family Study data in the present context of the 2018 Chair Gutenberg project aimed at identifying the dietary patterns of the two adolescent, European populations and further investigating their associations with cardiometabolic indices and their combined interactions with VEGF-A-related variants.

Regarding the dietary intake observed in the two populations, both cohorts reported a mediocre energy intake (TEENAGE: 1741.00 kcal/d and STANISLAS: 2056.03 kcal/d), based on the present dietary guidelines for adolescents [39], a generally good health status with BMI values within the normal range (18.5–25 kg/m^2). Five dietary patterns were extracted for in each population. Two of the identified patterns adhered to the principles of MD, namely the Greek "eggs and fibers" and the French "prudent snacking" patterns, explaining 7.47% and 10.44% of the respective total variances and including non-refined cereals, vegetables, eggs, and vegetable fats. Another MD-like pattern was observed in the Greek teenagers with the consumption of legumes, olives, olive oil and nuts in the "legumes and good fat" pattern. The influences of WD were apparent in French teenagers, who demonstrated high consumption of energy-dense food groups, such as red meat, animal fat and milk and yogurt in the "high protein and animal fat" pattern and soft drinks and sugary snacks in the "sugary snacks" pattern.

Accordingly, the predominant pattern in both populations (the "western breakfast" pattern) was related to WD-related food groups [404], such as cheese, processed meat and food items high in carbohydrates. The "western breakfast" pattern reflected a higher percentage (15.61%) of the variance explained in the Greek population, in comparison to the French one (10.58%). Breakfast habits were highlighted in the first 5-year follow-up in the STANISLAS Cohort, which underlined the importance of the household environment in dietary habits by finding a household variance of 42.5 to 52.9% in the energy intake observed in breakfast [405]. The importance of breakfast consumption and its contribution to daily energy intake of French children and families, is also supported by another, recent cross-sectional survey [406]. Although the western diet has been associated with elevated inflammation biomarkers [407], the cohort of the Greek teenagers reported no comorbidities and we found no associations between adherence to the "western breakfast" pattern and respective CRP levels. Interestingly enough, the "chicken and sugars" pattern identified in the Greek cohort was significantly associated with lower levels of logCRP (Model 1: $\beta = -0.051$, p-value = 0.006, Model 2: $\beta = -0.057$, p-value = 0.004 and Model 3: $\beta = -0.050$, p-value = 0.008). An inverse association between the consumption of poultry and CRP levels in teenagers has previously been reported, in the general context of adherence to the Dietary Approach to Stop Hypertension (DASH) diet regime [408], although a recent umbrella review showed Nutrients 2021, 13, 198 14 of 19 no association between the DASH diet and CRP levels in adults [409]. On the contrary, an inverse association between consumption of sweets and CRP levels is not supported by other studies. In fact, consumption of sugars and especially sugar-sweetened beverages has previously been associated with higher CRP levels in adults [410,411]. In adolescents, a different review has shown a positive association between sugar consumption and CRP [412], whereas another review found

greater consumption of sugars by normal weight adolescents in comparison with overweight ones, but did not find any association between sugar consumption and CRP [413]. A cross-sectional study investigating the relation between food intake and CRP levels in children also found that consumption of milk, citrus, melons and berries displayed associations with lower levels of CRP, potentially due to the general high content of fruits and vegetables in antioxidants and the association of dairy consumption with greater satiety and potential adherence to a generally healthier diet [414].

Furthermore, our study found that the "high protein and animal fat" pattern displayed significant associations with higher logtriglyceride and logBMI levels ($p < 0.01$), for French teenagers. The latter is in accordance with various cross-sectional studies that have researched the dietary habits of adolescents and their potential associations to BMI. A study by Gutiérrez-Pliego et al. unveiled three major dietary patterns in a population of 373 Mexican teenagers including a pattern high in refined "unhealthy" products, such as snacks, sugars and sweets, a pattern with high protein/high fat content and a pattern including high consumption of fruits, vegetables, nuts and whole grains. The study found a strong relationship ($p < 0.01$) between higher consumptions of the first two dietary patterns and higher BMI [415]. In the same context, a different study in Northeastern Brazil investigated data from 1247 adolescents. The study identified two dietary patterns, one referring to high consumption of sugars, sweets and cakes, amongst others, and one correlated with high consumption of fruits and vegetables. Higher adherence to the dietary pattern including "unhealthy" products, was, again, positively correlated with higher values of BMI ($p = 0.018$) [416].

Furthermore, a different study on the dietary habits of female adolescents showed that higher adherence to a "Western" pattern referring to increased consumption of fat and mediocre consumption of protein, among others, was associated with higher levels of BMI, waist circumference, as well as total cholesterol levels [417]. Although dietary patterns with a higher consumption of fat have generally been positively associated with cardiometabolic risk factors in teenagers [418,419], certain diets, including consumption of specific food groups, such as the DASH diet [420], have been related with a better metabolic profile [421]. Indeed, higher adherence to the DASH diet has been shown to relate to a reduced prevalence of metabolic syndrome and increased blood pressure during adolescence [404], as well as lower levels of HbA1c and systolic blood pressure, in young adults with type 1 and type 2 diabetes, respectively [422]. Better adherence to the components of the DASH diet was even associated with a lower risk of being a metabolic unhealthy obese, in children and adolescents with increased body weight [423]. Additionally, other high protein diets, such as the ketogenic diet and the Modified Atkins diet, have been associated with better effects on adolescents with epilepsy [424,425], with the ketogenic diet to have been related to reduced weight and fasting insulin and HOMA-IR levels in obese teenagers [426]. However, the aforementioned diets also usually include consumption of vegetables fats and fats derived from nuts, seeds, white meat (such as poultry and fish), as well as food groups like grains, vegetable fats, fruits and vegetables, which are not met when referring to dietary patterns centered on high protein or animal fat consumption.

Furthermore, the aforementioned beneficial associations have been primarily observed in adults or obese adolescent populations, who could potentially benefit from the adherence to a structured diet with the above food groups. This could potentially explain why our study demonstrated positive associations between the high consumption of protein and animal fats with BMI and triglyceride levels in adolescents mostly displaying BMI of a normal range.

Moreover, the present study evaluates the adherence to each dietary pattern, without comparing them with the respective adherence to the rest of the patterns extracted. The identification of dietary patterns of adolescents has generally been a subject of interest in recent literature. Gonzalez-Gil et al. investigated the dietary patterns of 5328 European adolescents in the context of the cross-sectional HELENA study [427]. The latter consisted of adolescent cohorts of 10 different European countries, including Greek teenagers from the cities of Athens and Heraklion, Crete. The study identified four dietary patterns in teenage boys and six dietary patterns in teenage girls. Patterns explaining greater total variance in boys referred to consumption of vegetables, pasta, rice, cheese and sweets among others, at the same time as dominant patterns in girls referred to consumption of Mediterranean-type food items, dairy and consumption of a healthy breakfast [428]. Additionally, when investigating the dietary habits of adolescents based on data collected in the 1995 Australian National Nutrition Survey, McNaughton et al. showed that a dietary pattern rich in fruit, salads, cereals and fish was found to be negatively associated with levels of diastolic blood pressure in teenagers older than 16 years of age [428]. Our study found no associations between the patterns containing fruit, vegetable and fish consumption and the levels of diastolic pressure in adolescents younger than 16 years of age. Furthermore, the I. Family Study investigated the association between the dietary patterns of 2451 pairs of European children and their parents, with regards to the existing food environment conditions. The study showed the role of food availability in the children's dietary choices, highlighting that the consumption of soft drinks was greatly dependent on their availability in the immediate food environment [429]. Moreno LA et al. also showed that increased consumption of sweet beverages was also associated with increased risk of adolescent obesity [430]. In our study, the "sugary snacks" pattern of the French population, which included consumption of sweetened beverages, was not related to logBMI values, but was associated with lower values of logHDL-C. However the effect disappeared when taking into account the adjusted threshold of statistical significance ($0.04 > 0.01$). A different study of German adolescents demonstrated that higher consumption of dietary patterns containing high-fat and high-carbohydrate, energy-dense foods was associated with lower socioeconomic levels and a lower intake of various vitamins and minerals [431].

Regarding the examination of the role of VEGF-A-related variants on adolescent cardiometabolic profile, two VEGF-A-related SNPs, namely the rs7043199 and the rs4416670 variants, presented significant relations with blood pressure indices in the TEENAGE adolescents. Moreover, the 9-SNP uGRS constructed out of risk variants for higher VEGF-A levels was associated with higher levels of logBMI and logSBP but lower levels of logHDL. Furthermore, the exploration of associations between the uGRS and the teenagers' dietary patterns revealed a significant relation between the adherence to the "Western Breakfast"

pattern and higher logDBP, as well as a nominal association for the “Eggs and Fibers” pattern and higher logGlucose.

In our sample, the negatively associated with VEGF-A levels C allele of the rs4416670 SNP was also negatively associated with logSBP levels. Debette et Visvikis-Siest et al. previously showed a positive relationship between the allele and increased pulse pressure in a healthy population [236]; this could potentially be attributed to the relation between lower levels of SBP which would subsequently signify greater values of pulse pressure. On the contrary, the A allele of the rs7043199 variant, which was previously negatively associated with VEGF-A [10,11], was hereby linked with higher levels of logSBP and logDBP. Although not as statistically strong (*p*-value = 0.004), this observed effect could possibly be attributed to the yet to be fully elucidated pleiotropic influence of the variant, the role of which has been previously investigated for overall risk for other disorders related to cardiometabolic profile, namely ischemic heart disease [432] and osteoporosis [433].

To the best of our knowledge, VEGF-A has not been extensively and exclusively examined in adolescents and the present constitute the first attempts in constructing a uGRS for teenagers using VEGF-A-associated variants. The present 9-SNP uGRS was linked to higher levels of logSBP (Model 1: β= 0.002, *p*-value = 0.03, Model 2: β= 0.019, *p*-value = 0.047, Model 3: β= 0.002, *p*-value = 0.037) and individuals with high GRS presented greater values compared to the ones with low GRS (*p*-value=0.027), showing that increased genetic predisposition to higher levels of VEGF-A is associated with higher blood pressure in adolescents. This finding is aligned with the well-known relationship between VEGF-A and hypertension, as current literature has shown that inhibition of VEGF-A receptors signifies higher levels of circulating VEGF-A which have, in turn, been associated with greater risk for hypertension [434-436]. In like manner and supporting the reciprocal relationship between the VEGF family and hypertension, Zorena et al showed that adolescents with type 1 diabetes and hypertension displayed greater levels of VEGF compared to healthy individuals or patients with type 1 diabetes but without hypertension [437].

Although this is an overall healthy population with most adolescents presenting normal weight, the accumulating effect of the 9 examined SNPs from Choi et al displayed a statistically significant, positive association with higher logBMI values. Additionally to the already underlined positive relationship between VEGF-B and VEGF-C levels and obesity presence [438,439], current literature further highlights the role of VEGF-A in obesity control [228,440,441]. In the presence of obesity and fat cell proliferation, VEGF-A expression increases as it participates in angiogenesis, cell differentiation and thermogenesis in the white and brown adipose tissues. In this context, VEGF-A contributes to the subsequent increase in energy expenditure and attempts to suppress further diet-induced increase and ameliorate insulin resistance in a compensatory effect [228,440,441]. However, as the increase of adipocytes progresses, VEGF-A is more produced and angiogenesis is further promoted in the white adipose tissue, thus allowing for further obesity establishment. This cascade of events creates a reciprocal circle where obesity presence induces VEGF-A expression and vice-versa. For that reason, the effect of VEGF-A on increased weight can be described as reciprocal and context-dependent, being mainly influenced by the potential pre-existence of increased body weight [227, 440]. Hereby, the positive association between the uGRS and logBMI was steadily maintained after adjustments for all three models

of confounding factors (Model 1: β = 0.0044, *p*-value =0.003, Model 2: β = 0.0043, *p*-value = 0.005, Model 3: β = 0.004, *p*-value = 0.009) and adolescents with high versus low genetic risk also presented higher values of logBMI suggesting an aggravating effect in BMI as genetic risk for higher VEGF-A increases. In a similar context to the present, Novikova et al showed that, compared to individuals of normal weight, adolescents with obesity presented a 12-fold increase in corresponding VEGF-A levels [442]. To boot, Loebig et al showed a similar positive association in heathy young men (aged 18-30 years old) under normal blood sugar conditions, where higher levels of VEGF-A were consistently associated with increased weight [443]. VEGF-A was also related with abdominal obesity in a sample of young individuals, as demonstrated by Guzman-Guzman et al when investigating relations with parameters of the metabolic syndrome [444]. Our present findings show that increased predisposition to higher levels of VEGF-A is related to higher BMI, however according to the aforementioned, it should be noted that the reciprocity of the relation remains significant, as increased VEGF-A levels can generally be observed due to increased BMI, thus potentially aggravating the positive predisposing genetic effect.

Another significant relation was observed between the uGRS and lower levels of logHDL (Model 1: β= 0.005, *p*-value = 0.032). Although this association was not maintained after correction for multiple confounding factors, when looking at individuals with higher versus lower genetic risk for increased VEGF-A, the former did present lower values of logHDL. When looking into potential associations between VEGF-A variants and HDL, both Debette et Visvikis-Siest and Stathopoulou et al showed that the negatively associated with VEGF-A A allele of rs6921438 SNP was related with lower HDL levels in healthy populations. The present finding denoting a positive association between increased VEGF-A and lower HDL levels can, thus, potentially be explained by the general overview of the role of elevated VEGF-A in worse lipidemic profile, rather than the direct effect of VEGF-A on HDL per se [445].

Furthermore, taking the biomarker's role on metabolism into account [228, 232, 233], we further attempted to unravel the meaning of the interplay between genetic predisposition for higher VEGF-A levels and multiple cardiometabolic indices by examining the potentially modifying role of dietary habits. In our sample, the interaction between the uGRS and the consumption of the "Western Breakfast" was associated with higher levels of logDBP (Model 1: β = 0.0060*, p*-value = 4.28e-05, Model 2: β = 0.00568, *p*-value = 0.000239). This finding can be explained by the fact that the "Western Breakfast" pattern consists of food groups with high fat content, namely cheese, dairy and processed meat [311], which have already been shown to associate with increased blood pressure in the literature [446]. Hojhabrimanesh et al showed similar significant associations between a "Western" dietary pattern and overall and systolic blood pressure in Iranian adolescents, as well as a positive but not statistically significant association for diastolic pressure [447] Although the pattern was not unilaterally associated with blood pressure measurements in our team's previous analyses [311], increased predisposition to higher VEGF-A appears to bring its aggravating effect to the forefront and vice-versa. This could be partly attributed to the positive effect of Western diet and red meat-derived protein which has been previously shown to elevate VEGF-A expression, but among patients with breast cancer [448].

What's more, although the 9-SNP uGRS was not alone associated to glucose in our sample, it did present a nominally significant interaction with the protein-rich

"Eggs and Fibers" dietary pattern (consisting of non-refined cereals, vegetables and eggs) in increasing logGlucose levels (Model 2: β = 0.00883, *p*-value = 0.0132). The involvement of VEGF-A on glucose homeostasis is well-known [234], as low levels of the biomarker are linked to insulin resistance, while its overexpression is associated with impaired insulin production and increased glucose levels [228,234]. Consequently, research in adolescent cohorts to date mainly surrounds diabetic individuals or related complications [446, 449] and has yet to yield significant results in healthy populations. Although fiber intake is generally regarded as having protective effects in the production of inflammatory biomarkers [447], the present finding could possibly refer to the reciprocal effect of dietary carbohydrate and protein intake on aggravating the genetic risk for VEGF-A levels and subsequent influence the elevated glucose levels.

Moreover, similar gene-diet interactions have also been explored in individuals with metabolic syndrome in studies examining target-SNPs for VEGF-A, rather than using a holistic genetic risk score approach. Ghazizadeh et al. showed that individuals with the AA genotype for the rs10738760 variant, which was also included in the present uGRS, and a higher adherence to foods with increased sugar and saturated fatty acids, among others, presented greater risk for metabolic syndrome [242]. It was further demonstrated that presence of the same A allele can significantly interact with even favorable dietary components (eg PUFAs) in ultimately elevating the risk for worse glycemic and lipidemic profile and, thus, metabolic syndrome [242]. Taking it one step further, Chedid et al., showed a significant association between BMI and the rs10738760 polymorphism in decreasing iron levels; an effect shown to be more prominent in individuals with obesity [244]. Finally, a different relation concerned the observed associations between presence of the 9-SNP-uGRS rs6921438 and rs6993770 included SNPs and micronutrient contents, namely high manganese, low zinc and low iron intakes in patients with metabolic syndrome [448, 4501, 451].

The strengths of the present study concern: i) its attempt to use previously unused nutritional data in identifying adolescent dietary habits and their associations with cardiometabolic profile; and ii) its hypothesis of investigating demonstrated effects of known VEGF-A variants on the cardiometabolic profile of healthy adolescents for the first time. The limitations of the present study are summarized in the following: i) presently analyzed phenotypic data for both populations were cross-sectionally collected, limiting the potential for generalized cause and effect conclusions to be drawn; ii) use of the PCA for the dietary pattern extraction, included subjective choices on the number of both analyzed food groups and extracted components; iii) comparisons between the two populations' dietary habits might have been affected by the different socio-economic conditions existing in the two countries during the mid-1990s for the STANISLAS and late 2000s for the TEENAGE study; iv) although attempted to be as maximized as possible, the variance explained by both populations' extracted dietary patterns concerned approximately half of each cohort; and v) the overall good health status of the populations which might not have allowed for the identification of distinct associations with cardiometabolic risk factors, as it would for example be in the case of patients with obesity.

3.3. Polygenic Risk Score for Body Mass Index on the NAFLD, THISEAS and OSTEOS studies

The following constitute information published under the publication J. Pers. Med. 2023, 13, 327, doi.org/10.3390/jpm13020327 and can be found in Appendix E.

3.3.1. Baseline Characteristics of the Studies' Populations

The anthropometric characteristics of the unified sample are described in Table 57. Overall, we used available data from 2083 participants, namely 342 participants from the NAFLD study, as well as 791 and 950 participants from the OSTEOS and THISEAS studies, respectively (Table 57). A total of 841 men and 1242 women were included, with a median age of 53 years (calculated at 2075 participants) and a median BMI of 27.38 kg/m2 . Within the respective databases, participants presented median BMIs in the spectrum of overweight for all three studies (NAFLD median BMI = 26.5 kg/m2 , OSTEOS median BMI = 26.91 kg/m2 and THISEAS median BMI = 27.81 kg/m2). BMI was not statistically significantly different between the NAFLD and OSTEOS studies but did present a statistically significant difference between the NAFLD and THISEAS as well as the OSTEOS and THISEAS studies ($p < 0.001$ for both pairs). Differences in age were also statistically significant between all studies ($p < 0.001$ for the Kruskal–Wallis test).

Table 57. Descriptive characteristics of the NAFLD, OSTEOS and THISEAS study populations.

	All			NAFLD			OSTEOS			THISEAS		
	All (n = 2075 for age, n = 2083 for BMI)	Men (n = 841)	Women (n = 1234 for age, n = 1242 for BMI)	All (n = 342)	Men (n = 140)	Women (n = 202)	All (n = 783 for age, n = 791 for BMI)	Men (n = 101)	Women (n = 682 for age, n = 690 for BMI)	All (n = 950)	Men (n = 600)	Women (n = 350)
	Med (IQR)											
Age	53 (18)	54 (19)	52 (19)	47 (18)	44 (17)	50 (16)	50 (18)	47 (28.5)	51 (16.25)	59 (19)	58 (18.75)	60 (21)
BMI (kg/m^2)	27.38 (6.18)	27.68 (5.34)	27.02 (7.10)	26.5 (6.23)	26.8 (4.54)	25.9 (6.98)	26.91 (6.81)	26.70 (5.13)	26.94 7.01)	27.81 (5.80)	27.88 (5.43)	27.77 (6.51)

3.3.2. Polygenic Risk Score for Body Mass Index

Summary statistics for the merged dataset were calculated with BMI phenotype as a response variable and using the extended (imputed based on the 1000 genomes external reference panel) and further filtered genotypic dataset. In order to properly estimate the effects of individual SNPs that potentially contributed to the BMI phenotype in the unified dataset, we applied four different frameworks for summary statistics estimation, namely a simple generalized linear model (GLM) as implemented in the R statistical language, the regression algorithm implemented in the R package statgen GWAS as well as the SNPTEST software and the more generalized PLINK framework. In all cases, the sex, age, NAFLD status and cardiovascular disease status of individuals were incorporated in the regression models as confounders, along with several automatically selected principal components to capture potential underlying population stratifications not reflected by the other confounders. The four sets of summary statistics were used as input to PRSice2 along with the target samples in an iterative PRS derivation procedure, as described in Materials and Methods. To evaluate the performance of each summary statistics estimation method, we used the PRS R^2 metric returned by PRSice2, which measures percentage of BMI variability explained by the PRS in the regression models. The PRS R^2 values for each method were averaged over 100 PRS derivation iterations and the method that yielded the highest PRS R^2 was selected to provide the summary statistics for final PRS derivation. In our case, SNPTEST yielded the highest average PRS R^2 (0.012 ± 0.006, pmin = 0.0002, pmedian = 0.0375, pmax = 0.3194), followed by GLM (0.011 ± 0.006, pmin = 0.0003, pmedian = 0.0697, pmax = 0.4251) and statgenGWAS (0.010 ± 0.006, pmin = 0.0005, pmedian = 0.0718, pmax = 0.3579). PLINK yielded the lowest average PRS R2 values but with the smallest variability across 100 iterations (0.009 ± 0.004, pmin = 0.0002, pmedian = 0.0802, pmax = 0.5282).

After completion of the 100 PRS derivation iterations, we assessed the stability of the extracted PRSs. In our case, PRS extraction process was highly dependent on source (training) dataset summary statistics. As a result, the SNP content of each PRS greatly varied between iterations, therefore affecting the performance of the latter and its contribution in explaining BMI. In order to mitigate the observed PRS instability, the 100 different SNP sets comprising the 100 different PRSs returned by PRSice2 with SNPTEST summary statistics were aggregated requiring that an SNP considered for inclusion in a PRS candidate should appear at least five times in the end of the iterative procedure. Subsequently, several PRS candidates were assembled with SNP content based on frequency of appearance of the latter across the aggregated SNP set, new regression models were created based on the initial target dataset splits used by PRSice2 and PRS R^2 values were assembled along with their respective significance when compared with the baseline PRSice2 PRS R^2 distribution. As our goals included derivation of a PRS with a less extended number of SNPs but of high predictive value, the new PRS R^2 values were further penalized based on the number of SNPs that each PRS candidate included. Then, using the resulting distribution of penalized PRS R^2 values, we detected local maxima, denoting both high predictive value and lower SNP content. The number of SNPs yielding an adequately high penalized PRS R^2 while maintaining significance when compared to the baseline PRS

R^2 distribution was found to be 343 (PRS R^2 = 0.1156 ± 0.0277). Notably, our iterative and aggregative PRS derivation process resulted in a PRS with ~10 times improved explanatory power (bootstrap p-value = 0) than using PRSice2 alone.

Next, we further evaluated the final 343-SNPs-selected PRS for BMI using the total merged dataset coupled with an iterative 10-fold cross-validation process, where, in each iteration of the process, we left out 5–50% of the total dataset samples, each time increasing the left-out samples by 5% and creating regression models including (full) and excluding (reduced) the PRS while maintaining the other covariates. Overall, the PRS increased the predictive power of the models by 31–33%, with the minimum PRS R^2 value observed at 0.3159 ± 0.0190 (p-value = 4×10^{-87}) when leaving out 50 of samples, with the maximum value at 0.3279 ± 0.0114 (p-value = 9×10^{-130}). A final regression model using the 343-SNP PRS for BMI with the total merged dataset yielded a PRS R^2 = 0.3241 (beta = 1.011, p-value = 4×10^{-193}). Finally, to evaluate the ability of the 343-SNP PRS to characterize close phenotypes, we created a regression model with the same covariates but using population weight instead of BMI. The model yielded PRS R^2 = 0.2313 (beta = 2.702, p-value = 4.15×10^{-158}).

The aforementioned 343-SNP PRS deriving from using SNPTEST displayed a statistically significant association for BMI (beta = 1.011, p-value = $4x10^{-193}$) and a positive correlation, where increased PRS values were associated with increased BMI levels. The examined population presented an overall median risk, with most observations met in the 0.25–0.50 range. Out of the 343 SNPs identified in the PRS (see Supplementary Table S2), automatically identified known associations included in the GWAS Catalog were displayed for 16 SNPs, namely rs2710804 (27 associations) and rs2955742 (five associations) (see Table 58).

Table 58. List of PRS SNPs with known associated traits in GWAS Catalog.

Consortial summary statistics (GWAS Catalog)					Known associated traits		Unified Cohort Summary Statistics	
SNP	Nearest gene	Position (Chr:bp)	Alleles	MAF	Effect Allele	Associated Traits	Effect allele	Beta *
rs11668205	IZUMO4	19:2096429-2099593	G/A	0.09 (A)	N/A	Abnormality of chromosome segregation	G	−0.32575
rs488248	LOC728192	13:105944370	C/A/T	0.23 (C)	T	Antineoplastic agent	C	−0.17048
rs480039	SLC35F3	1:234290732	G/A/C/T	0.37 (A)	N/A	Gut microbiome measurement	G	−0.17361
rs2288061	RPL18P13	16:76135833	G/A/C	0.34 (A)	G	Delta-5 desaturase measurement	G	−0.17776
rs2807854	HLX-AS1	1:220856499	T/C/G	0.25 (T)	T	LDL, apoB measurements	T	−0.13816
rs2955742	TMEM266	15:76153791	G/A	0.10 (A)	A	Serum urea, cystatin c, creatinine, urate, glomerular filtration measurement	G	−0.19108
rs2710804	SEPT7,EEPD1	7:36044919	T/C	0.23 (C)	#N/A	Fibrinogen measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Serum alanine aminotransferase measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	KIAA1706	7:36044919	T/C	0.23 (C)	C	C-reactive protein measurement	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Leukocyte count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Myeloid white cell count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	N/A	Leukocyte count	T	−0.1356

rs2710804	SEPT7, EEPD1	7:36044919	T/C	0.23 (C)	N/A	Fibrinogen measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	T	Platelet count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Leukocyte count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Serum albumin measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	C-reactive protein measurement	T	−0.1356
rs2710804	EEPD1	7:36044919	T/C	0.23 (C)	C	Fibrinogen measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2710804	LOC101928618	7:36044919	T/C	0.23 (C)	T	Serum alanine aminotransferase measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Myeloid white cell count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Platelet crit	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2251188	ZNF12, ZNF316	7:6664701	A/C/G/T	0.16 (A)	G	Basophil count, neutrophil count	A	0.13807
rs7589592	ENSG00000237720	2:2709171	T/A/C	0.41 (C)	N/A	Diffuse plaque measurement	T	0.11391
rs1010304	CHD6, EMILIN3	20:41473007	A/G	0.30 (G)	A	Memory performance, word list delayed recall measurement	A	−0.28657
rs12673506	CHN2	7:29382170	G/A	0.24 (A)	A	Gut microbiome measurement	G	−0.185
rs17662327	HNRNPA1P41,JAK2	9:4967587	T/C/G	0.16 (C)	T	Wellbeing measurement	T	0.14714

rs2485662	MEX3A/LMNA	1:156113677	T/C	0.31 (T)	N/A	Triacylglycerol 48:1, triacylglycerol 50:2 measurements	T	0.11601
rs4718965	AUTS2	7:70575462	C/A/T	0.08 (C)	C	Cortical surface area measurement	C	0.19049
rs9847987	intergenic/CFAP20DC-DT	3:59432807	C/T	0.20 (T)	T	Neuritic plaque measurement	C	0.26274
rs10252228	DPY19L1, NPSR1	7:34900427	A/G	0.29 (G)	G	Exercise	A	0.12063

SNP: single nucleotide polymorphism; Chr: chromosome; bp: base pairs; MAF: minor allele frequency; beta: effect size for BMI

*Results were derived via linear regressions after adjusting for sex, age, NAFLD status and number automatically selected PCs for population stratifications. Effect sizes (betas) and ORs shown for the corresponding SNP and effect sizes (betas) are reported for the respective effect allele.

3.3.3. Discussion

The present study sought to investigate application of an automated pipeline for PRS extraction using data from the three Greek studies of NAFLD, OSTEOS and THISEAS. In this population of Greek adults, the constructed PRS displayed a statistically significant association for BMI, with an R2 of 0.3241 (beta = 1.011, p-value = 4 × 10−193). The iterative pipeline presented here attempts to address various matters on PRS extraction, namely selection of an appropriate threshold for SNP inclusion and prediction accuracy [452] as well as stability of the SNP content of PRS candidates across different training and test dataset splits. In attempting to strengthen PRS construction methodology [453], this pipeline proposes implementation of iterative processes through repetitive steps of sample splitting, aggregating SNP frequency and effect size as well as comparative use of summary statistic metrics and consideration of lifestyle and genetic covariates. As a result, the suggested PRS includes a less extended number of variants but of high explanatory power. In this spectrum, this effort aims at facilitating construction of high-validity PRSs and subsequently promoting their use as a diagnostic tool accounting for various individual characteristics in daily practice. Use of the information of increased or reduced genetic risk for elevated BMI values, as demonstrated by the PRS, can potentially be translated in clinical practice to intensify (in the case of increased risk) or modify and personalize recommendations on lifestyle parameters to combat overweight and obesity. To the best of our knowledge, the present study constitutes the first attempt to develop a PRS for BMI using data from a Greek population and a previous attempt for construction of a PRS has only been referred to once before in the current literature, exploring Parkinson's disease in older Greek adults [454]. Implementation of the suggested aggregated methodology refers, among others, to (a) repetitive splitting of the overall sample; (b) comparative use of different summary statistics in an attempt to reduce population size and SNP selection bias, respectively. Thus, future work will concern attempts in replicating the proposed PRS in wider populations of different ancestry. Other attempts to create PRSs for BMI in populations of European ancestry are extensively described in the current literature, with an overall number of 56 BMI-related entries in the PGS Catalog [165]. All referred entries include parts of populations of European ancestry but present a wide range in the numbers of PRS-included variants, from a few tens up to several thousand or millions, with these numbers possibly limiting their effective usage in research or clinical settings. Although the PRS proposed here includes only 343 SNPs, the yielded R2 of 0.3241 is substantially comparable, and, in some cases, higher, than the ones presented in other PRSs from BMI, which include thousands of SNPs [165]. An overall advantage is also observed when comparing the present results to other attempts in European populations, which have a priori calculated the effect of literature-based PRSs using a limited number of SNPs. Use of our proposed pipeline is an advanced tool due to the notion that the aggregated approach of splitting processes strengthens identification of appropriate and sometimes novel SNPs increases the validity of the results and makes up for the need to have a very large sample size. In the current study, we observe links for various indices related to cardiovascular profile for twelve out of the sixteen variants with GWAS-Catalog-identified associations. The latter could be explained by inclusion of data for THISEAS participants with diagnosed cardiovascular disease (19.58% of the participants). Although the mediating effect of

BMI is usually accounted for when investigating the effect of genetic or polygenic risk scores on indices of cardiovascular disease, the reciprocal relation between variation in cardiometabolic indices levels and BMI levels has not been extensively demonstrated through BMI-PRS-included, CVD-related variants. Out of the associated SNPs, the C allele of the rs2710804-included variant presents the majority of reported associations, namely with cell count types (platelets, leukocytes, lymphocytes) and even measurements of C-reactive protein. In this context, the negative effect of the T allele observed in our study (β = −0.1356) could denote a positive relation of the C allele with metabolic pathways of inflammation and disturbed immunological responses in the subsequent increasing effect of BMI values. Interestingly and among this PRS's novel associations, we find two variants previously linked to gut microbiome measurements in populations of European ancestry. More specifically, Rühlemann MC et al. previously associated the rs480039 SNP with a 0.082571946 unit increase in P_Bacteroidetes abundance among German individuals [455]. Similarly, a 0.1019 unit increase in the abundance of parabacteroides in stools of individuals of Finnish ancestry for the A allele of the rs12673506 SNP was shown by Qin et al. [456]. Comparably, our study showed that the G allele of the rs480039 and rs12673506 variants was negatively related to BMI levels (β = −0.1736 and β = −0.1850, respectively). This is not the first time that the Parabacteroides genus has been linked to body weight. The majority of studies denote a higher Firmicutes:Bacteroidetes ratio and a generalized reduction in species variation in individuals with increased body weight or obesity [457], and different studies have found positive associations between genus and normal weight or weight loss in mice, as well as fat loss in humans [456-462]. It is plausible that the corresponding SNPs are further linked to BMI through the genus's role in gut production of bile acids and succinate, which have, in turn, been associated with reduction in body weight [461]. When referring to SNPs related to lifestyle, our suggested PRS included one variant related to well-being (variant rs17662327) and one variant associated with exercise (rs10252228). More specifically, in our sample, presence of the T allele of the former SNP was linked to a 0.1471 change in BMI levels. Previously, Okbay et al. demonstrated a 0.0182 unit increase in sentiment of life satisfaction or emotional well-being of adults for the T allele [462]. Our study further showed that presence of the A allele of the rs10252228 SNP was related to higher BMI values (β = 0.1206). This finding could be in accordance with the 0.027 unit increase in exercise associated with leisure time shown for the SNP's G allele in Japanese adults [463], meaning that the positive effect of the A allele on BMI could be mediated by individuals' low exercise levels. One of the great strengths of the present study entails implementation of our novel methodology for extraction of PRS, which enables effective management and analysis of the vast amounts of genetic data required for such analyses. The automated pipeline enables practical application of our suggested holistic approach for extensive examination of thousands of SNPs, leading to identification of various novel associations. Through the methodological approach of applying a repetitive process of continuous adjustment of the R 2 measure for the number of each-time-associated SNPs, the pipeline aims to facilitate integration of PRS use in daily healthcare practice, for example as part of widely distributed consumer reports. It should be stressed that, as this methodology is based on the highest R2 values of the aggregate PRS candidates, it ensures high explanatory power of the reduced signature. At the same time, it mitigates any

computational and data management burden imposed by PRSs with large (up to millions) numbers of SNPs. Limitations of the present study mainly concern power given the restrained participant sample size available for conducting analyses. Another limitation refers to use of a unified database of participants from three different studies. It is possible that variation in participant characteristics and bias accompanying use of a large analogic sample size of participants with cardiovascular disease played a considerable part in identifying associations between BMI and SNPs related to regulation of cardiovascular indices. However, we determined that much of the potential variability introduced by the fact of joining three databases was successfully captured by one of the PCs incorporated in the model. In addition, although the hypothesized pathways through which the identified SNPs potentially affect BMI levels provide insight for novel relations, there is little evidence to establish direct causal relationships. However, the present analysis sets a foundation for the suggested causal SNPs, and further research is also needed to explore the possibility of relations through their role as proxies for different associated variants.

4. Conclusive Remarks

The present thesis sought to investigate the effects of genetic and lifestyle determinants on obesity-related characteristics and the modification of corresponding, cardiometabolic risk factors. The aims of the nodes of this Dissertation concerned the conduct of the first-ever dietary intervention examining the effects of different-macronutrient-content-hypocaloric diets in adults with overweight or obesity; the examination of genetic and lifestyle factors affecting adolescent health and cardiometabolic status; and the overall assessment of genetic and gene-diet influences in anthropometric and obesity-related traits and modifications.

The iMPROVE study provides results in line with the ones of similar attempts conducted in European and international projects. The study yielded significant reductions in body weight and body composition indices after an intervention period of 3 months and showed no differentiation between the responses of adults following a low-fat, high-carbohydrate or a high-protein group. What's more, the study showed multiple nominal associations between genetic variants and anthropometric or lifestyle factors, denoting significant effects of genetic makeup on the modification of related indices. More specifically, genetic predisposition can affect changes in weight or quality of life, but populations of bigger sample size are needed to allow for more elaborate conclusions to be drawn.

Furthermore, dietary habits appear to influence adolescent health and cardiometabolic status, with more balanced regimens associated with better glycemic and lipidemic profile. Gene-diet interactions in this crucial-for-development life stage show significant influences in the modifications of obesity-related traits, with VEGF-A variants yielding significant interactions with teenage profiles for the first time. Lastly, in line with international consortia, examining the genetic effect in the form of a constructed PRS appears to also provide significant results in the Greek population, with a newly constructed PRS accounting for a percentage of its BMI variance.

Strengths of the present thesis are summarized in its attempts to respond to previously untreated research questions and fill existing gaps in current literature. This Dissertation attempts to treat a wide range of research questions in an effort to holistically understand and assess the effect of genetic makeup, lifestyle, as well as their respective interplay in factors of cardiometabolic nature. In this way, and building on key-findings in the field of nutrigenetics, the present study aims at providing new and somewhat innovative findings, even in this period of vast scientific achievement in the field. This study yielded results for multiple initiatives taking place for the first time in the examined populations, such as the dietary intervention in the Greek adults, the comparative analyses in the adolescent populations, the examination of the role of VEGF-A on adolescent parameters and the creation of the PRS for adult BMI. Nevertheless, the study presents significant limitations, the central one concerning the limited sample size of the iMPROVE population; a consequence mainly attributed to its timeframe coinciding with the COVID-19 pandemic and consecutive quarantines.

In addressing the effect of gene and lifestyle factors on obesity characteristics, the present thesis lays the ground for further research in the field, building and elaborating on its findings. Future directions can concern: i) the investigation of other types of hypocaloric diets in the observed weight loss of Greek adults and subsequent examination of gene-diet interactions; ii) potential attempts in replicating the

constructed GRSs in other populations of different characteristics; and iii) conduct of further GWAS in the populations of the TEENAGE and STANISLAS cohorts. Assessment of the holistic interplay of gene-lifestyle interactions is of vital importance for the in-depth understanding of nutrigenetic influences in cardiometabolic modifications, as well as in the general context of promoting and maintaining a good health status. In the future, integration of nutrigenetic information in obesity-prevention and treatment strategies might prove greatly beneficial in successful policy-making and tackling of all sorts of coinciding NCDs.

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Appendix A: Scientific Publications Related to Thesis Introduction

Review article

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Polygenic risk scores and personalized approaches to cardiometabolic disease prevention and treatment: A short review

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ABSTRACT

Accounting for the role of genetic variants in disease is increasingly gaining ground as a major contributing factor to the maximization of successful precision medicine and personalized nutrition approaches. An aggregated technique to quantifying genetic effect refers to the development and use of disease-specific Polygenic Risk Scores (PRSs) deriving from the sum of the weighted effects of multiple disease-related Single Nucleotide Polymorphisms (SNPs), mainly from Genome-Wide association studies (GWAS). Integration of PRS use in medical and nutritional practice is largely discussed in current literature, with special attention to: i) disease prediction accuracy after PRS consideration and their potential utility; ii) the role of current methodological approaches used to derive reliable results and the effect of limitations such as ancestry or population size; iii) the familiarization of healthcare professionals with the meaning of genetic information; and iv) the context-based interpretations of PRS results in the formation of personalized advice. In this context, the present short review aims to summarize current findings on PRS use and utility in cardiometabolic, weight-related disorders and discuss future directions for their potential integration in the practice of personalized nutrition.

KEY WORDS: *Polygenic risk scores, cardiometabolic disease, weight management, personalized nutrition*

INTRODUCTION

Deciphering disease etiology by quantifying the impact of genetic predisposition constitutes the focal point in the conduct of research surrounding genetics during the last years. Identifying and investigating the effect of disease-associated single nucleotide polymorphisms (SNPs), as well as using them to create aggravated genetic scores, provided encouraging results in the field of cardiovascular (CVD) and cardiometabolic disease[1,2]. Those findings shed a quantifiable light on the role of genetic makeup while expanding the horizons for the potential creation of new and personalized treatment approaches. The construction of polygenic risk scores (PRSs) thus quickly expanded to the notion of potentially contributing to determining

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disease risk and subsequently contributing to effective disease prevention, diagnosis and even treatment[1-3]. The need for more extensive research resulted in the gradual evolution of continuously enhanced methodological approaches for PRS extraction[4]. As the latter examine the effect of multiple variants on the outcome of interest based on a large SNP pool in populations of increased size, their creation and use were extensively investigated through genome-wide association studies (GWAS) in large consortia. The increasing presence of PRSs for multiple phenotypes in the current literature ultimately led to the creation of PGS catalog, an inclusive database comprising of all PRS entries created to date[5].

Discussion and research around PRS use as a prediction and treatment tool has recently yielded encouraging results, with studies reporting beneficial effects in cardiovascular and cardiometabolic disease[1,2]. Provision of lifestyle recommendations appeared to significantly contribute to obesity treatment[2] and coronary artery disease (CAD) prediction and greatly benefit individuals with high PRS across the spectrum of CVD, with PRSs constructed even for stroke and hypertension[1,3]. In like manner, the American Heart Association recently focused on the potential utility of PRS in CVD and other cardiometabolic disorders such as type 2 diabetes (T2D), underlining the need for the conduct of additional research to strengthen PRS inclusion in current practice[2]. Subsequently, discussion around the integration capacity of PRSs as a way to promote precision medicine and personalized nutrition is ongoing, with special attention on ameliorating relevant challenges, namely the differentiational influencing capacity following interaction with environmental stimuli, the diverse methodological approaches in PRS extraction and the understanding of the true meaning of genetic information both from professionals and patients alike.

PRS and weight-related parameters

Evaluation of genetic risk in the form of summed risk scores primarily treated CVD danger but quickly expanded to other disorders of cardiometabolic profile[2]. The conduct of extensive GWAS was accompanied by the development and expansion of the Genetic Investigation of Anthropometric Traits (GIANT) consortium[6]. This led to the identification of multiple Body Mass Index (BMI)-associated loci with the milestone discovery of the first 97 loci accounting for about 2.8% of the marker's variation[7]. Nowadays, approximately 6% of BMI variance is explained by 785 near-independent genome-wide significant SNPs[8,9]. Thus, the beginning approaches of quantifying genetic predisposition mainly involved the literature-based, a priori selection of disease-related variants and the subsequent investigation of the impact of their added effects. Therefore, various genetic risk scores of tens of SNPs were created and used in the examination of associations between increased genetic risk and disease manifestation or severity. In like manner, research on personalized approaches for combatting cardiometabolic and weight-related disorders primarily focused on examining the combined effect of target SNPs with different dietary regimens. In this context, the first large initiatives such as the FOOD4ME project and the POUNDS lost clinical trial[10,11], attempted to unveil the interactive role of genetic makeup and nutritional habits in overweight and obesity. Focusing on target SNPs and macronutrient content, the projects provided limited, but encouraging, evidence on the effect of gene-diet interactions on anthropometric traits.

Based on GIANT-derived information or the conduct of independent GWAS, different teams proceeded to the development of PRSs for BMI in populations of various sizes. To date, PRSs associated to anthropometric traits and body measurements account for 154 of the database entries[5]. Indeed, nowadays, attempting to decipher the multifactorial obesity etiology using genetic information has become central in research surrounding BMI, with efforts made to explain the polygenic prediction of weight formation throughout the life course[12,13] Khera et al. highlighted the role of including a multi-variant PRS in explaining weight variance in populations ranging from birth cohorts to middle-aged individuals[12]. Correspondingly, Shi et al recently constructed a different BMI PRS to investigate potential associations with overall cardiometabolic health from early age to adulthood. The study revealed significant associations between the score and other indices of cardiometabolic profile, namely fasting glucose and systolic blood pressure[13]. Building on the data and the role of genetic makeup in overweight or obesity presence, current research also focuses on the potential influence of genetic markers on weight loss. A study by de Toro-Martín investigating the extent of the genetic effect on the success of bariatric surgery, showed an increase in the prediction model accuracy when including PRSs, as well as significant interactions between the scores and the reduction in post-surgery recovery and surgery type[14]. In the same context, Katsareli et al showed that adults with increased genetic risk score for obesity noted a decrease in post-bariatric surgery loss of excess weight, with each unit of the score being associated with a 4.618% decrease in the 12-month observed weight loss[15].

In the same spectrum and building on the findings of previous key projects, emphasis should also be given on studies looking into the potential interactions between genetic scores and macronutrient content[16]. Moreover,

studies focusing on the genetic influence on the observed weight loss after lifestyle interventions to combat overweight and obesity even outside of a clinical environment are also needed. Research on this field could unravel the gene-diet interactions surrounding weight management and loss and ultimately maximize the impact of individualized recommendations using genetic data to determine optimal treatment strategies. As a result, effectively unravelling the genetic proportion of body weight variance could progressively allow for the formation of more inclusive strategies to its management.

PRS Interactions with Lifestyle Determinants

In addition to accounting for the risk attributed to genetic makeup, the impact of PRS interactions with lifestyle factors such as diet, ultimately influencing weight management have also been studied. In a 2021 study by Wang et al, a 60-SNP PRS was constructed using variants found to be associated with birth weight and later-life disease. The interactions between the genetic score and dietary parameters showed that healthy habits during early life, such as breastfeeding, were beneficial in reducing the risk for worse lipidemic profile in adult life in participants with higher genetic risk[17]. The significant modifying effect of diet was also demonstrated by Tan et al, who showed that individuals with higher PRS for obesity indeed presented higher levels of C-reactive protein but those levels appeared reduced in the presence of high dietary protein intake[18]. Similarly, middle-aged individuals with a higher genetic risk score for thinness presented lower body weight; an association aggravated with high protein and low carbohydrate intake, among others[19]. The multidisciplinary character of genetic risk-associated interactions is evident throughout the reciprocal interplay between the formation of anthropometric characteristics' levels and the formation of the lifestyle choices surrounding them. In adult populations, Dashti et al. showed that adults with higher genetic risk for obesity were less likely to make healthier food choices at workplace and more likely to purchase more food and adhere to unhealthy dietary habits such as delaying or skipping breakfast and homemade meals[20]. However, Lee et al showed that BMI PRSs were related to body weight in Korean adults, but not to their respective caloric or macronutrient intake[21]. Similarly, Konttinen et al highlighted that elevated genetic risk was more correlated with increased weight gain during a 7-year period in individuals not demonstrating restrained eating than those who adhered to it. However, the study attributed the effect to the role of previous processes entailing weight gain and nutritional habits, rather than a separate factor which will influence future weight gain[22]. Extended associations have also been explored, with Park et al showing that individuals with a high genetic risk for BMI, early menarche and attrition to an unhealthy diet (i.e. high consumption of fried foods and low consumption of fruits and vegetables) presented an increased obesity risk compared to those with late menarche and attrition to a healthier diet[23]. A different study focusing on European children and adolescents, underlined the modifying effect of diet, where genetic influence was attenuated by fiber intake in participants presenting higher genetic risk for obesity[24].

To boot, PRS-lifestyle interactions constitute a focal point across the spectrum of understanding more weight-related diseases. The emphatic effect of nutrition is underlined in studies of approximately 70000 participants of the UK Biobank, where adherence to a healthier diet was associated with reduced risk for cardiovascular disease, even in individuals with a high genetic risk score. Similarly, adoption of a healthier lifestyle was linked to lower CVD risk and overall mortality, again irrespective of genetic danger[25,26]. Moreover a different large study with data for almost 340000 UK Biobank participants showed that increased genetic risk for type 2 diabetes (T2D) was associated with higher chances for CVD manifestation; an effect reduced in individuals with better quality of lifestyle[27]. With regards to T2D alone, increased values of a PRS for the disease and attrition to the Western dietary pattern were associated with higher levels of fasting glucose[28]. Likewise, López-Portillo et al demonstrated that fasting glucose levels were higher in non-diabetic individuals with increased genetic risk for T2D and higher consumption of sugary beverages, compared to those with lower genetic risk scores and reduced intakes of the latter[29]. Biochemical interactions have also been studied, where PRS for T2D have been found to significantly interact with triglyceride and cholesterol levels in the subsequent formation of fasting glucose levels[30]. Merino et al showed the dominating effect of unhealthy diet in increasing T2D risk even by 30%, again irrespective of genetic risk[31]. Additionally, although Zhang et al did not show significant interactions between genetic risk and adherence to the plant-forward EAT-Lancet diet for T2D onset, their study did note that individuals with increased genetic risk and lower attrition to the dietary pattern did present the highest risk for T2D presence during a 24-year follow-up period[32]. Correspondingly, PRS-diet interactions have been evident in more disorders, such as cancer and dementia, where an increased diet quality lower the

chances for disease onset, even in individuals of high genetic risk[33-35]. In a similar context, lifestyle can also indirectly affect the gravity of genetic risk on actual disease manifestation via increase in weight-related anthropometric measurements alone. Esteve-Luque et al showed that higher values of BMI significantly interacted with genetic risk in increasing triglyceride levels and the subsequent risk for hypertriglyceridemia[36]. A different study underlined that obesity presence led to higher risk for T2D, even in individuals with lower genetic risk and better lifestyle quality[37].

PRS Utility in Personalized Recommendations

Research around the potential role of PRS use in clinical practice has shown that inclusion of PRSs in models for cardiometabolic disorders such as cardiovascular disease (CVD) can account for risk prediction in a manner similar to established contributing factors such as cholesterol levels[38-40]. The Task Force of the International Common Disease Alliance has further underlined the importance of PRS inclusion in increasing the accuracy of predicting CVD disease risk and severity, throughout one's lifetime[41], and the weighted contribution of PRS to maximizing patient outcomes[41]. Given the potential increase in accuracy observed in prediction models after the addition of PRS, testing their potential utility has also expanded to the field of anthropometrics. Choe et al showed that a BMI PRS was associated not only with longitudinal BMI change, but also other cardiometabolic phenotypes, such as fatty liver[42]. A similar attempt was made by Padilla-Martinez et al., who displayed significant associations between PRSs for T2D and obesity and manifestations of prediabetes and other disrupted cardiometabolic parameters[43].

In this context, PRS use could be seen as a useful tool to increase disease prevention through successful prediction and/or early detection. This notion carries both favorable effects for public health and financial parameters of healthcare systems, as well as optimizing individual understanding and ability to choose and decide optimal combatting strategies[44]. Although the inclusion of PRSs and relevant interactions can explain cardiometabolic disease risk[45], the conversation around its clinical validity underlines the importance of real-time context on PRS information evaluation and decision-making in order to avoid confusion with genetic determinism[40,41]. This sheds a light on the vital role of both development of valid methodologies to increase PRS reliability, transferability and accuracy, as well as the professionals' familiarization with the interpretation of its information. This is also why the education of healthcare professionals is put in the center of integrating genetic information into daily practice.

Furthermore, taking PRS information into account can prove beneficial on its own accord in patients with extremely high genetic risk[41] and, thus, PRS utility is also discussed at personal level[38]. PRS information can be differentially valuable to each individual, according to both their personal interest and understanding of the information, as well as relevant genetic risk in outcomes of interest. The latter might not always correlate to matters of clinical importance, but do account for increasing awareness on genetic predisposition for various matters significant to the individual. It is therefore why, a reliable approach to PRS calculation for various traits, with easily understandable and interpretable results is central in future research surrounding PRS use[38]. Especially in cases regarding cardiometabolic disorders such as overweight, obesity and type 2 diabetes, finding ways to efficiently include PRS prediction in easily applicable risk tools is considered a priority for the maximization of PRS efficacy.

Challenges in PRS Construction and Interpretation

Although inclusion of PRSs in disease prognosis can be beneficial, several considerations arise when discussing the methodological aspect of PRS construction, the efficacy of the various PRS development methodologies presented in current literature and the real-time interpretation capacity in clinical and non-clinical settings. Firstly, the fundamental limitation of PRS' universal application concerns the underrepresentation of data used from populations of different genetic ancestry[44]. To date, although several attempts for PRS construction using data from various populations have been made, PRSs presented in literature mainly focus on European ancestry. The lack of existent PRSs deriving from large cohorts of global populations affects their translational capacity in less frequently examined populations where contextually phenotype-associated variants, SNP linkage disequilibrium (LD) or allele frequency may vary. Therefore, a preceding necessity of developing more PRSs using data from populations around the globe is formed before discussing their maximum use, in order to ensure universal application capacity.

Another pillar of PRS development refers to the biases of the different methodological approaches undertaken in calculating the scores[44]. Diverse current practices consist of: i) the replication of simple aggravations of the risk-alleles for phenotype-associated variants using their respective effect sizes from current literature (i.e. consortia such as the GIANT one or data from large studies such as

the UKBiobank[46] of the Twins Early Development Study -TEDS[47]) ; and ii) the conduct of novel GWAS in populations of sufficiently large sample sizes, extraction of summary statistics, subsequent identification of phenotype-associated variants and their risk alleles' aggravation in a holistic score. As PRS development and phenotype examinations are ongoing, research may simultaneously focus on the identification of novel phenotype-associated variants and the replication of previously identified ones. As a result, the statistical design and assessment may significantly differ across studies and the final choice for the optimal model to be used may lie in the discretion of the researcher according to the needs of the research question at hand. Additionally, differences in samples sizes significantly matter in effective PRS validation. Although the effect of using target-SNPs outside of reference populations can be limited, current discussion around the role of population size has shown that cohorts with a few thousands of participants can be of use in replicating results and using SNPs from PRSs deriving from even larger populations[48]. Moreover, the additional variety in statistical methods (i.e. p-value thresholds, clumping, Bayesian or lasso-based penalization), packages (eg PRScs, LDpred2) and assessment applied can largely affect the end product which may be ultimately differentiated across studies. It is therefore highlighted that standardization of the PRS extraction process[49] is central to facilitating their validation and sequentially increasing their predictive ability. Additionally, in this context, attempts to practically compare PRS results and methodology[4,50-52] can provide useful data for the next steps in the need for a unified, applicable approach to allow for PRSs capable of yielding rapid but reliable results and effective comparisons of findings between populations of different characteristics.

Moreover, familiarization with the true meaning deriving from the information of the PRS is vital in its correct interpretation. Understanding the potentially indirect effects of SNPs included in the models and weighing the environmental factor in are key considerations in constructing future PRSs as reliable disease prediction risk tools. Apart from the technical aspects, a different cornerstone of practical PRS use appertains to the familiarization of healthcare professionals with the field. Proper assuefaction with the practical meaning of PRS information is critical for professionals to address disease risk and convey the appropriate message to patients. The delicate understanding of individual risk and its practical meaning in ultimate disease manifestation can be challenging in cases where the risk is small or the patient is not properly acquainted with the details of their genetic profile. Both professional and patient education and perceptions around PRS utility are integral in its successful use as a disease screening and treatment tool[40,44].

PRS and Nutrigenetics/Nutrigenomics in Future Healthcare Practice

Although there is a limited number of studies investigating and discussing the extent of PRS effective translation to date, future directions can be encouraging on the incorporation of PRS methodologies in the daily practice[1-3]. PRS inclusion in disease screening and the formation of personalized recommendations could potentially offer a solution to the growing pressure applied to healthcare systems for more inclusive strategies and efficient use of financial resources[49]. In the field of nutrigenetics (i.e. the impact of SNPs on certain nutrient interaction or role in metabolic pathways) and nutrigenomics (i.e. the impact of nutrients on gene expression), PRS use can be considered as a promising tool in the advancement of personalized nutrition.

Understanding the connective links between research conduct and translation is substantial in order to be able to reinforce PRS practical use. An integral part to such an effort would be the effective translational communication between bioinformatics and healthcare sectors in order to enhance proper PRS use and interpretation[49]. Especially when referring to the use of PRSs in cardiometabolic and weight-related disorders, understanding, quantifying and translating the contribution of genetic predisposition is vital in interpreting genetic impact. Incorporating genetic information in medical and nutritional advice can maximize the success of the proposed strategies, while informing the individuals in main aspects of their genetic profile. In this spectrum, PRS interpretation in weight-related disorders can only be effective when conducted and evaluated alongside the effect of other lifestyle determinants (Figure 1). This can allow for increased motivation on behavioral change and lifestyle adaptations[41] to the proposed measures, which can subsequently strengthen the disorders' effective management.

In an attempt to dissect the steps of including genetic details in current practice and promote personalized nutrition, in 2022 the Academy of Nutrition and Dietetics published the creation of a *Nutrigenomics Care Map* specifying the timeline of nutrigenetic information integration in nutritional assessment[53]. The map puts professional formation on the forefront of the practice, by inserting the sufficient nutrigenomics training prerequisite as the first out of the four steps of the process. Patient screening, genetic testing and communication of genetic profiling results as part of the nutritional assessment and the setting

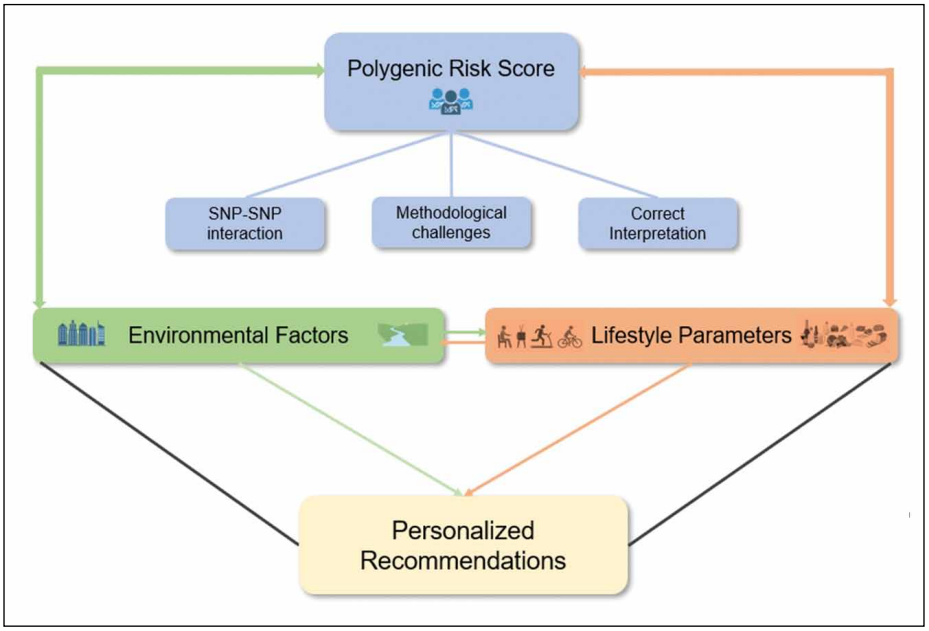

FIGURE 1. Polygenic Risk Score (PRS) in Personalized Recommendations (created with BioRender.com).

of SMART (specific, measurable, attainable, relevant and time-based) goals complete the suggested procedure[53]. Such an approach aims to maximize nutritional consulting by actively involving the patient in the formation of goals and dietary regimens optimally corresponding to their genetic profile. Integration of PRSs in this effort could allow the practice to move forward from personalized advice provided only based on specific genotypes of key genes associated to body weight or obesity[54,55]. As a result, more research in the form of Randomized Clinical Trials (RCTs) is needed, regarding the interactions between BMI PRSs and dietary regimens in order to establish the evidence-based approaches required for the nodes of individualized advice. Such efforts would subsequently enhance our understanding and forming of optimal recommendations, each-time targeting the outcome of interest and adopting the literature-based, corresponding strategy (eg advice on adherence to a dietary regimen of specific macronutrient content for the achievement of weight loss in individuals with specific PRS for obesity). Due to the current increase observed in the offer of nutrigenetic services, establishment of scientific, quality guidelines for directing healthcare professionals is vital[56].

Furthermore, on principle, the meaning of PRS information differentiates itself according to the nature of the disorder in reference. For example, a PRS will be differently interpreted in cases of monogenic rather than polygenic diseases, such as the cardiometabolic and weight-related ones. The multidisciplinary character of those disorders therefore reciprocally affects the creation of the appropriate framework in which it will be communicated. This interplay between genetic information communication and healthcare setting factors centrally affects both the formation and the influencing capacity of public health policies in precision medicine and nutrition[38-40]. The latter, thus, re-enforces the need for sectors simultaneously operating on unravelling the relations between the creation, interpretation and communication of genetic information across healthcare professionals. These could, in turn, be incorporated into screening tools for multiple traits and contribute to the creation of individualized disease prevention or treatment strategies.

CONCLUSIONS

Future incorporation of PRS information in the daily healthcare practice could present considerable advantages to advancing precision medicine and personalized nutrition. Creation of sound methodologies, accounting for the extent of the impact for environmental stimuli and simultaneously able to allow for the effective inclusion of PRS results in disease prediction, diagnosis and prognosis is deemed vital in bringing PRS research and application forward. PRS information on cardiometabolic and weight-related disorders can increase the prognostic validity of already existent tools and the fruitful formation and implementation of individualized recommendations. However, sufficient familiarization of healthcare professionals with the meaning and contextual translation of PRS results plays a major part in its proper communication where attention must be given in the role of the interac-

tions between SNPs, environment and lifestyle determinants in ultimate disease manifestation. Future initiatives should aim at uniformly enhancing both methodology development and educational formation in attempting to firmly establish, integrate and distribute PRS use as a daily practicum.

Conflict of Interest

The authors declare no conflict of interest

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ΠΕΡΙΛΗΨΗ

Πολυγονιδιακοί Δείκτες Κινδύνου και προσωποποιημένες προσεγγίσεις στην πρόληψη και την αντιμετώπιση καρδιομεταβολικών ασθενειών: Σύντομη Ανασκόπηση

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Η διερεύνηση του ρόλου των γενετικών παραλλαγών στην εμφάνιση ασθενειών χρησιμοποιείται ολοένα και περισσότερο με στόχο τη βελτιστοποίηση των επιτυχών προσεγγίσεων στα πεδία της ιατρικής ακριβείας και της προσωποποιημένης διατροφής. Μια συγκεντρωτική τεχνική για την ποσοτικοποίηση της γενετικής επίδρασης αφορά στην ανάπτυξη και τη χρήση ειδικών-για-κάθε-ασθένεια Πολυγονιδιακών Δεικτών Κινδύνου (ΠΔΚ), μέσω τους αθροίσματος της επίδρασης συσχετιζόμενων μονονουκλεοτιδικών πολυμορφισμών (ΜΝΠ) προερχόμενων από μελέτες σάρωσης του γονιδιώματος. Η σύγχρονη βιβλιογραφία πραγματεύεται εκτενώς την ενσωμάτωση της χρήσης των ΠΔΚ στην ιατρική και διαιτολογική πρακτική, με ιδιαίτερη έμφαση: i) στην προβλεπτική ακρίβεια εμφάνισης ασθενειών έπειτα από την ενσωμάτωση της πληροφορίας των ΠΔΚ σε αντίστοιχα μοντέλα, ii) στο ρόλο των σύγχρονων μεθοδολογικών προσεγγίσεων για την εξαγωγή αξιόπιστων αποτελεσμάτων και την αντίστοιχη επίδραση περιορισμών όπως η γενεαλογική καταγωγή και το μέγεθος του πληθυσμού αναφοράς, iii) στην εξοικείωση των επαγγλματιών υγείας με τη σημασία της γενετικής πληροφορίας και iv) στην κάθε φορά προσαρμοσμένη στα υπάρχοντα πλαίσια ερμηνεία των αποτελεσμάτων των ΠΔΚ στη δημιουργία προσωποποιημένων συστάσεων. Σε αυτό το πλαίσιο, η παρούσα σύντομη ανασκόπηση συνοψίζει τα σύγχρονα ευρύματα της βιβλιογραφίας σχετικά με τη χρήση και τη χρησιμότητα των ΠΔΚ σε καρδιομεταβολικές νόσους και νόσους που σχετίζονται με το σωματικό βάρος, καθώς και πραγματεύεται τις μελλοντικές κατευθύνσεις για την πιθανή ενσωμάτων των ΠΔΚ στην πρακτική της προσωποποιημένης διατροφής.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: *Πολυγονιδιακοί δείκτες κινδύνου, καρδιομεταβολικές ασθένειες, διαχείριση βάρους, προσωποποιημένη διατροφή*

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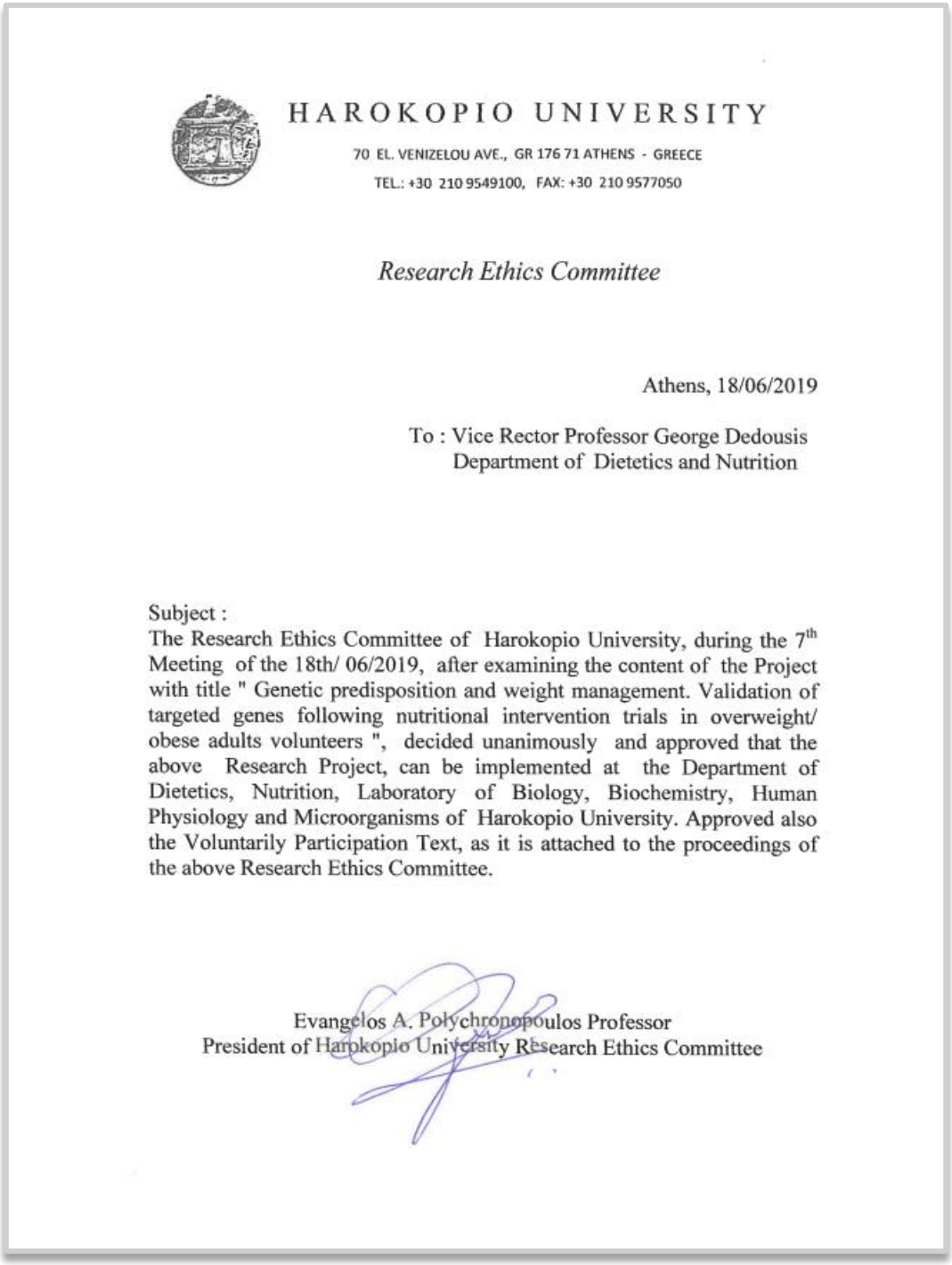
Appendix B: iMPROVE Study-related documents

Appendix B. iMPROVE Study-related documents

B1. Ethics Approval for the iMPROVE study.



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Research Ethics Committee

Athens, 18/06/2019

To : Vice Rector Professor George Dedousis
Department of Dietetics and Nutrition

Subject :
The Research Ethics Committee of Harokopio University, during the 7th Meeting of the 18th/ 06/2019, after examining the content of the Project with title " Genetic predisposition and weight management. Validation of targeted genes following nutritional intervention trials in overweight/ obese adults volunteers ", decided unanimously and approved that the above Research Project, can be implemented at the Department of Dietetics, Nutrition, Laboratory of Biology, Biochemistry, Human Physiology and Microorganisms of Harokopio University. Approved also the Voluntarily Participation Text, as it is attached to the proceedings of the above Research Ethics Committee.

Evangelos A. Polychronopoulos Professor
President of Harokopio University Research Ethics Committee

Figure 30. Conduct approval for the iMPROVE study by the Research Ethics Committee of Harokopio University of Athens.

B2. Original iMPROVE Study Questionnaires

How often do you consume:	Frequency of consumption (servings/week or otherwise stated)					
All cereals (bread, pasta, rice, etc.)	Never	1-6	7-12	13-18	19-31	> 32
	0	1	2	3	4	5
Potatoes	Never	1-4	5-8	9-12	13-18	> 18
	0	1	2	3	4	5
Fruits	Never	1-4	5-8	9-15	16-21	> 22
	0	1	2	3	4	5
Vegetables	Never	1-6	7-12	13-20	21-32	> 33
	0	1	2	3	4	5
Legumes	Never	< 1	1-2	3-4	5-6	> 6
	0	1	2	3	4	5
Fish	Never	< 1	1-2	3-4	5-6	> 6
	0	1	2	3	4	5
Red Meat Products	≤ 1	2-3	4-5	6-7	9-10	> 10
	5	4	3	2	1	0
Poultry	≤ 3	4-5	5-6	7-8	9-10	> 10
	5	4	3	2	1	0
Full fat dairy products (cheese, yogurt, milk)	≤ 10	11-15	16-20	21-28	29-30	> 30
	5	4	3	2	1	0
Use of Olive Oil (times/week)	Never	Rare	< 1	1-3	3-5	Daily
	0	1	2	3	4	5
Alcohol (ml/day)	0	< 300	400	500	600	> 700
	5	4	3	2	1	0

Figure 31. Mediterannean Diet Score Questionnaire by Panagiotakos et al [313]

Center for Epidemiologic Studies Short Depression Scale (CES-D-R 10)

Below is a list of some of the ways you may have felt or behaved.

Please indicate how often you have felt this way during the past week by checking the appropriate box for each question.

	Rarely or none of the time (less than 1 day)	**Some or a little of the time** (1-2 days)	**Occasionally or a moderate amount of time** (3-4 days)	**All of the time** (5-7 days)
1. I was bothered by things that usually don't bother me.	☐	☐	☐	☐
2. I had trouble keeping my mind on what I was doing.	☐	☐	☐	☐
3. I felt depressed.	☐	☐	☐	☐
4. I felt that everything I did was an effort.	☐	☐	☐	☐
5. I felt hopeful about the future.	☐	☐	☐	☐
6. I felt fearful.	☐	☐	☐	☐
7. My sleep was restless.	☐	☐	☐	☐
8. I was happy.	☐	☐	☐	☐
9. I felt lonely.	☐	☐	☐	☐
10. I could not "get going."	☐	☐	☐	☐

Figure 32. The Center for Epidemiologic Studies Short Depression Scale (CES-D-R-10) [314]

SF-12 Health Survey

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. **Answer each question by choosing just one answer**. If you are unsure how to answer a question, please give the best answer you can.

1. **In general, would you say your health is**:

☐1 Excellent ☐2 Very good ☐3 Good ☐4 Fair ☐5 Poor

The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	YES, limited a lot	YES, limited a little	NO, not limited at all
2. **Moderate activities** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf.	☐1	☐2	☐3
3. Climbing **several** flights of stairs.	☐1	☐2	☐3

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	YES	NO
4. **Accomplished less** than you would like.	☐1	☐2
5. Were limited in the **kind** of work or other activities.	☐1	☐2

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	YES	NO
6. **Accomplished less** than you would like.	☐1	☐2
7. Did work or activities **less carefully than usual**.	☐1	☐2

8. **During the past 4 weeks, how much did pain interfere with your normal work (including work outside the home and housework)?**

☐1 Not at all ☐2 A little bit ☐3 Moderately ☐4 Quite a bit ☐5 Extremely

These questions are about how you have been feeling during the past 4 weeks.
For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks…

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
9. Have you felt calm & peaceful?	☐1	☐2	☐3	☐4	☐5	☐6
10. Did you have a lot of energy?	☐1	☐2	☐3	☐4	☐5	☐6
11. Have you felt down-hearted and blue?	☐1	☐2	☐3	☐4	☐5	☐6

12. **During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?**

☐1 All of the time ☐2 Most of the time ☐3 Some of the time ☐4 A little of the time ☐5 None of the time

Patient name: Date: PCS: MCS:

Visit type (circle one)
Preop 6 week 3 month 6 month 12 month 24 month Other:_____

Figure 33. The 12-item Short Form Survey [SF-12] [315]

ATHENS INSOMNIA SCALE

This scale is intended to record your own assessment of any sleep difficulty you might have experienced. Please, check (by circling the appropriate number) the items below to indicate your estimate of any difficulty, provided that it occurred at least three times per week during the last month.

1. SLEEP INDUCTION *(time it takes you to fall asleep after turning-off the lights)*

0	1	2	3
No problem	Slightly delayed	Markedly delayed	Very delayed or did not sleep at all

2. AWAKENINGS DURING THE NIGHT

0	1	2	3
No problem	Minor problem	Considerable problem	Serious problem or did not sleep at all

3. FINAL AWAKENING EARLIER THAN DESIRED

0	1	2	3
Not earlier	A little earlier	Markedly earlier	Much earlier or did not sleep at all

4. TOTAL SLEEP DURATION

0	1	2	3
Sufficient	Slightly insufficient	Markedly insufficient	Very insufficient or did not sleep at all

5. OVERALL QUALITY OF SLEEP *(no matter how long you slept)*

0	1	2	3
Satisfactory	Slightly unsatisfactory	Markedly unsatisfactory	Very unsatisfactory or did not sleep at all

6. SENSE OF WELL-BEING DURING THE DAY

0	1	2	3
Normal	Slightly decreased	Markedly decreased	Very decreased

7. FUNCTIONING (PHYSICAL AND MENTAL) DURING THE DAY

0	1	2	3
Normal	Slightly decreased	Markedly decreased	Very decreased

8. SLEEPINESS DURING THE DAY

0	1	2	3
None	Mild	Considerable	Intense

Figure 34. The Athens Insomnia Scale (AIS) [316]

Ερωτηματολόγιο Συχνότητας Κατανάλωσης Τροφίμων:

Τρόφιμα και ροφήματα, μεγέθη μερίδων και συχνότητα κατανάλωσης

Φαγητό ή ρόφημα	Μέγεθος μερίδας αναφοράς	Συχνότητα Κατανάλωσης
ΓΑΛΑΚΤΟΚΟΜΙΚΑ		
Πλήρες γάλα/ γιαούρτι	1 κούπα (240ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Χαμηλό σε λίπος γάλα/γιαούρτι	1 κούπα (240ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Κίτρινο τυρί / κρεμώδες τυρί	30g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Λευκό τυρί (πχ τυρί φέτα)	30g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Χαμηλό σε λίπος τυρί (light/cottage)	30g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Αυγό (βραστό, τηγανητό, ομελέτα)	50g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΔΗΜΗΤΡΙΑΚΑ, ΑΜΥΛΟΥΧΑ ΤΡΟΦΙΜΑ		
Λευκό ψωμί / τοστ	1φέτα (30g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ολικής άλεσης ψωμί / φρυγανιά	1φέτα (30g), 2 τεμάχια	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Burger ψωμί	1τεμάχιο (60g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Κράκερς	2 λεπτά τεμάχια (20g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Δημητριακά/ μπάρες δημητριακών	1/2κούπα (20g), 1τεμάχιο	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ρύζι άσπρο	1κούπα	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ρύζι καστανό	1κούπα	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ζυμαρικά / αποφλοιωμένο κριθάρι	1κούπα	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ζυμαρικά ολικής άλεσης	1κούπα	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Πατάτες βραστές/ ψητές/ πουρές	1 μέτρια	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Τηγανητές πατάτες	½ μερίδα	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΚΡΕΑΣ		
Μοσχάρι (μπριζόλα, φιλέτο)	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μπιφτέκι/ κεφτεδάκια/ κιμάς	120g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Κοτόπουλο/γαλοπούλα (όλα τα είδη)	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Χοιρινό (μπριζόλα, φιλέτο)	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Αρνί/ κατσίκι/ κυνήγι/ παϊδάκια	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ

Αλλαντικά	1φέτα (30g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Λουκάνικο/ μπέικον	1 μέτριο τμχ., 2φέτες (30g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Τύπου light/ χωρίς λίπος αλλαντικά	30g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΨΑΡΙΑ		
Μικρά ψάρια	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μεγάλα ψάρια	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Θαλασσινά (χταπόδι, καλαμαράκια, γαρίδες)	150g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΟΣΠΡΙΑ, ΠΑΡΑΔΟΣΙΑΚΑ ΦΑΓΗΤΑ		
Όσπρια (φακές, φασόλια, ρεβίθια, φάβα)	1 μερίδα (300g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Σπανακόρυζο / λαχανόρυζο	1μερίδα (250g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Παστίτσιο/ μουσακάς/ παπουτσάκια	1μερίδα (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΛΑΧΑΝΙΚΑ		
Αρακάς, φασολάκια, μπάμιες, αγκινάρα	200g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ντομάτα, αγγούρι, καρότο, πιπεριά	100g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μαρούλι, λάχανο, σπανάκι, ρόκα (ωμά)	80g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μπρόκολο, κουνουπίδι, κολοκυθάκι	100g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Χόρτα, σέλινο, σπανάκι (βρ)	90g	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΦΡΟΥΤΑ, ΞΗΡΟΙ ΚΑΡΠΟΙ		
Πορτοκάλι	1μέτριο (170g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μήλο, αχλάδι	1 μέτριο (140g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Άλλα χειμερινά φρούτα	1 κομμάτι , ½ κούπα (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μπανάνα	1μέτρια (100g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Άλλα καλοκαιρινά φρούτα	1κομμάτι, ½ κούπα (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Χυμοί φρούτων	1 ποτήρι (240g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Αποξηραμένα φρούτα	¼ ποτηριού (35g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ξηροί Καρποί	1 κούπα του καφέ (50g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
SNACKS		
Σπιτικές πίτες (πχ. Τυρί, σπανάκι)	1 κομμάτι (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ

Πίτες (άλλες, εκτός σπιτιού)	1 κομμάτι (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Τοστ, σάντουιτς	1 κομμάτι (200g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΓΛΥΚΑ, ΑΛΜΥΡΑ SNACKS		
Γλυκά ταψιού	1 κομμάτι (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Γλυκά του κουταλιού, κομπόστα φρούτων, ζελέ φρούτων	1μερίδα (100g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Πάστα, τάρτα	1κομμάτι (150g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Κρουασάν, γκοφρέτα, κέικ, μπισκότα	1τεμάχιο, 1 κομμάτι, 3- 4τμχ	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Σοκολάτα	1μέτρια (60g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Παγωτό, milk-shake, κρέμα, ρυζόγαλο	1τμχ	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Πατατάκια, ποπ-κορν	1σακουλάκι (70g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μέλι, μαρμελάδα, ζάχαρη	1κγ (5g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ελιές	10 μικρές/ 5 μεγάλες	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΡΟΦΗΜΑΤΑ		
Κρασί	1 ποτήρι (125ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μπίρα	1 ποτήρι (240ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Άλλα αλκοολούχα ποτά	1 ποτήρι (30ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Αναψυκτικά	1 κουτάκι (330ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Αναψυκτικά light	1 κουτάκι (330ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Καφές	1 κούπα (240ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Τσάι / άλλα αφεψήματα	1 κούπα (240ml)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
ΛΙΠΗ		
Μαγιονέζα, σως	1κ.σ (15g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Light μαγιονέζα, light σως	1κ.σ (15g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Ελαιόλαδο	3κ.σ (45g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Σπορέλαιο	3κ.σ (45g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Μαργαρίνη	1κ.σ (15g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ
Βούτυρο	1κ.σ (15g)	Ποτέ/σπάνια, 1-3φ/μήνα, 1-2φ/εβδ, 3-6 φ/εβδ, 1φ/ημ, ≥ 2φ/ημ

φ/μήνα: φορές στο μήνα; φ/εβδ: φορές στην εβδομάδα; φ/ημ: φορές στην ημέρα; τμχ: τεμάχιο

Figure 35. 69-item FFQ n Greek [317]

Σύντομο Τηλεφωνικό IPAQ Τελευταίων 7 Ημερών

Πρόκειται να σας ρωτήσω σχετικά με το χρόνο που δαπανήσατε όντας σωματικά δραστήριος τις τελευταίες 7 ημέρες. Παρακαλώ απαντήστε κάθε ερώτηση ακόμα και αν δεν θεωρείτε τον εαυτό σας δραστήριο άτομο. Σκεφτείτε τις δραστηριότητες που πραγματοποιείτε στην εργασία σας, ως μέρος των εργασιών του σπιτιού και του κήπου, για να πάτε από το ένα μέρος στο άλλο, και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή αθλητισμό.

1. **Κατά τη διάρκεια των τελευταίων 7 ημερών, πόσες ημέρες κάνατε έντονες σωματικές δραστηριότητες;** [Οι έντονες δραστηριότητες μας κάνουν να αναπνέουμε πολύ πιο έντονα από το κανονικό και μπορεί να περιλαμβάνουν άρση βαρέων αντικειμένων, σκάψιμο, αεροβική άσκηση, ή γρήγορη ποδηλασία. Σκεφτείτε μόνο εκείνες τις σωματικές δραστηριότητες που πραγματοποιείτε για τουλάχιστον 10min κάθε φορά. Σε απάντηση μηδέν, άρνηση ή «δεν γνωρίζω», >> Ερώτηση 3]

   ___ Ημέρες ανά εβδομάδα [VDAY; Range 0-7, 8,9]

   8. Δεν γνωρίζω/Δεν είμαι σίγουρος

   9. Αρνήθηκε

2. **Πόσο χρόνο δαπανήσατε συνήθως κάνοντας έντονες σωματικές δραστηριότητες σε μία από αυτές τις ημέρες;** [Σκεφτείτε μόνο εκείνες τις σωματικές δραστηριότητες που πραγματοποιήσατε για τουλάχιστον 10min κάθε φορά.

   ___Ώρες ανά ημέρα [VDHRS; Range 0-16]

   ___ Λεπτά ανά ημέρα [VDMIN; Range 0-960, 998, 999]

   998. Δεν γνωρίζω/Δεν είμαι σίγουρος

   999. Αρνήθηκε

   [Αν ο ερωτώμενος δεν μπορεί να απαντήσει επειδή το μοτίβο του δαπανηθέντος χρόνου ποικίλει σημαντικά από μέρα σε μέρα]

   Πόσο χρόνο συνολικά δαπανήσατε τις τελευταίες 7 ημέρες κάνοντας έντονες σωματικές δραστηριότητες;

   ___ Ώρες ανά εβδομάδα [VWHRS; Range 0-112]

   ___ Λεπτά ανά εβδομάδα [VWMIN; Range 0-6720, 9998, 9999]

   9998. Δεν γνωρίζω/Δεν είμαι σίγουρος

   9999. Αρνήθηκε

3. **Κατά τη διάρκεια των τελευταίων 7 ημερών, πόσες ημέρες πραγματοποιήσατε μέτριες σωματικές δραστηριότητες;** [Οι μέτριες σωματικές δραστηριότητες μας κάνουν να αναπνέουμε κάπως πιο έντονα από το κανονικό και μπορεί να περιλαμβάνουν μεταφορά ελαφρών φορτίων, ποδηλασία σε κανονικό ρυθμό, ή διπλό τένις. Μην συμπεριλάβετε το περπάτημα. Και πάλι, σκεφτείτε μόνο εκείνες τις σωματικές δραστηριότητες που πραγματοποιήσατε για τουλάχιστον 10 λεπτά κάθε φορά. Σε απάντηση μηδέν, άρνηση ή «δεν γνωρίζω», >> Ερώτηση 5].

___ Ημέρες ανά εβδομάδα [MDAY; Range 0-7, 8,9]

8……. Δεν γνωρίζω/Δεν είμαι σίγουρος

9……. Αρνήθηκε

4. **Πόσο χρόνο δαπανήσατε συνήθως κάνοντας μέτριες σωματικές δραστηριότητες σε μία από αυτές τις ημέρες;**

___ Ώρες ανά ημέρα [MDHRS; Range 0-16]

___ Λεπτά ανά ημέρα [MDMIN; Range 0-960, 998, 999]

998. Δεν γνωρίζω/Δεν είμαι σίγουρος

999. Αρνήθηκε

[Αν ο ερωτώμενος δεν μπορεί να απαντήσει επειδή το μοτίβο του δαπανηθέντος χρόνου ποικίλει σημαντικά από μέρα σε μέρα]

Πόσο χρόνο συνολικά δαπανήσατε τις τελευταίες 7 ημέρες κάνοντας μέτριες σωματικές δραστηριότητες;

___ Ώρες ανά εβδομάδα [MWHRS; Range 0-112]

___ Λεπτά ανά εβδομάδα [MWMIN; Range 0-6720, 9998, 9999]

9998. Δεν γνωρίζω/Δεν είμαι σίγουρος

9999. Αρνήθηκε

5. **Κατά τη διάρκεια των τελευταίων 7 ημερών, πόσες ημέρες περπατήσατε για τουλάχιστον 10 λεπτά κάθε φορά;** [ο χρόνος αφορά σε αυτόν που δαπανήσατε περπατώντας σε εργασία και σπίτι, περπάτημα για μετακίνηση, άλλο περπάτημα που έχετε πραγματοποιήσει για ψυχαγωγία, άσκηση, ή στον ελεύθερο χρόνο. Σε απάντηση μηδέν, άρνηση ή «δεν γνωρίζω», >> Ερώτηση 7]

___ Ημέρες ανά εβδομάδα [WDAY; Range 0-7, 8,9]

8. Δεν γνωρίζω/Δεν είμαι σίγουρος

9. Αρνήθηκε

6. Πόσο χρόνο δαπανήσατε περπατώντας σε μία από αυτές τις ημέρες;

___ Ώρες ανά ημέρα [WDHRS; Range 0-16]

___ Λεπτά ανά ημέρα [WDMIN; Range 0-960, 998, 999]

998. Δεν γνωρίζω/Δεν είμαι σίγουρος

999. Αρνήθηκε

[Αν ο ερωτώμενος δεν μπορεί να απαντήσει επειδή το μοτίβο του δαπανηθέντος χρόνου ποικίλει σημαντικά από μέρα σε μέρα]
Πόσο χρόνο συνολικά δαπανήσατε περπατώντας τις τελευταίες 7 ημέρες;

____ Ώρες ανά εβδομάδα [WWHRS; Range 0-112]

___ Λεπτά ανά εβδομάδα [WWMIN; Range 0-6720, 9998, 9999]

9998. Δεν γνωρίζω/Δεν είμαι σίγουρος

9999. Αρνήθηκε

7. Κατά τη διάρκεια των τελευταίων 7 ημερών, πόσο χρόνο δαπανήσατε *καθισμένος* (τυπική καθημερινή ημέρα της εβδομάδας); [ο χρόνος που δαπανήσατε καθισμένος, ή ξαπλωμένος όχι κοιμώμενος: στην εργασία, στο σπίτι, κάνοντας εργασίες, και κατά τη διάρκεια του ελεύθερου χρόνου, σε γραφείο, σε επίσκεψη φίλων, διαβάζοντας, καθισμένος ή για να δείτε τηλεόραση.

___ Ώρες ανά ημέρα [SDHRS; Range 0-16]

___ Λεπτά ανά ημέρα [SDMIN; Range 0-960, 998, 999]

998. Δεν γνωρίζω/Δεν είμαι σίγουρος

999. Αρνήθηκε

[Αν ο ερωτώμενος δεν μπορεί να απαντήσει επειδή το μοτίβο του δαπανηθέντος χρόνου ποικίλει σημαντικά από μέρα σε μέρα]
Πόσο χρόνο συνολικά δαπανήσατε *καθισμένος* την περασμένη Τετάρτη;

___ Ώρες την Τετάρτη [SWHRS; Range 0-16]

___ Λεπτά ανά εβδομάδα [SWMIN; Range 0-960, 998, 999]

998. Δεν γνωρίζω/Δεν είμαι σίγουρος

999. Αρνήθηκε

Figure 36. Short Form IPAQ in Greek [31

Appendix C: Scientific Publications on the iMPROVE Study

nutrients

MDPI

Article

The iMPROVE Study; Design, Dietary Patterns, and Development of a Lifestyle Index in Overweight and Obese Greek Adults

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Abstract: Background: Dietary and lifestyle habits constitute a significant contributing factor in the formation of anthropometric and biochemical characteristics of overweight and obese populations. The iMPROVE study recruited overweight and obese Greek adults and investigated the effect of gene–diet interactions on weight management when adhering to a six-month, randomized nutritional trial including two hypocaloric diets of different macronutrient content. The present paper displays the design of the intervention and the baseline findings of the participants' dietary habits and their baseline anthropometric and biochemical characteristics. Methods: Baseline available data for 202 participants were analyzed and patterns were extracted via principal component analysis (PCA) on 69-item Food-Frequency Questionnaires (FFQ). Relationships with indices at baseline were investigated by multivariate linear regressions. A Lifestyle Index of five variables was further constructed. Results: PCA provided 5 dietary patterns. The "Mixed" pattern displayed positive associations with logBMI and logVisceral fat, whereas the "Traditional, vegetarian-alike" pattern was nominally, negatively associated with body and visceral fat, but positively associated with HDL levels. The Lifestyle Index displayed protective effects in the formation of logBMI and logGlucose levels. Conclusions: Dietary patterns and a Lifestyle Index in overweight and obese, Greek adults highlighted associations between diet, lifestyle, and anthropometric and biochemical indices.

Keywords: overweight; obesity; adults; dietary patterns; lifestyle index; health status; online assessment tool; nutritional intervention; weight management

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1. Introduction

The past decades have marked a noticeable increase in adult overweight and obesity. Current epidemiological evidence suggests that more than half of the adult in the European population presents a body mass index (BMI) of above 25 kg/m^2 and is, therefore, classified in the category of overweight [1]. Factors relating to increased body weight vary, including genetic predisposition, lifestyle habits, and environmental conditions, as well as their respective interplay.

The role of dietary habits in overweight and obesity development has been extensively studied in populations of various ages and ethnicities [2]. It is widely established that consumption of energy-dense foodstuffs and/or products with high sugar or fat content is positively correlated with increased weight and weight management [3]. Apart from habitual dietary preferences, adherence to specific dietary patterns, such as the Mediterranean or the Western diets, has also been associated with long-term weight management. In their 2017 review, Mu et al. demonstrated that diets rich in fruits and vegetables are correlated

with lower values of BMI, whereas higher consumption of meats and high-fat products are associated with increased BMI [2]. In the same context, Cena and Calder examined the components of constructing a healthy diet and its relation to general health status. Their 2020 review concluded that diets including fruits, vegetables, and plant-based foods, among others, such as the Mediterranean and Asian diets, reduce the risk of non-communicable diseases (NCDs) development [4].

Additionally, the identification of adherence to specific dietary patterns has been shown to be related to lifestyle parameters, namely depression and sleep characteristics. Li et al. showed that adherence to balanced dietary patterns including whole-grain products, fish, fruit, and vegetables was related to decreased risk of depression appearance, in contrast with adherence to western-diet alike patterns with elevated content in processed products and red meat [5]. Moreover, Godos et al. (2021) showed that attrition to similar, balanced dietary patterns was associated with enhanced sleep habits and sleep quality [6].

The effect of dietary habits has recently been incorporated in the attempt to collectively evaluate various lifestyle characteristics, via the construction of Lifestyle Indices (LI). Creation of LI is gaining more and more ground in recent literature and allows for the evaluation of the interconnected effect of multiple variables on phenotypic traits, such as obesity, cognitive abilities [7], development of other NCDs, such as cardiovascular disease [8] and overall mortality rates. Research studies including dietary habits in lifestyle indices are currently gaining more ground. Navarro et al., demonstrated the relationship between a maternal healthy lifestyle index, including calculation of the Healthy Eating Index (HEI), increased physical activity, pre-pregnancy BMI, smoking and alcohol drinking habits and the development of obesity in their offspring. Increased values of the index were negatively associated with obesity development during childhood [9]. Moreover, higher scores of a lifestyle index comprising of age, sex, smoking, drinking and exercise habits, sleep quality and BMI were associated with increased absolute mortality risk of older adults in Europe, United States, and the United Kingdom [10].

In this context, the present analyses represent the baseline results deriving from the iMPROVE study. The iMPROVE study, as a whole, attempts to evaluate gene–diet interactions in observed weight management, weight loss, body composition, and the lifestyle characteristics of a sample of overweight and obese Greek adults, adhering to one out of two hypocaloric dietary regimens of different macronutrient content, for a total duration of 6 months. The present article seeks to: (a) display the design, as well as the aims and objectives of the iMPROVE study and (b) evaluate the sample population's baseline dietary habits and potential relations with biochemical biomarkers. These analyses constitute the first step in assessing the effect of dietary habits on the participants' observed weight loss and further associate them with characteristics of genetic predisposition.

2. Materials and Methods

The Greek iMPROVE study constitutes a six-month randomized clinical trial, a nutritional intervention focusing on the investigation of gene–diet interactions on body weight regulation and lifestyle parameters. More specifically, the study aims at evaluating the role of target-genes in overweight and obese, Greek adults, under different dietary interventions. The study was approved by the Research Ethics Committee of Harokopio University of Athens (Protocol Number: 1800/13-06-2019) and was conducted at the premises of Harokopio University during the period 2020–2021. Recruitment took place in spring 2020 and handling and analysis of the data was, then, carried out during 2021. Moreover, the study was registered with the ClinicalTrials.gov database of the United States of America National Health Institute's (NIH) National Library of Medicine (ClinicalTrials.gov Identifier: NCT04699448). Due to the inclusion of human participants throughout its entirety, the trial was conducted adhering to the principles outlined in the Declaration of Helsinki. The study consisted of a sample of 202 overweight and obese, Greek adults, residing at the region of Attica at the time of recruitment. In this context, the present article summarizes the

baseline characteristics of the study population and the associations observed between the participants' dietary habits and biomarkers of glycemic and lipidemic control at baseline.

2.1. Nutritional Intervention Design and Study Population

The overall aims of the iMPROVE study are summarized in the following: (a) To investigate the effect of adhering to two hypocaloric dietary regimens of different macronutrient content on the observed weight loss of overweight and obese adults; (b) to investigate gene–diet interactions concerning the weight management and various lifestyle characteristics of the study population; and (c) to investigate the utility of an original, online assessment tool as a way of long-distance evaluation of the volunteers' progress. In this spectrum, the objectives of the study were formed as follows: (a) To design a six-month, randomized clinical trial including overweight and obese adults, who were blindly recruited to following one out of two different hypocaloric dietary regimens and who were subject to two in-person follow-up meetings, conducted at three and six months (middle and end) after the beginning of the intervention; and (b) to create an original assessment tool to further monitor the monthly, self-reported progress of the volunteers throughout their participation in the intervention. The design of the study is shown in Figure 1.

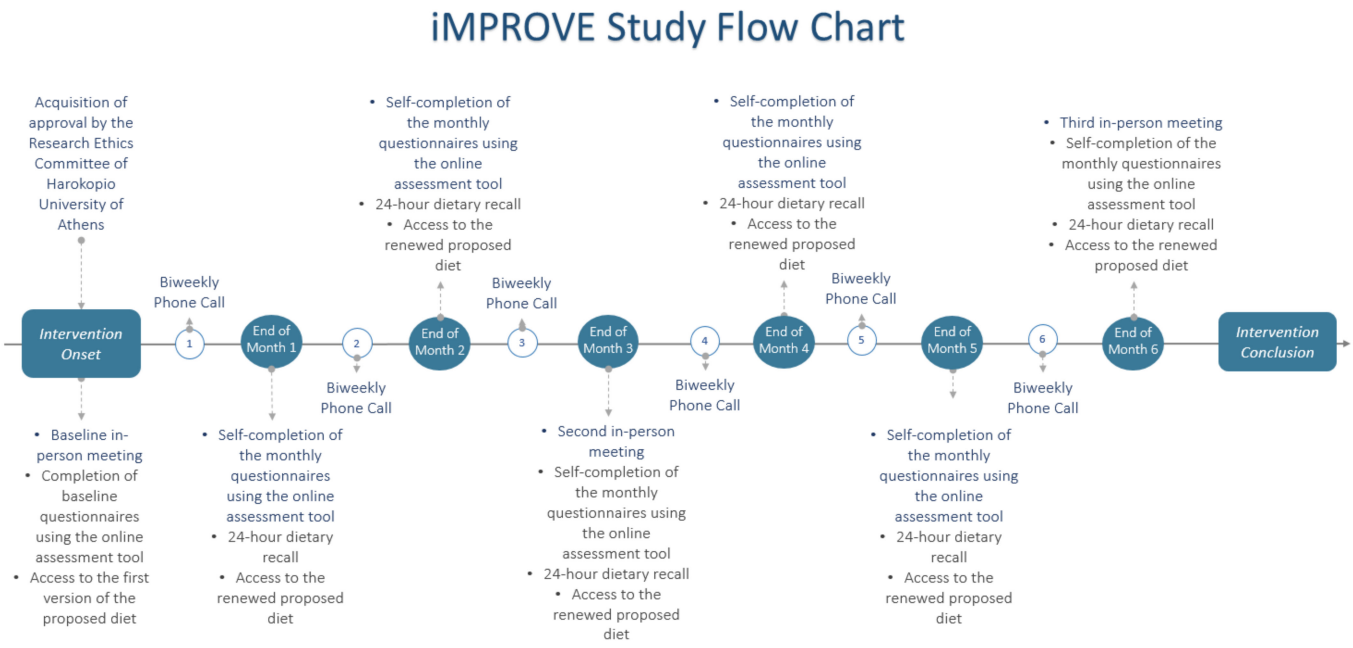
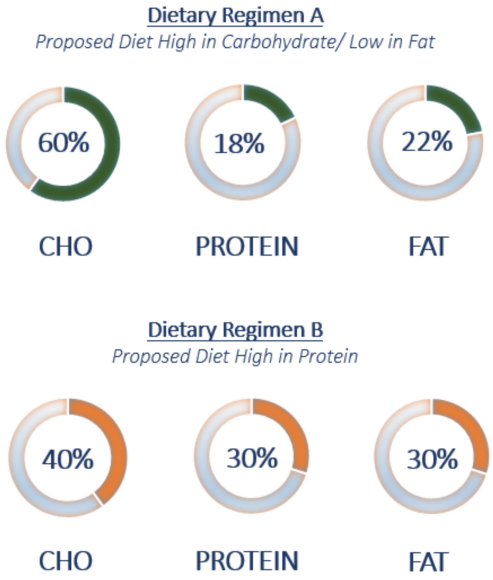


Figure 1. Flow chart of the iMPROVE Study. After completion of the baseline meeting, each participant was randomly allocated to one of the two proposed dietary intervention groups. Those referred either to a dietary regimen high in carbohydrates/low in fat, with 60% of energy intake deriving from carbohydrates, 18% deriving from protein, and 22% deriving from fat, or a dietary regimen high in protein, with 40% of the total energy intake coming from carbohydrates, 30% deriving from protein, and 30% deriving from fat. Nutritional needs for each participant were calculated using the Mifflin equation and adjusted for their reported physical activity level (PAL). According to the guidelines of the National Institutes of Health (NIH) [11], optimal weight loss should take place aiming at a mean weight loss of 0.5 to 1 kg per week, which is translated to a 7500 kcal reduction in the individual's weekly energy intake, or a 500 kcal reduction on a daily basis. Therefore, daily individual nutritional needs for each participant were further reduced by 500 kcal. The team of Harokopio University of Athens created three categories of proposed hypocaloric diets with a mean energy content of 1500 kcal, 1800 kcal, or 2000 kcal. Six different versions of the proposed diets, one for each intervention month, were created to allow for monthly renewal of the proposed diets, based on the participants' observed weight loss and dietary habits and/or tastes. The proposed diets concerned a 7-day pattern to be repeated for each month and mainly adhered to the Mediterranean pattern, including the daily consumption of 3 to 5 portions of fruit and vegetables and 1 to 2 portions of dairy products and the weekly consumption of 2 to 3 portions of grains, fish, and chicken, while limiting the consumption of red meat to 1 meal per week.

Inclusion criteria for participation in the study consisted of: (a) age above 18 years old at the time of recruitment; (b) existence of a body mass index (BMI) of above 25 kg/m^2; (c) no reporting of extreme weight loss in the 3 to 6 months prior to the beginning of the intervention; and (d) maintenance of a stable level of physical activity prior to and throughout the duration of the intervention. Similarly, the exclusion criteria included: (a) For women, the existence of pregnancy or lactation, or intention of becoming pregnant in the 6 months during the intervention period; (b) the existence of diagnosed comorbidities related to increased body weight or disturbed dietary intake (i.e., diagnosed type 1 or 2 diabetes mellitus, cardiovascular disease, gastrointestinal disorders, mental illness or disorders related to dietary intake); (c) parallel intake of dietary supplements aiming at body weight loss; and (d) parallel participation in a different research study related to weight management and/or dietary intake.

Prospective participants received oral information on the proceedings and the aims of the trial and provided written consent prior to enrolment. All volunteers were asked to visit the premises of Harokopio University of Athens in order to attend the baseline assessment session and the two subsequent, in-person follow-up meetings at the middle and end of the intervention period. All meetings were conducted in the presence of a nutrition health-care professional (dietitian/nutritionist). All in-person sessions included clinical examination, anthropometric measurements, and collection of fasting blood samples. Participants were expected to use the originally created online assessment tool in order to complete questionnaires regarding anthropometric and lifestyle parameters at baseline and at the end of each month. Following successful completion of all monthly questionnaires, the participants were allowed access to the proposed dietary regimen at the beginning of each intervention month.

Furthermore, each participant was allocated a nutrition-expert contact who monitored their adherence to the patterns (Scheme 1) and progress by: (a) Conducting biweekly follow-up phone calls and monthly 24-h dietary recalls in order to discuss the potential concerns and provide advice, as to ensure maximum adherence to the proposed diet, (b) monitoring the monthly completion of all online questionnaires; (c) evaluating the participant's self-reported monthly weight; and (d) renewing and allowing access to the proposed diet at the online platform.



Scheme 1. Macronutrient distribution of the proposed diets.

2.2. Online Assessment Tool

For the purposes of the study, the team of Harokopio University of Athens created an online assessment tool including all the questionnaires that required completion from the

volunteers, both at baseline and at a monthly level. The proposed diet was administered to the participant via access to the platform and was renewed monthly, once all questionnaires of the preceding month were checked by the nutrition expert. Access to the original, online assessment tool was granted with unique usernames and passwords for each volunteer. During the baseline session, each volunteer was taken on a virtual tour of the online assessment tool and was explicitly shown how to access and use it, by the nutrition expert.

At baseline, participants were required to complete questionnaires providing information on: (a) their medical history; (b) demographic characteristics; (c) feeling of satiety, by completing a 5-scale short questionnaire; (d) adherence to the Mediterranean dietary pattern, by completing the questionnaire of the MedDiet Score [12]; (e) depression characteristics, by completing the DEPR-S-10 Questionnaire [13]; (f) characteristics of quality of life and health status, by completing the short version of the SF-12 Questionnaire [14]; (g) characteristics of quality of sleep, by completing the Athens Insomnia Scale Questionnaire [15]; (h) dietary habits, by completing a 69-item Food Frequency Questionnaire (FFQ) [16]; and (i) physical activity habits, by completing the short version of the IPAQ Questionnaire [17]. Completion of the SF-12, sleep and IPAQ Questionnaires at a monthly basis were also a prerequisite prior to the renewal of the proposed diet. Moreover, at the end of each month participants were further called to insert information on their anthropometric measurements (i.e., current weight, waist and hip circumference measurements), feeling of satiety and self-reported adherence to the proposed diet during the past month.

2.3. Clinical Examination and Anthropometric Measurements

During all three in-person meetings, clinical examination and anthropometric measurements were carried out by the trained dietitians or health-care or nutrition experts, using suitable equipment and standardized techniques. Clinical examination of the participants included: (a) evaluation of their physical status; and (b) blood pressure measurements, conducted after ensuring that the participant was at a calm state and at their bare, left upper arm, while sitting in an upright position with elevated feet and the arm supported at heart level.

The anthropometric data collected included: (a) Height measurements to the nearest 0.1 cm, using a portable stadiometer, where the participant was barefoot, with relaxed shoulders and looking straight ahead; (b) weight measurements to the nearest 0.1 kg, using the scales of the Tanita BC-418 Segmental Body Composition Analyzer, where the participant was barefoot and maintaining light clothing; (c) waist measurements (between the twelfth rib and the iliac crest), using a non-extensible soft tape; and (d) hip measurements, at the widest point of the hips, using a non-extensible soft tape. Participants were further shown and taught how to properly conduct the waist and hip circumference measurements, in order to monitor their progress and report it in the monthly anthropometric measurements' online questionnaire. Body composition analysis for individual participants was conducted via bioelectrical impedance analysis and more specifically, by using the Tanita BC-418 Segmental Body Composition Analyzer. The participants were refrained from food or water intake for at least two hours prior to the measurement conduct, be barefoot and maintain light clothing without any metal objects. Body composition data were acquired for each volunteer including body fat percentage, amount of body fat in kilos, distribution of body fat in the trunk and the limbs, body water percentage, and fat free mass. Body mass index (BMI) was calculated via dividing the weight (kg) by the square height (in m^2) for each participant and subjects were classified to the categories of: overweight ($25\ kg/m^2 \leq BMI < 30\ kg/m^2$), obese with class I obesity ($30\ kg/m^2 \leq BMI < 35\ kg/m^2$), obese with class II obesity ($35\ kg/m^2 \leq BMI < 40\ kg/m^2$), or obese with class III obesity ($BMI \geq 40\ kg/m^2$).

2.4. Biochemical Analyses

Upon arrival to an in-person meeting and after following an 8-h fasting, a blood sample of 23 mL was collected from the antecubital vein of the participant by a trained health-care

professional, following blood pressure measurements. Hematological biomarkers and biomarkers of biochemical profile were analyzed. All remaining samples were stored at −80 °C for future analyses. Low-density cholesterol (LDL-C) was calculated using the Friedewald Equation.

2.5. Genotyping Analyses

The buffy coat samples isolated for each participant were used for DNA extraction, via use of the Invitrogen iPrep Purification Instrument and the Invitrogen iPrep PureLink gDNA Blood Kit [18]. Isolated samples were stored at −20 °C for a period of up to two months after extraction and prior to genotyping. Samples were stored at −80 °C, for a longer period and future analyses. All samples were sent for genome-wide sequencing using the Axiom Precision Medicine Diversity Research Array [19], which provided data for over 850,000 SNPs, deletions, and CNVs.

2.6. Dietary Assessment

Assessment of dietary intake at baseline took place by completing the validated 69-item Food Frequency Questionnaire (FFQ) in the online assessment tool. Dietary assessment and evaluation of subsequent adherence to the proposed diet was conducted monthly via: (a) A 24-h dietary recall, carried out by the nutrition expert; and (b) completion of the 5-scale self-reported adherence questionnaire, via use of the online assessment platform.

2.7. Physical Activity Assessment

Physical activity levels at baseline were assessed by completing the short version of the International Physical Activity Questionnaire (IPAQ) on the online platform. The same questionnaire was completed at the end of each intervention month.

2.8. Statistical Analysis

Data analyses were conducted using the Statistical Package for Social Sciences (SPSS), version 23 [20], as well as the R statistical package [21]. Dietary patterns were extracted by conducting principal component analysis (PCA) on 32 food groups deriving from the data of the FFQ. The Varimax orthogonal rotation was used and the KMO and Bartlett's test was implemented to evaluate data adequacy. Five dietary patterns were set to be extracted with Eigen values bigger than 1. Variable distribution was evaluated via the Shapiro–Wilk and Kolmogorov–Smirnov tests. Differences in mean/median values of variables within the two sexes were evaluated using the Mann–Whitney test. We further tested for potential associations between the extracted patterns and a variety of anthropometric and biochemical indices, via multivariate linear regressions. Non-normally distributed variables were log-transformed. We further examined the potential associations between the extracted patterns and several indices, by separating them into tertiles and testing for associations using the parametric ANOVA test and the non-parametric Kruskal–Wallis test, depending on the distribution of the examined variable. After analyzing the data for the entirety of the sample, a novel Lifestyle Index was constructed including variables found to be correlated with logBMI and body fat percentage, based on Pearson's chi-square test values. Further association tests (i.e., multivariate regressions) were conducted to assess the potential relationship between the Lifestyle Index and clinical and biochemical biomarkers. The level of statistical significance for all analyses was set at $\alpha = 0.05$ and results were also interpreted for the adjusted cut-off value of a = 0.05/number of patterns extracted (i.e., a = 0.05/5 = 0.01).

3. Results

3.1. Descriptive Statistics

The entirety of the study population's anthropometric, clinical, dietary, and lifestyle characteristics are presented in Tables 1 and 2. Median ± IQR is presented for all non-normally distributed variables and mean ± standard deviation (SD) is presented for the

variables following the normal distribution. Out of the 235 volunteers expressing interest to participate in the study, data are shown for 202 eligible subjects who successfully attended the baseline meeting, completed the majority of the baseline questionnaires using the online tool, and were recruited in one of the two intervention arms. The sample size of the 202 individuals assures adequate power to detect statistical significance. Our baseline sample consisted of 142 women (70.29%) and 60 men (29.7%), with a median age of 47 years old. The majority of participants were married (60.9%), with more than half of our sample reporting having higher education (61.9%) and less than 3% reporting having no acquired education at all. The vast majority of the participants were reported as non-smokers (151 non-smokers vs. 50 smokers, out of 201 participants with available data). The estimated physical activity level showed that roughly half of the subjects were leading a moderately active way of life (104 out of the 199 participants with available data), with about 32% reporting a sedentary lifestyle. All 202 eligible volunteers were blindly recruited in the intervention groups, with 46.5% following the high-carbohydrate/low-fat diet and 53.5% adhering to the high-protein diet.

Table 1. Characteristics of the iMPROVE cohort by sex.

n	Smoking		Physical Activity Categories			Diet Group	
	Smokers	Non-Smokers	Low	Moderate	Vigorous	High Carbohydrate/Low-Fat	High Protein
Total	50	151	64	104	31	94	108
Women	38	103	43	76	30	74	68
Men	12	48	21	28	11	20	40

Table 2. Anthropometric, clinical, dietary characteristics and characteristics of depression, quality of sleep, and health status in the iMPROVE cohort, by sex.

Variable	Total			Women			Men			*p*-Value
	n	Median	IQR	n	Median	IQR	n	Median	IQR	
Age	202	47	15	142	47.50	14	60	45.78 *	17 *	$p > 0.05$ **
SBP (mmHg)	196	121.00	21	139	117.00	19	57	131.00	21	$p < 0.001$ **
DBP (mmHg)	196	80.84 *	9.86 *	139	78.68 *	9.05 *	57	86.09 *	9.85 *	$p < 0.001$ **
Pulse Rate (Beats per minute)	196	74.99	11.38	139	74.00	16	57	73.00	14	$p > 0.05$ **
Anthropometric Characteristics										
Weight (kg)	202	87.10	26	142	82.80	18	60	100.65	29	$p < 0.001$ **
BMI (kg/m^2)	202	31.34	6.9	142	31.34	6.9	60	31.33	7.1	$p > 0.05$ **
Body fat (%)	202	37.95 *	7.8 *	142	41.50 *	5.4 *	60	29.55 *	5.8 *	$p < 0.001$ **
Body fat (kg)	202	32.95	13.3	142	34.85	12.8	60	29.05	14.5	$p < 0.002$ **
Fat free mass(kg)	202	52.05	18	142	48.50	7	60	71.20	15	$p < 0.001$ **
Total body water (kg)	202	38.05	13	142	36.35 *	4.03 *	60	52.10	11	$p < 0.001$ **
Visceral fat	202	10.00	6	142	9.74	3.16	60	14.50	5.69	$p < 0.001$ **
Upper body fat (%)	201	36.60	10	141	38.50	8	60	32.10	8	$p < 0.001$ **
Upper body fat (kg)	201	17.60	7	141	17.30	7	60	18.00	7	$p > 0.05$ **
Upper body fat-free mass (kg)	201	28.80	9	141	27.67 *	2.75 *	60	38.00	7	$p < 0.001$ **
Waist circumference (cm)	185	99.00	17	130	96.50	15	55	105.00	17	$p < 0.001$ **
Hip circumference (cm)	185	114.50	14	130	115.75	16	55	114.00	10	$p > 0.05$ **
WHR	185	0.85	0.12	130	0.83	0.09	55	0.92	0.09	$p < 0.001$ **
Biochemical Biomarkers										
Fasting glucose (mg/dL)	193	92.00	10.50	135	92.00	10.00	58	95.00	16.25	$p < 0.001$ **
Urea (mg/dL)	193	28.00	9.00	135	27.00	9.00	58	30.12 *	6.01 *	$p < 0.001$ **
Creatinine (mg/dL)	193	0.68	0.21	135	0.62 *	0.10 *	58	0.85 *	0.12 *	$p < 0.001$ **
Uric acid(mg/dL)	193	4.70	1.45	135	4.30	1.10	58	5.75 *	1.00 *	$p < 0.001$ **
Total cholesterol (mg/dL)	193	177.96 *	33.57 *	135	179.32 *	31.98 *	58	174.79 *	37.13 *	$p > 0.05$ **
HDL cholesterol (mg/dL)	193	49.00	16.50	135	52.00	17.00	58	42.00	14.50	$p < 0.001$ **

Table 2. *Cont.*

Variable	Total			Women			Men			*p*-Value
	n	Median	IQR	n	Median	IQR	n	Median	IQR	
LDL cholesterol (mg/dL)	193	105.20	38.70	135	105.00	38.40	58	108.00	42.05	$p > 0.05$ **
Triglycerides (mg/dL)	193	90.00	5.00	135	86.00	51.00	58	105.50	86.50	$p < 0.001$ **
Total bilirubin (mg/dL)	193	0.37	0.23	135	0.35	0.23	58	0.45	0.29	$p < 0.001$ **
Direct bilirubin (mg/dL)	193	0.16	0.08	135	0.15	0.09	58	0.17	0.08	$p < 0.001$ **
Serum protein (g/dL)	193	6.70	0.50	135	6.70	0.45	58	6.60	0.60	$p > 0.05$ **
Serum albumin (g/dL)	193	4.20	0.30	135	4.40	0.50	58	4.20	0.40	$p < 0.001$ **
SGOT/AST (IU/L)	193	16.00	6.00	135	15.00	5.00	58	18.00	5.25	$p < 0.001$ **
SGPT/ALT (IU/L)	192	15.00	11.75	134	13.00	9.00	58	22.00	16.25	$p < 0.001$ **
Lifestyle Characteristics										
AIS Score ***	140	5.00	7.00	97	5.00	7.00	43	4.00	7.00	$p > 0.05$ **
CESD-R-10 Scale	201	6.00	5.00	141	6.00	4.00	60	5.00	5.75	$p < 0.001$ **
SF PCS 12 Score	145	51.98	12	99	50.37	11	46	53.82	8	$p < 0.001$ **
SF MCS 12 Score	145	49.37	15	99	49.44	16	46	46.62 *	8.36	$p > 0.001$ **

* The selected variables follow the normal distribution and are presenting as mean ± standard deviation. ** Statistically significant differences between the sexes were assessed via calculation of the Mann–Whitney test. *** The Athens Insomnia Scale Score was calculated only for participants reporting the referred characteristics ≥3 times/week for the past month. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WHR: waist-to-hip ratio; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SGOT/AST: glutamic oxaloacetic transaminase/aspartate transaminase; SGPT/ALT: glutamate pyruvate transaminase blood/alanine transaminase; AIS: Athens Insomnia Scale; CESD-R-10: Center for Epidemiologic Studies Depression Scale Revised—10; SF PCS 12: Short Form (Health Survey) Physical Component Score 12; SF MCS 12: Short Form (Health Survey) Mental Component Score 12.

Overweight participants constituted 38.6% of our overall sample, with the remaining 61.4% spreading across the three different obesity categories (35.1%, 14.9%, and 11.4% of the participants classified as Class I, II, or III obese, respectively). Median BMI was calculated at 31.34 kg/m^2 and did not differ between men and women, whereas body composition data displayed statistically significant differences between the two sexes, with men reporting higher levels of fat-free mass (71.20 kg vs. 48.50 kg, $p < 0.001$) and total body water ($p < 0.001$) and women presenting increased body fat values (41.40% vs. 29.55%, $p < 0.001$). Although men were found to have increased waist-to-hip ratio (WHR) (0.92 vs. 0.83, $p < 0.001$) waist circumference in comparison to women (105 cm vs. 96.5 cm, $p < 0.001$), hip circumference did not present significant differences. Similar differences were further observed in the clinical characteristics, with men reporting higher levels of blood pressure, fasting glucose (95 mg/dL vs. 92 mg/dL, $p < 0.001$), serum urea (30.12 mg/dL vs. 27.00 mg/dL, $p < 0.001$), creatinine (0.12 mg/dL vs. 0.10 mg/dL, $p < 0.001$), uric acid levels (5,75 mg/dL vs. 4.30 mg/dL, $p < 0.001$), total triglycerides (105.5 mg/dL vs. 86 mg/dL, $p < 0.001$), serum bilirubin (0.45 mg/dL vs. 0.35 mg/dL, $p < 0.001$), SGOT (18 IU/L vs. 15 IU/L, $p < 0.001$), and SGPT levels (22.00 IU/L vs. 13 IU/L, $p < 0.001$). Women presented higher levels of HDL cholesterol (52.00 mg/dL vs. 42.00 mg/dL, $p < 0.001$) and serum albumin levels (4.40 g/dL vs. 4.20 g/dL, $p < 0.001$).

3.2. Lifestyle Characteristics

The 8-item Athens Insomnia Scale (AIS) score on evaluation of sleep qualities was calculated for participants who reported the selected outcomes more than three times per week in the month leading to the beginning of the intervention. The AIS score presented a median of 5 out of the scale maximum scoring of 24 and did not display statistically significant differences between the sexes. Overall, participants did not express significant irregularities neither in sleep quality, including sleep induction, total sleep duration, and awakenings at night and expressed delayed sleep induction, nor in effects of sleep on aspects of the next day (i.e., well-being, overall functioning, and sleepiness).

Contrary to the AIS score, the CESD-R-10 scale score on depression characteristics showed that women displayed higher median values (scoring of 6 vs. 5, $p < 0.001$). The majority of the participants did not display depression characteristics, such as feelings of fear and helplessness, with the overall sample presented a mean CESD-R-10 score of 6, with the scale maximum scoring calculated at 18. Moreover, the physical component of

the SF-12 score on self-reported quality of life underlined increased levels in men than in women (53, 82 vs. 50, 37, $p < 0.001$), whereas the mental component did not show important dissimilarities. No statistically significant differences were found when the participants were classified into the four different BMI groups (Figures 2 and 3).

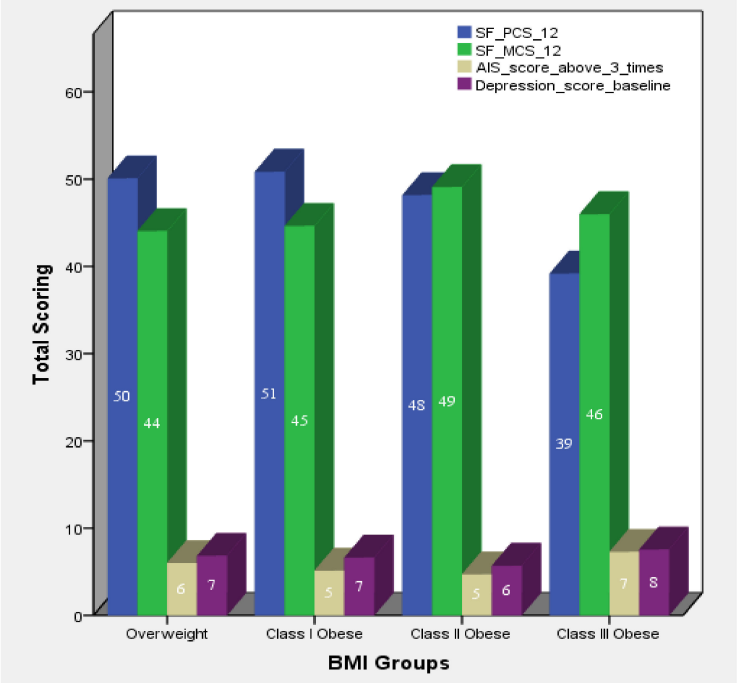


Figure 2. Baseline scoring of the four lifestyle questionnaires, based on BMI categories.

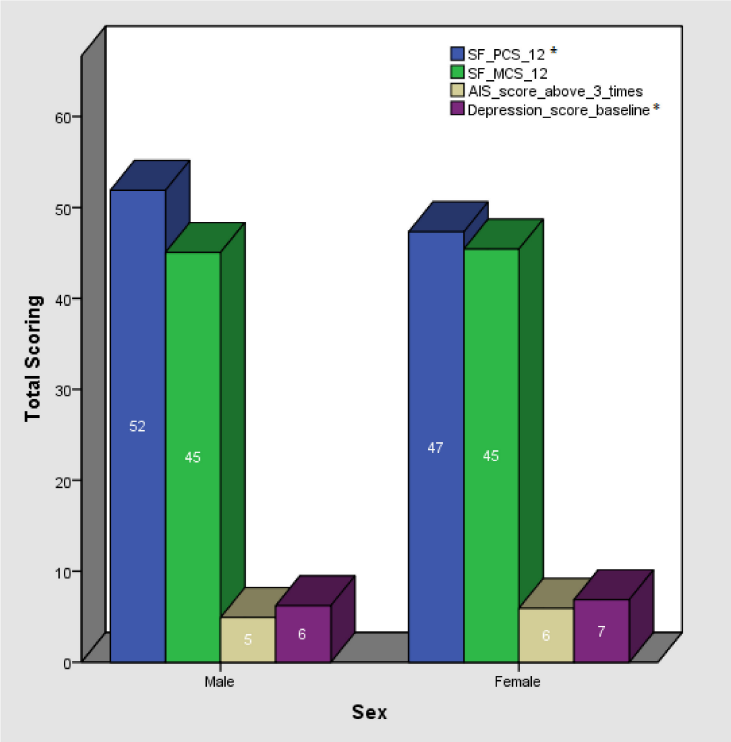


Figure 3. Baseline scoring of the four lifestyle questionnaires, based on sex (* The presented variables were statistically significantly different between the sexes.).

As shown in Table 3. we further investigated the potential effect of the aforementioned lifestyle aspects on logBMI and %body fat baseline levels. After adjusting for confounding factors including age, sex, smoking habits, physical activity level and education years, Only the physical component of the SF-12 questionnaire (SF PCS 12 Score) was found to be associated with the characteristics of interest, displaying a negative effect on both logBMI and %body fat values ($\beta = -0.003$, $p < 0.001$ and $\beta = -0.218$, respectively).

Table 3. Multivariate linear regression analyses on the relation between lifestyle characteristics and BMI and body fat baseline values.

Variable	Model 1 *		
	β	*SE*	*p*-Value
logBMI			
Athens Insomnia Scale Score	0.001	0.001	0.612
CESD-R-10 Scale	0.002	0.001	0.189
SF PCS 12 Score	−0.003	0.001	<0.001
SF MCS 12 Score	0.001	0.001	0.217
Body fat (%)			
Athens Insomnia Scale Score	0.188	0.128	0.143
CESD-R-10 Scale	0.175	0.100	0.083
SF PCS 12 Score	−0.218	0.057	<0.001
SF MCS 12 Score	0.049	0.054	0.371

* Model 1: Adjusting for age, sex, smoking, physical activity level and education years.

3.3. Dietary Patterns

PCA on the available data of the 202 participants' FFQs resulted in the identification of five dietary patterns accounting for 40.34% of the sample's total variance. The KMO and Bartlett's Test ($p < 0.001$) presented a Kaiser–Meyer–Olkin Measure of 0.726, indicating sufficient data adequacy. All factor loadings for each component were above or approaching a value of 0.3. As shown in Table 4, the 32 food groups formed based on the 69-item questions, included in the analysis reflected the variety of foodstuffs consumed by the sample population, including both widely consumed food categories such as meat and cereals, as well as traditional Greek recipes (i.e., pastitsio, spinach rice, and homemade pies). Alcohol and beer reported servings were included in the analysis, due to the sample's low median values (2 and 16 mL/d, respectively) and the lack of heavy drinkers.

Table 4. Mean consumption and PCA factor loadings of the 32 FFQ-derived food groups.

			Components				
	Mean Consumption (Median. IQR)	**Food Group**	**1**	**2**	**3**	**4**	**5**
Croissant (g/d)	5.2 (11.56)	Sweets	0.705				
Chocolate (g/d)	12.85 (8.85)						
Tarts (g/d)	10.00 (10.00)						
Ice cream (g/d)	7.66 (24.64)						
Mayonnaise (g/d)	1.11 (2.02) *	Mayonnaise	0.664				
White bread (g/d)	19.28 (17.28)	Refined Cereals	0.643				
Cereals (g/d)	4.28 (4.28)						
White rice (g/d)	10.53 (23.32)						
Barley (g/d)	9.33 (30.00)						
Burger bread (g/d)	3.00 (10.44) *						
Chips (g/d)	4.66 (4.66)	Salty Snacks	0.628				
Crackers (g/d)	1.33 (4.28)						
Honey (g/d)	1.07 (4.66)	Sugary Snacks	0.596				
Soft drinks (mL/d)	28.69 (72.42) *						
Fruit compost (g/d)	7.58 96.66) *						
Tray Sweets (g/d)	10.00 (10.00)	Tray Sweets	0.584				
Pastitsio (g/d)	10.00 (10.00)	Pastitsio	0.493				
Potatoes (boiled. cooked. not fried) (g/d)	11.53 (25.53)	Potatoes (boiled, cooked, not fried)	0.469				
Chicken (g/d)	32.14 (0.00)	Chicken	0.388				
Seed oil (g/d)	3.23 (8.09) *	Seed oil, margarine, butter	0.374				
Margarine (g/d)	1.03 (2.46) *						
Butter (g/d)	0.50 (1.00)						
Light mayonnaise (g/d)	0.71 (1.84) *	Light Products	0.367				
Light cold cuts (g/d)	2.00 (6.42)						
Light soft drinks (g/d)	22.00 (70.71)						
Sausage (g/d)	1.08 (1.45)					−0.342	
Tomatoes (g/d)	64.28 (42.85)	Vegetables		0.640			
Lettuce (g/d)	34.28 (34.28)						
Broccoli (g/d)	21.42 (14.76)						
Spinach (g/d)	6.00 (13.28)						
Full fat milk (mL/d)	43.46 (71.26)	Dairy		0.568			
Low fat milk	51.42 (154.28)						
White cheese (g/d)	6.42 (17.28)						
Eggs (g/d)	10.71 (7.38)	Eggs		0.562			
Oranges (g/d)	36.42 (97.95)	Fruits		0.525			
Apples (g/d)	30.00 (80.66)						
Bananas (g/d)	21.42 (57.61)						
Winter fruit (g/d)	32.14 (86.42)						
Summer fruit	32.14 (64.28)						

Table 4. *Cont.*

			Components				
	Mean Consumption (Median. IQR)	**Food Group**	**1**	**2**	**3**	**4**	**5**
Whole bread (g/d)	19.28 (17.28)	Non-refined cereals		0.443			
Brown rice (g/d)	6.72 (14.73) *						
Whole pasta (g/d)	8.54 (9.33)						
Large fish (g/d)	10.00 (22.14)	Large fish		0.432			
Olive oil (g/d)	45.00 (45.00)	Olive oil		0.345			
Dried fruit (g/d)	3.35 (6.88) *	Dried fruit		0.330			
Coffee (mL/d)	240.00 (240.00)	Caffeinated Beverages				−0.504	
Tea (mL/d)	16.00 (51.42)						
Seafood (g/d)	10.00 (10.00)	Seafood			0.685		
French Fries (g/d)	4.83 (15.53)	French Fries			0.648		
Homemade pies (g/d)	10.00 (0.00)	Pies			0.510		
Other pies (g/d)	10.00 (10.00)						
Beef (g/d)	10.00 (22.14)	Red Meat			0.499		
Minced beef	25.71 (17.71)						
Pork (g/d)	10.00 (22.14)						
Lamb (g/d)	5.83 (13.84)						
Alcohol (mL/d)	2.00 (6.42)	Alcohol and Beer			0.398		
Beer (mL/d)	16.00 (51.42)						
Legumes (g/d)	64.28 (44.28)	Legumes				0.698	
Spinach and Rice (g/d)	16.66 (53.57)	Traditional, Greek recipes				0.695	
Green Peas (g/d)	42.85 (29.52)						
Olives	1.00 (3.21)	Olives					0.645
Small fish (g/d)	10.00 (32.14)	Small fish					0.584
Nuts (g/d)	3.33 (28.81)	Nuts					0.343
Fruit Juice (g/d)	16.00 (51.42)	Fruit Juice					0.311
Total Variance Explained (%)			14.74%	9.87%	6.26%	4.96%	4.49%

* The selected variables are presented as mean ± standard deviation (SD).

The dietary patterns provided are summarized in the following (Table 5): (a) The "Mixed" pattern (total variance explained: 14.74%) which reported the consumption of a variety of food groups including both light products and processed products high in fat and sugars (i.e., sweets, mayonnaise, refined cereals, salty snacks, sugary snacks, tray sweets, the Greek pastitsio, potatoes, chicken, seed oil, margarine, butter, light products, and sausage); (b) the "Mediterranean-proxy" (or Med-proxy) pattern (total variance explained: 9.87%), including the consumption of food groups usually found in the Mediterranean diet, such a vegetables, dairy, eggs, fruits, non-refined cereals, large fish, olive oil, dried fruit, and caffeinated drinks, such as coffee and tea; (c) the "Eating out" pattern (total variance explained: 6.26%), consisting of food group combinations frequently consumed outside the household, i.e., seafood, French fries, pies, red meat and alcohol; (d) the "Traditional, vegetarian-alike" pattern (total variance explained: 4.96%), reporting consumption of legumes and traditional Greek recipes (i.e., spinach rice and cooked green peas); and (e) "High in unsaturated fats and fruit juice consumption" pattern (total variance explained: 4.49%), consisting of olives, small fish, nuts and fruit juice, with the first, high in unsaturated fats and fruit juice consumption, groups presenting the greatest factor loadings.

Table 5. Multivariate linear regressions between the extracted dietary patterns and indices of anthropometric and biochemical characteristics.

	Model 1			Model 2			Model 3		
	β	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**
LogBMI									
Mixed Pattern	0.019	0.005	<0.001	0.017	0.005	0.001	0.015	0.005	0.009
Med-proxy Pattern	−0.002	0.005	0.758	<0.001	0.005	0.937	−0.001	0.006	0.867
Eating-out Pattern	0.004	0.005	0.366	0.004	0.005	0.432	0.001	0.005	0.780
Traditional, vegetarian-alike Pattern	−0.005	0.005	0.272	−0.008	0.005	0.132	−0.008	0.005	0.132
High in unsaturated fats and fruit juice consumption Pattern	−0.005	0.005	0.338	−0.006	0.005	0.269	−0.006	0.005	0.281
Body fat %									
Mixed Pattern	1.179	0.392	0.003	−0.149	0.230	0.516	−0.195	0.259	0.451
Med-proxy Pattern	−0.010	0.423	0.982	0.261	0.234	0.266	0.351	0.255	0.171
Eating-out Pattern	0.316	0.399	0.430	0.005	0.217	0.982	−0.045	0.232	0.848
Traditional, vegetarian-alike Pattern	−0.770	0.387	0.048	−0.476	0.221	0.032	−0.402	0.236	0.090
High in unsaturated fats and fruit juice consumption Pattern	−0.335	0.400	0.404	0.054	0.220	0.808	0.167	0.236	0.480
LogVisceral Fat									
Mixed Pattern	0.032	0.009	0.001	−0.002	0.004	0.633	<0.001	0.004	0.980
Med-proxy Pattern	−0.002	0.010	0.808	0.002	0.004	0.629	0.004	0.004	0.302
Eating-out Pattern	0.011	0.010	0.266	0.002	0.004	0.548	0.002	0.004	0.642
Traditional, vegetarian-alike Pattern	−0.018	0.009	0.058	−0.008	0.004	0.032	−0.007	0.004	0.088
High in unsaturated fats and fruit juice consumption Pattern	−0.007	0.010	0.450	0.002	0.004	0.522	0.006	0.004	0.139
logCreatinine(mg/dL)									
Mixed Pattern	0.008	0.005	0.136	0.008	0.006	0.143	0.012	0.006	0.048
Med-proxy Pattern	−0.008	0.006	0.150	−0.010	0.006	0.066	−0.013	0.006	0.029
Eating-out Pattern	−0.001	0.005	0.897	0.001	0.005	0.848	0.001	0.006	0.875
Traditional, vegetarian-alike Pattern	−0.003	0.005	0.549	−0.008	0.005	0.155	−0.008	0.006	0.149
High in unsaturated fats and fruit juice consumption Pattern	0.002	0.005	0.648	0.001	0.005	0.802	0.001	0.006	0.876
logHDL Cholesterol (mg/dL)									
Mixed Pattern	−0.020	0.007	0.006	−0.013	0.007	0.079	−0.016	0.009	0.057
Med-proxy Pattern	−0.005	0.008	0.528	−0.009	0.008	0.257	−0.010	0.008	0.245
Eating-out Pattern	0.009	0.007	0.229	0.011	0.007	0.135	0.013	0.008	0.082
Traditional, vegetarian-alike Pattern	0.013	0.007	0.054	0.017	0.007	0.017	0.016	0.008	0.039
High in unsaturated fats and fruit juice consumption Pattern	0.005	0.007	0.483	0.006	0.007	0.445	0.003	0.008	0.688
logTriglycerides(mg/dL)									
Mixed Pattern	0.038	0.014	0.007	0.021	0.015	0.155	0.033	0.016	0.048
Med-proxy Pattern	−0.008	0.015	0.579	−0.003	0.015	0.850	−0.002	0.016	0.908
Eating-out Pattern	<0.001	0.014	0.998	−0.003	0.014	0.816	−0.007	0.015	0.622
Traditional, vegetarian-alike Pattern	0.009	0.014	0.509	0.006	0.014	0.662	0.015	0.015	0.316
High in unsaturated fats and fruit juice consumption Pattern	0.002	0.014	0.875	<0.001	0.015	0.976	−0.004	0.016	0.788
logTotal Bilirubin(mg/dL)									
Mixed Pattern	−0.002	0.015	0.913	0.001	0.017	0.971	0.013	0.018	0.472
Med-proxy Pattern	−0.036	0.016	0.025	−0.043	0.017	0.010	−0.042	0.018	0.019
Eating-out Pattern	−0.005	0.015	0.735	−0.001	0.015	0.943	−0.005	0.016	0.772
Traditional, vegetarian-alike Pattern	0.032	0.015	0.031	0.030	0.016	0.059	0.033	0.016	0.047

Table 5. *Cont.*

	Model 1			Model 2			Model 3		
	β	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**
High in unsaturated fats and fruit juice consumption Pattern logSerum protein(g/dL)	0.018	0.016	0.255	0.015	0.016	0.357	0.016	0.017	0.356
Mixed Pattern	0.004	0.002	0.036	0.003	0.002	0.187	0.005	0.002	0.029
Med-proxy Pattern	−0.001	0.002	0.575	−0.001	0.002	0.720	<0.000	0.002	0.829
Eating-out Pattern	−0.001	0.002	0.603	−0.001	0.002	0.599	−0.002	0.002	0.273
Traditional, vegetarian-alike Pattern	0.002	0.002	0.164	0.002	0.002	0.217	0.003	0.002	0.136
High in unsaturated fats and fruit juice consumption Pattern LogSGOT/AST(IU/L)	0.001	0.002	0.525	0.001	0.002	0.530	<0.001	0.002	0.794
Mixed Pattern	0.024	0.011	0.029	0.024	0.012	0.043	0.028	0.012	0.022
Med-proxy Pattern	0.003	0.012	0.797	0.004	0.012	0.759	0.001	0.012	0.911
Eating-out Pattern	−0.011	0.011	0.317	−0.009	0.011	0.393	−0.013	0.011	0.216
Traditional, vegetarian-alike Pattern	0.003	0.011	0.811	−0.003	0.011	0.799	−0.004	0.011	0.735
High in unsaturated fats and fruit juice consumption Pattern logSGPT/ALT (IU/L)	0.006	0.011	0.603	0.004	0.012	0.735	0.003	0.011	0.824
Mixed Pattern	0.052	0.014	<0.001	0.049	0.016	0.002	0.070	0.016	<0.001
Med-proxy Pattern	−0.009	0.016	0.570	−0.005	0.016	0.768	−0.006	0.017	0.740
Eating-out Pattern	−0.003	0.015	0.816	−0.005	0.015	0.714	−0.009	0.015	0.555
Traditional, vegetarian-alike Pattern	0.002	0.014	0.910	<0.001	0.015	0.996	<0.001	0.015	0.978
High in unsaturated fats and fruit juice consumption Pattern	0.005	0.015	0.724	0.006	0.016	0.701	−0.002	0.016	0.919

Model 1: Adjusting for age and sex. Model 2: Adjusting for age, sex, smoking habits, physical activity level and logBMI (except for logBMI values). Model 3: Adjusting for age, sex, smoking habits, physical activity level, logBMI, education years, family and professional status.

Nominal associations ($p < 0.05$) are described as follows: consumption of the "Mixed" pattern was correlated with: (a) increased logBMI values, after adjustment for the confounding factors of all models (Model 1:β = 0.019, $p < 0.001$, Model 2:β = 0.017, $p < 0.001$, Model 3: β = 0.015, p-value = 0.009; (b) increased levels of body fat percentage, in model 1 (β = 1.179, $p = 0.003$); (c) increased levels of the logVisceral fat in Model 1 (β = 0.032, $p = 0.001$); (d) increased levels of logCreatinine, in Model 3 (β = 0.012, $p = 0.048$) (e) decreased values of HDL cholesterol in Model 1 (β = −0.020, $p < 0.006$); (f) increased levels of logTriglycerides, in Models 1 (β = 0.038, $p = 0.007$) and 3 (β = 0.033, $p = 0.048$); (g) increased levels of logSerum protein in Models 1 (β = 0.004, $p = 0.036$) and 3 (β = 0.005, $p = 0.029$); and (h) increased levels of logSGOT/AST (Model 1: β = 0.024, $p = 0.029$, Model 2: β = 0.024, $p = 0.043$, Model 3: β = 0.028, p-value = 0.022) and SGPT/ALT (Model 1:β = 0.052, $p < 0.001$, Model 2: β = 0.049, $p = 0.002$, Model 3: β = 0.070, $p < 0.001$). The "Med-proxy" pattern was found related with lower values of logCreatinine, in Model 3 (β = −0.013, $p = 0.029$) and lower values of logTotal Bilirubin, in all models (Model 1: β = −0.036, $p = 0.025$, Model 2: β = −0.043, $p = 0.010$, Model 3: β = −0.042, $p = 0.019$). The "Traditional, vegetarian-alike" pattern was associated with: (a) reduced levels of body fat, in Models 1 (β = −0.770, $p = 0.048$) and 2 (β = −0.476, $p = 0.032$); (b) decreased logVisceral fat values, in Model 2 (β = −0.008, $p = 0.032$); and (c) increased levels of logHDL cholesterol in models 2 and 3 (Model 2: β = 0.017, $p = 0.017$, Model 3: β = 0.016, $p = 0.039$). After evaluation for the adjusted threshold of statistical significance (i.e., a = 0.05/5 = 0.01), statistically significant associations remained for: (a) the "Mixed" pattern and increased logBMI, body fat and logSGPT/ALT values; (b) the "Mixed" pattern and decreased logHDL cholesterol values; and (c) the "Med-proxy" pattern and decreased levels of logTotal Bilirubin. Further associations are displayed in Appendix A.

After extracting the dietary patterns, we further explored the effect of their respective adherence to each anthropometric and biochemical biomarker, by separating them into tertiles. As shown in Figures 4–7, increased adherence to the "*Mixed*" pattern was associated with: (a) increased levels of logBMI ($p = 0.003$), (b) decreased levels of logHDL cholesterol ($p = 0.007$), (c) increased levels of logSerum protein ($p = 0.008$). Additionally, categorization in the higher tertile of the "*Med-proxy*" pattern was associated with lower levels of logCreatinine ($p = 0.011$).

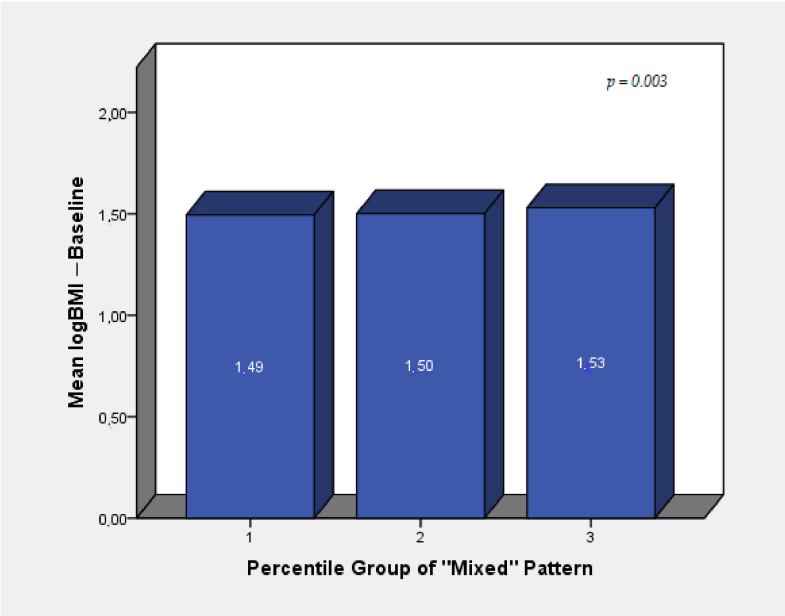


Figure 4. Percentile distribution and associations between the "Mixed" Pattern and logBMI values.

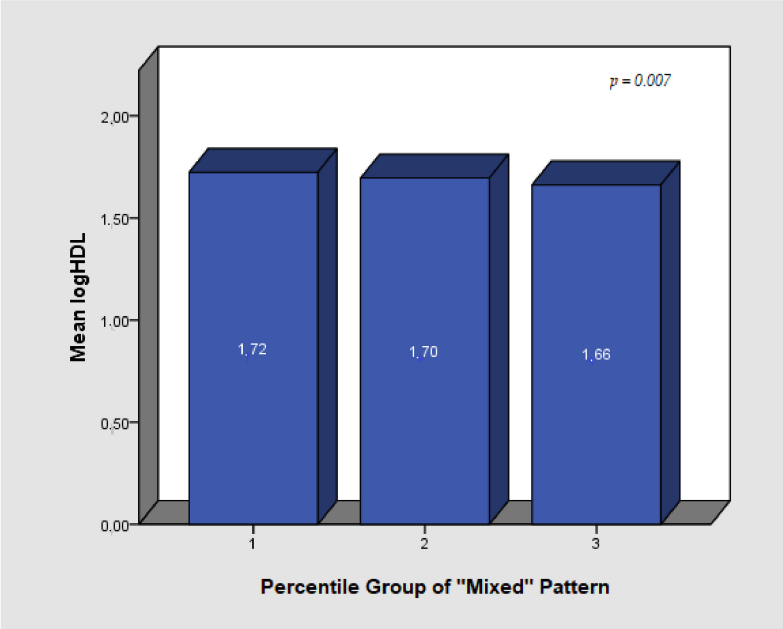


Figure 5. Percentile distribution and associations between the "Mixed" Pattern and logHDL values.

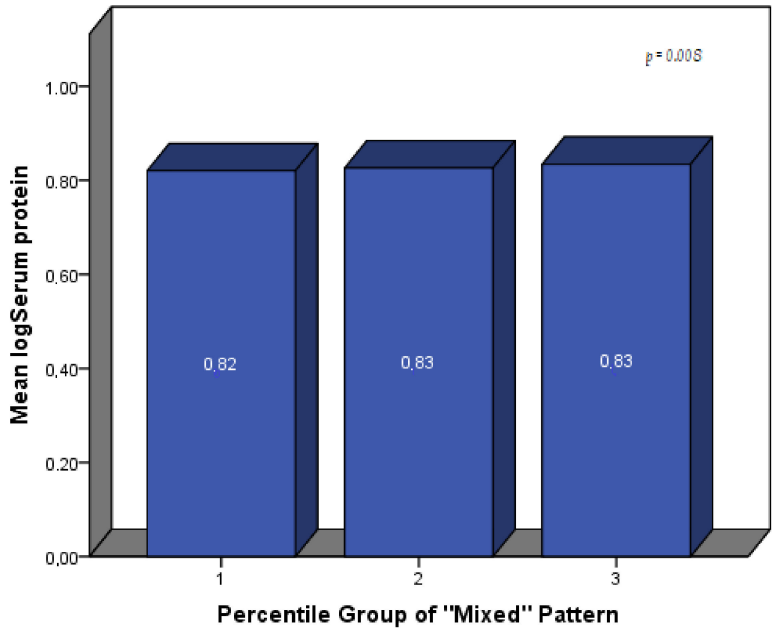


Figure 6. Percentile distribution and associations between the "Mixed" Pattern and logSerum Protein values.

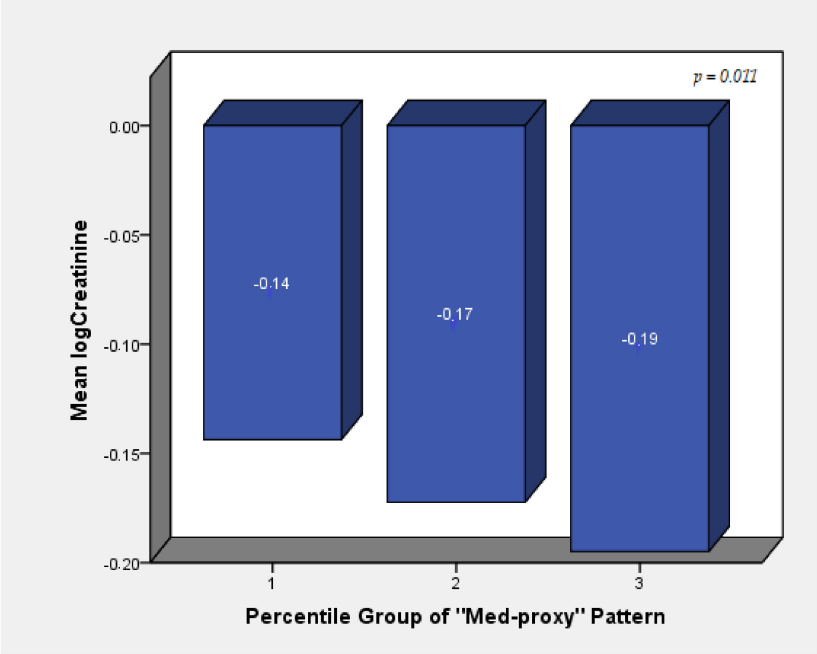


Figure 7. Percentile distribution and associations between the "Med-proxy" Pattern and logCreatinine values.

3.4. Lifestyle Index (LI) Construction

Following extraction of the dietary patterns, we examined the potential relations between different sets of variables, in order to evaluate the construction of a Lifestyle Index. We examined potential correlations between the reported lifestyle questionnaire scores, the extracted dietary patterns, and basic aspects, such as smoking and physical activity habits, with anthropometric indices, such as BMI and body fat percentage. All variables under examination were divided into categories, with higher values indicating favorable effects. Continuous and nominal variables displaying positive correlations were dichotomized

based on the sample's reported median values (attribution of a value of 1 for scores below the sample's median and a value of 2 for scores above the observed median).

Variables displaying statistically significant ($p < 0.05$), positive correlations with either logBMI of body fat percentage values included: the "Mixed" and "Med-proxy" dietary patterns, the CESD-R-10 depression scale score, and the physical component of the SF-12 scored questionnaire. Subsequently, a Lifestyle Index was created, based on the sum of the aforementioned, dichotomized variables and physical activity categories, as shown in Equation 1. Smoking habits were not included in the Index creation, due to roughly 75% of our sample being reported as non-smokers. Maximum score of the Lifestyle Index was calculated at the value of 11. The Index was calculated for 141 participants and the sample presented a median score of 8 and an IQR of 2.

$$\begin{aligned} LifestyleIndex \; &= Physical\ Activity\ Category + "Mixed" pattern\ dichotomized\ score + "Med \\ &- proxy" dichotomized\ score + CESD - R - 10\ dichotomized\ score + SF \\ &- PCS\ dichotomized\ score \end{aligned} \quad (1)$$

Variables displaying statistically significant ($p < 0.05$), positive correlations with either logBMI of body fat percentage values included: the "Mixed" and "Med-proxy" dietary patterns, the CESD-R-10 depression scale score and the physical component of the SF-12 scored questionnaire. Subsequently, a Lifestyle Index was created, based on the sum of the aforementioned, dichotomized variables and physical activity categories, as shown in Equation 1. Smoking habits were not included in the Index creation, due to roughly 75% of our sample being reported as non-smokers.

As depicted in Table 6, the Lifestyle index presented strong associations, including an inverse correlation with: (a) logBMI ($\beta = -0.010$. p=0.019), (b) logFasting glucose (Model 1: $\beta = -0.009$, $p = 0.007$. Model 2: $\beta = -0.007$. $p = 0.036$); and (c) logSGPT ($\beta = -0.027$, $p = 0.049$). When looking at the sex-stratified analyses. Women displayed negative associations between the Index's values and body fat percentage (Model 1: $\beta = -0.911$, $p = 0.030$). logFasting glucose (Model 1: $\beta = -0.011$, $p = 0.003$. Model 2: $\beta = -0.010$, $p = 0.007$). logSGOT/AST (Model 1: $\beta = -0.017$, $p = 0.026$. Model 2: $\beta = -0.018$, $p = 0.023$) and logSGPT/ALT (Model 1: $\beta = -0.039$. $p = 0.003$, Model 2: $= -0.038$, $p = 0.005$). Men also showed negative associations with logBMI ($\beta = -0.015$, $p = 0.045$) and body fat percentage (Model 1: $\beta = -1.123$, $p = 0.048$).

Table 6. Multivariate linear regressions between anthropometric and clinical characteristics and the constructed lifestyle index.

	Model 1									**Model 2**								
	Total *			**Women**			**Men**			**Total ****			**Women**			**Men**		
	β	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**
logBMI																		
Lifestyle Index	−0.010	0.004	0.019	−0.007	0.005	0.183	−0.015	0.007	0.045	–	–	–		–			–	
Body fat (%)																		
Lifestyle Index	−0.867	0.451	0.056	−0.911	0.414	0.030	−1.123	0.551	0.048	−0.307	0.393	0.436	−0.446	0.231	0.056	−0.446	0.339	0.572
logFasting glucose (mg/dL)																		
Lifestyle Index	−0.009	0.003	0.007	−0.011	0.003	0.003	−0.005	0.006	0.429	−0.007	0.003	0.036	−0.010	0.004	0.007	<0.001	0.006	0.953
logSGOT (IU/L)																		
Lifestyle Index	−0.006	0.009	0.534	−0.017	0.007	0.026	0.017	0.020	0.397	−0.006	0.010	0.518	−0.018	0.008	0.023	0.015	0.021	0.484
LogSGPT (IU/L)																		
Lifestyle Index	−0.027	0.014	0.049	−0.039	0.013	0.003	<0.001	0.023	0.988	0.292	0.277	0.293	−0.038	0.013	0.005	0.003	0.024	0.895

* Model 1: Adjusting for age. ** Model 2: Adjusting for age and logBMI baseline values.

4. Discussion

The present analyses display the design and the baseline population characteristics and dietary habits of the iMPROVE study. Overall, our baseline sample of 202 volunteers displayed satisfactory levels of lifestyle quality, with the majority of participants not reporting depression symptoms or heavily disrupted sleep quality.

Five dietary patterns were identified, including: (a) a "Mixed" pattern; (b) a pattern including food groups similar to those of the Mediterranean diet, entitled "Med-proxy" pattern; (c) the "Eating-out" pattern consisting of food combinations usually found in restaurants or fast-food environments (i.e., pies); (d) the "Traditional, vegetarian-alike" pattern, characterized by plant-based, Greek, traditional recipes; and (e) the "High in unsaturated fats and fruit juice consumption" pattern, including foods groups with high unsaturated fats and magnesium content (i.e., small fish and nuts) and highlighting representative habits of healthy snacking across Greek adults (i.e., olives, nuts and fruit juice). Interestingly, while the "Mixed" pattern included a vast majority of processed foods with added sugars and high fat content (i.e., chocolate, croissants, tray sweets, soft drinks, chips, seed oil, margarine, and butter), it was also characterized by light products and chicken and potatoes' consumption. This can be potentially attributed to the representative consumption of specific food groups by overweight and obese Greeks, who tend to adhere to short-term, self-imposed attempts to follow a more balanced diet. The latter do not result in successful weight management and/or weight loss efforts, but are exactly characterized by increased consumption of light products and simple food combinations, such as chicken and potatoes. The "Med-proxy" and the "Traditional, vegetarian-alike" patterns are representative of the dietary habits of the Greek population, evidently influenced by the Mediterranean diet and its increased content in fruit, vegetables, and legumes. Apparently, due to its high sugar and fat content, the "Mixed" pattern was associated with higher levels of anthropometric and biochemical characteristics. On the other hand, the plant-based, traditional recipes presented negative associations with body fat and positive relations with increased levels of HDL cholesterol. We further evaluated the within-group tertile categorization of adherence to each pattern, showing that higher tertiles were related to stronger associations for specific patterns, such as the positive relationship between adherence to the "Mixed" pattern and logBMI levels and the negative relationship between the increased adherence to the same pattern and logHDL values.

The concept of obese adults and the effect of dietary intake in the formation of their cardiometabolic profile display great interest, with current literature to be reporting similar findings to the ones outlined in the present study. Interestingly, the majority of studies aiming at identifying dietary patterns in overweight/obese populations, usually provide results for dietary habits adhering to the Western diet (including food groups with increased content of processed foods and/or foods in high fat and sugar content) or to a more balanced dietary pattern including fruit and vegetables, relating to higher and lower values of BMI, respectively [2]. Such patterns may include food combinations each time representative of the region of living, while maintaining a strong influence of the dietary habits and combinations usually found in the Western and/or the Mediterranean diet. A 2021 study by Saghafi-Asi et al. investigating the relationship between dietary patterns and biochemical biomarkers of 151 healthy obese Iranian adults, also underlined a positive association between a "Western" dietary pattern with high fat and sugar content and BMI and body fat levels [22]. Additionally, a different study in Romanian obese adults also underlined the identification of a "high meat/high fat", a "Western," and a "Prudent" pattern [23]. Similar findings were reported in a cohort of 410 Polish participants of a case-control study, where adherence to a pattern influenced by the Western one was related with higher levels of fat tissue and waist circumference, in contrast with the adherence to a "Healthy" pattern [24]. In their 2019 longitudinal study, Neri-Sanchez et al. also underlined the positive association between adherence to a "Risky" dietary pattern, including high fat and high sugar content, with the presence of central obesity in Mexican adults [25]. A different pattern consisting of poultry, vegetables, red meat, and red meat products,

among others, was also associated with obesity in male, Chinese adults, in a 2021 study of 1739 adults by Wang et al. [26].

Additionally, a different cross-sectional study of our group identified similar associations between dietary patterns and biochemical biomarkers in the adults of the POMAK population. More specifically, the dietary pattern including increased consumption of products high in sugars was related to low levels of HDL cholesterol [27]. Similar trends were also noted when investigating the dietary patterns of adolescent populations, where a dietary pattern with high protein and animal fat content was associated with elevated levels of logBMI and logTriglycerides, in French teenagers [28].

Furthermore, the development of the novel Lifestyle Index using the data deriving from the study sample, allowed for further investigation of the quality of life characteristics on the anthropometric and biochemical indices. Consisting of five variables, including two of the present dietary patterns extracted, the Index displayed negative associations with logBMI and body fat levels, as well as levels of the log-transformed variables of fasting glucose, SGOT, and SGPT. Thus, LI confirmed that higher quality of dietary intake and higher levels of physical activity reduced depression symptoms and improved self-reported conception of health status and may display a protective effect on body composition, as well as a favorable influence on improved glycemic profile.

Overall, development of lifestyle indices as a means of quantifying and evaluating the potential influence of specific lifestyle aspects on body weight is mounting, as analyzed in the beginning of the paper, lifestyle indices can also incorporate dietary information via calculation of diet quality indices. A 2017 systematic review of 34 studies by Asghari et al., sought to investigate the effect of diet quality indices in obesity-related traits, showing that Healthy Eating Index (HEI) displayed an inverse association with obesity. The same review also concluded that different dietary scores, in general, did not efficiently assess diet quality, with most significant findings being presented in populations of the United States [29]. Furthermore, different research groups have investigated the effect of lifestyle characteristics, such as sedentary behavior and screen-time, in adolescent populations [30–32]. In adults, current research refers to potential associations between constructed lifestyle indices and specific diseases or disease-related outcomes, namely cardiovascular disease [8], cancer [33], and type 2 diabetes [34]. Lenz et al., showed that creation of a Lifestyle Index for evaluation of life quality in adults at risk for cardiovascular disease can be a useful tool [35]. Furthermore, in a similar effort to evaluate the lifestyle aspects and weight characteristics, Roda et el in 2016, also investigated the potential effect of sleep qualities, screen time, and dietary intake, among others, highlighting a strong positive association between sedentary behavior and overweight [36].

A major advantage of the present study is the use of the online assessment tool, as a means enabling long-distance communication and monitoring, during the time of social distancing, due to the novel coronavirus disease 19 (COVID-19) pandemic. On the other hand, limitations of the present study include: (a) the substantial impact of the COVID-19 pandemic on volunteer recruitment rates. More specifically, conduct of the study's volunteer recruitment took place exactly in the midst of the COVID-19 pandemic, which resulted in a limited recruitment capacity due to: (a) The social-distancing protocols implemented in recruitment sites, properly adhering to the state guidelines, which resulted in a restricted number of participants visiting the premises; (b) the limited expression of interest for participation in the study, due to fear of in-person meetings and subsequent spread of the COVID-19 disease; (c) the long-distance maintenance of an increased adherence rate to the proposed diets, due to the extended time period between the in-person follow-up meetings; and (d) the proper use of the online assessment tool by older adults who had both limited access and knowledge on the use of state-of-the-art technological devices and online tools.

5. Conclusions

Results from the present study suggest that the iMPROVE overweight and obese, adult cohort displays a satisfactory level of lifestyle characteristics and dietary behaviors

representative of the overweight and obese Greek population. Assessment of the constructed Lifestyle Index, further solidifies the validity of our findings, highlighting the protective effect of increased lifestyle quality in the formation of elevated body weight. In this spectrum, the findings of the present paper enhance the understanding of overweight/obesity lifestyle determinants in our sample population and lay the ground for the next analysis steps of the iMPROVE study, which focus on assessing the impact of the proposed intervention and the role of candidate genes in various weight-related indices. Assessment of the holistic interplay of gene-lifestyle interactions is of vital importance for the in-depth understanding of nutrigenetic influences in weight loss, as well as in the general context of weight management and/or weight loss maintenance.

Author Contributions: Conceptualization, G.V.D. and A.C.K.; methodology, G.V.D. and A.C.K.; software, I.V.; validation, M.K. and I.P.K.; formal analysis, M.K. and I.P.K.; investigation, M.K., I.P.K., E.A.K.; resources, G.V.D.; data curation, M.K., E.A.K., S.L.; writing—original draft preparation, M.K., I.P.K.; writing—review and editing, I.P.K., M.K.; visualization, G.V.D. and A.C.K.; supervision, G.V.D., A.C.K.; project administration, G.V.D.; funding acquisition, G.V.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Research Ethics Committee of Harokopio University of Athens (Protocol Number: 1800/13-06-2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to participants' privacy and ethical restrictions.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Continuation of Table 5, with analyses of variables that did not display statistically significant changes (to be uploaded as Table A1).

Table A1. Multivariate linear regressions between the extracted dietary patterns and indices of anthropometric and biochemical characteristics.

	Model 1			**Model 2**			**Model 3**		
	β	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**
logGlucose (mg/dL)									
Mixed Pattern	0.006	0.004	0.127	0.001	0.004	0.803	0.001	0.004	0.803
Med-proxy Pattern	<0.001	0.004	0.980	0.002	0.004	0.560	0.002	0.004	0.560
Eating-out Pattern	0.002	0.004	0.635	0.001	0.004	0.862	0.001	0.004	0.862
Traditional, vegetarian-alike Pattern	0.003	0.004	0.462	0.001	0.004	0.871	0.001	0.004	0.871
High in unsaturated fats Pattern	−0.003	0.004	0.380	−0.005	0.004	0.222	−0.005	0.004	0.222
logUrea (mg/dL)									
Mixed Pattern	0.004	0.007	0.566	0.002	0.008	0.814	0.001	0.009	0.932

Table A1. *Cont.*

	Model 1			Model 2			Model 3		
	β	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**	***β***	***SE***	***p*-Value**
Med-proxy Pattern	0.010	0.008	0.204	0.012	0.008	0.145	0.012	0.009	0.196
Eating-out Pattern	−0.005	0.007	0.498	−0.005	0.007	0.467	−0.008	0.008	0.349
Traditional, vegetarian-alike Pattern	<0.001	0.007	0.989	−0.001	0.008	0.847	−0.004	0.008	0.622
High in unsaturated fats Pattern logUric Acid(mg/dL)	−0.002	0.007	0.758	−0.002	0.008	0.791	−0.004	0.008	0.637
Mixed Pattern	0.006	0.006	0.377	−0.006	0.007	0.361	−0.004	0.007	0.628
Med-proxy Pattern	−0.004	0.007	0.585	−0.001	0.007	0.854	<0.001	0.007	0.963
Eating-out Pattern	0.003	0.006	0.603	0.002	0.006	0.749	−0.002	0.006	0.805
Traditional, vegetarian-alike Pattern	−0.002	0.006	0.739	−0.004	0.006	0.506	−0.004	0.007	0.502
High in unsaturated fats Pattern Total Cholesterol (mg/dL)	−0.006	0.006	0.336	−0.006	0.006	0.358	−0.007	0.007	0.331
Mixed Pattern	−0.259	2.402	0.914	1.283	2.575	0.619	0.922	2.915	0.752
Med-proxy Pattern	−3.271	2.534	0.198	−2.864	2.622	0.276	−3.629	2.860	0.206
Eating-out Pattern	−0.080	2.394	0.973	−0.274	2.414	0.910	−0.631	2.601	0.809
Traditional, vegetarian-alike Pattern	2.297	2.297	0.323	3.766	2.463	0.128	4.722	2.618	0.073
High in unsaturated fats Pattern logLDL Cholesterol (mg/dL)	−0.202	2.445	0.934	0.819	2.519	0.746	0.103	2.711	0.970
Mixed Pattern	−0.007	0.010	0.492	0.005	0.010	0.613	0.002	0.011	0.878
Med-proxy Pattern	−0.008	0.010	0.430	−0.005	0.010	0.642	−0.008	0.011	0.491
Eating-out Pattern	−0.003	0.010	0.751	−0.004	0.009	0.659	−0.006	0.010	0.557
Traditional, vegetarian-alike Pattern	−0.006	0.009	0.539	0.002	0.010	0.816	0.005	0.010	0.625
High in unsaturated fats Pattern logAlbumin(g/dL)	−0.010	0.010	0.321	−0.003	0.010	0.755	−0.003	0.011	0.746
Mixed Pattern	0.002	0.004	0.665	0.003	0.004	0.412	0.003	0.002	0.199
Med-proxy Pattern	−0.002	0.004	0.691	−0.002	0.004	0.584	0.002	0.002	0.289
Eating-out Pattern	<0.001	0.004	0.904	0.001	0.004	0.759	<0.001	0.002	0.977
Traditional, vegetarian-alike Pattern	0.003	0.004	0.368	0.003	0.004	0.508	0.003	0.002	0.115
High in unsaturated fats Pattern	−0.002	0.004	0.606	−0.003	0.004	0.441	0.001	0.002	0.733

Model 1: Adjusting for age and sex. Model 2: Adjusting for age, sex, smoking habits, physical activity level, and logBMI (except for logBMI values). Model 3: Adjusting for age, sex, smoking habits, physical activity level, logBMI, education years, family, and professional status.

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Appendix D: Scientific Publications on the 2018 Gutenberg Project

nutrients

MDPI

Article

Dietary Patterns, Blood Pressure and the Glycemic and Lipidemic Profile of Two Teenage, European Populations

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Abstract: The present study sought to retrospectively investigate the dietary habits of two adolescent, European populations from the cross-sectional Greek TEENAGE Study and French STANISLAS Family Study. We aimed to explore the relation between the populations' dietary patterns and blood pressure, glycemic and lipidemic profile. Dietary patterns were extracted via Principal Component Analysis (PCA), based on data collected from two 24 h dietary recalls for the TEENAGE study and a 3-day food consumption diary for the STANISLAS study. Multiple linear regressions and mixed models analyses, adjusting for confounding factors, were employed to investigate potential associations. A total of 766 Greek teenagers and 287 French teenagers, were included in analyses. Five dietary patterns were extracted for each population accounting for 49.35% and 46.69% of their respective total variance, with similarities regarding the consumption of specific food groups (i.e., western-type foods). In the TEENAGE Study, the "chicken and sugars" pattern was associated with lower CRP levels, after adjusting for confounding factors (p-value < 0.01). The "high protein and animal fat" dietary pattern of the STANISLAS Family Study was related to higher BMI (p-value < 0.01) and higher triglycerides levels (p-value < 0.01). Our findings summarize the dietary habits of two teenage, European populations and their associations with cardiometabolic risk factors.

Keywords: dietary patterns; teenagers; European populations; blood pressure; glucose; cholesterol; triglycerides; cardiometabolic risk factors

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1. Introduction

Adolescence constitutes a period of increased nutritional needs, required to support the physical growth that accompanies puberty [1,2]. Healthy eating is of vital importance during adolescence [3,4], in order to ensure the sufficient macronutrient and micronutrient intake needed for proper physical development [1], cognitive performance [5–7] and good mental health [8]. Dietary habits during the adolescent years directly influence body weight regulation and play a major role in the healthy development that comes with adolescence [9]. Adherence to "unhealthy" eating habits during this period increases the risk of obesity development [10,11], which has, in turn, been long associated with an increased risk of non-communicable disease manifestation, such as type 2 diabetes, both in adolescence and later on in adult life [9,10]. Indeed, the presence of adolescent obesity has been associated with severe obesity in late adulthood [12,13] and a greater risk for type 2 diabetes development in early adulthood [13]. In addition, higher Body Mass Index (BMI) values during adolescence have been associated with higher BMI values during adulthood, as well as a 30 to 40% increased risk in adult mortality [14].

The causes of overweight, obesity and non-communicable disease development in adolescent populations are related to the consumption of energy-dense foods, reduction of physical activity, as well as socioeconomic factors, such as food availability and food preference, influenced by geographic factors [9,11]. Energy-dense foods have been related both directly and indirectly, via their positive association, with overweight and obesity development, in the development of non-communicable diseases [11]. Indeed, poor eating habits have regularly been associated with a high consumption of foods with high fat and/or sugar contents [9].

Adolescent dietary habits are also directly linked to the teenagers' metabolic profile and the interplay between biomarkers of glycemic and lipidemic control [15]. It has been shown that adherence to an "unhealthy" dietary pattern is associated with a higher risk for metabolic syndrome presence [15]. Their importance is further highlighted by the increased incidence of type 2 diabetes in young children and teenagers [16]. Consumption of energy-dense foods in children and teenagers with a family history of type 2 diabetes, plays a central role in the formation of a worse glycemic profile and potentially, subsequent development of type 2 diabetes (T2D) [16]. T2D in children is associated with a deteriorated lipidemic profile (i.e., dyslipidemia), as a direct effect of the observed insulin resistance [17]. A different study showed that Greek children with dyslipidemia and unfavourable dietary habits, such as consuming only one meal per day, displayed higher levels of various biomarkers of lipidemic control, namely total cholesterol and low-density lipoprotein cholesterol (LDL-C) [18].

Another cardiometabolic risk factor receiving more and more attention is the development of hypertension and the elevated levels of arterial pressure in adolescents. Indeed, high blood pressure can be met in teenagers, with boys reporting higher levels of blood pressure than girls [19].

The present analyses constitute the first step in the context of the 2018 Gutenberg Chair project, aimed at firstly investigating the role of dietary habits in the anthropometric and biochemical profile of two adolescent, European populations and subsequently exploring the potential role of nutrition as a modifier of genetic make-up in adolescence. The latter will take place via an investigation into the relationship between the populations' dietary habits and their glycemic and lipidemic profile and inflammation markers with genetic risk scores created for anthropometric indices, biomarkers of glycemic and lipidemic control and inflammation markers.

In this context, the aim of the present study is to investigate the dietary habits of the two populations from the Greek TEENAGE Study and the French STANISLAS Family study and their potential associations with blood pressure, biomarkers of glycemic and lipidemic control and levels of C-reactive protein (CRP). Therefore, the objectives of the study are formed as follows: (a) to identify the dietary patterns of adolescents in the Greek and French cohorts; and (b) to investigate potential, respective associations between said patterns and blood pressure, anthropometric indices, biomarkers of glycemic and lipidemic control and CRP levels.

2. Materials and Methods

2.1. The TEENAGE Study Cohort

The TEENAGE (TEENs of Attica: Genes and Environment) study constitutes a cross-sectional study conducted during the period 2008–2010 in the region of Attica, Greece [20,21]. The study was approved by the Institutional Review Board of Harokopio University and the Greek Ministry of Education and Religious Affairs and took place adhering to the guidelines of the Declaration of Helsinki [22]. The study consisted of a sample of healthy, Greek adolescent students residing in the Attica region during the period of recruitment.

All students and their parents received written information on the aims and the procedures of the study prior to enrolment and all participants provided written consent. All students enrolled participated in an assessment session with either a nutrition or

a pediatric health-care professional, which included clinical examination, collection of blood samples, conduct of a 24 h dietary and physical activity recall and collection of anthropometric and lifestyle data. A second 24 h dietary and physical activity recall was conducted via telephone, 3 to 10 days after the in-person meeting. Overall, data for an original sample of 857 adolescent students from 1440 schools in the region of Attica, aged 13 to 15 years old, were cross-sectionally collected.

Collection of anthropometric data during the in-person meeting consisted of height (measured to the nearest 0.1 cm), weight (measured to the nearest 0.1 kg), waist and hip circumference and skinfold measurements (measured to the nearest 0.1 mm). Height was measured using a portable stadiometer, where participants were barefoot, looking ahead and with relaxed shoulders. Weight was measured via use of scales, where participants were barefoot and with light clothing. BMI was calculated as weight divided by height (kg/m^2). Waist and hip circumference were measured using a soft tape, the former between the twelfth rib and the iliac crest and the latter at the widest point of the hips. Two skinfold measurements were collected for each of the triceps, subscapular and suprailiac skinfolds, using the Lange skinfold calipers.

Assessment of dietary habits took place via the collection of the two non-consecutive 24 h dietary recalls, which were conducted on different days of the week. Analysis of the data collected took place via use of the Nutritionist Pro software, version 2.2 [23]. The ratio of reported energy intake to BMR was calculated for each student, in order to assess potential under-reporting. BMR was calculated using the Schofield equations [24,25] and cut-off points [26] were adapted to the ones reported for children and adolescents [27]. Participants who had previously reported dieting in the past or never dieting, were excluded.

For the purposes of the present study, we used the available anthropometric, biochemical and dietary data of 766 adolescent students (as shown in Table 1). Dietary pattern extraction was based on the mean consumption of food groups, derived from the two non-consecutive 24 h dietary recalls.

Table 1. Anthropometric, biochemical and dietary characteristics of the TEENAGE Study population.

	TEENAGE Study						
	All		**Boys**		**Girls**		***p*-Value ***
	n	**Median (IQR)**	***n***	**Median (IQR)**	***n***	**Median (IQR)**	
Age (years)	766	13.30 (1.31)	349	13.36 (1.38)	417	13.26 (1.25)	<0.001
Weight (kg)	766	55.00 (14.00)	349	56.00 (16.00)	417	54.00 (13.00)	0.001
Body Mass Index (BMI) (kg/m^2)	766	20.88 (4.38)	349	20.85 (4.45)	417	20.93 (4.37)	0.517
Waist-to-hip ratio (WHR)	763	0.76 (0)	349	0.79 (0)	414	0.73 (0)	<0001
Systolic Blood Pressure (SBP) (mmHg)	743	119.00 (16)	335	120.67 (11.93) **	408	118.00 (15)	0.001
Diastolic Blood Pressure (DBP) (mmHg)	743	70.00 (12)	335	71.00 (12)	408	70.00 (12)	0.825
Energy Intake (kcal/day)	766	1741.00 (760)	349	1939.00 (779)	417	1574.00 (609)	<0.001
Glucose (mg/dL),	611	80.00 (12)	283	81.00 (11)	328	79.00 (12)	<0.001
HOMA-IR	539	2.28 (2)	255	2.12 (2)	284	2.37 (2)	<0.001
Insulin (mg/dL)	539	11.00 (7)	255	10.00 (7)	284	12.00 (8)	<0.001
Total Cholesterol (mg/dL)	611	157.00 (33)	283	156.49 (25.18) **	328	157.50 (31)	0.210
Low density lipoprotein Cholesterol (LDL-C) (mg/dL)	611	54.00 (16)	283	90.57 (21.78) **	328	88.40 (26)	0.651
High Density Lipoprotein Cholestrol (HDL- C) (mg/dL)	611	89.20 (27)	283	53.00 (16)	328	56.00 (17)	0.001
Triglycerides (mg/dL)	611	56.00 (24)	283	55.00 (25)	328	57.00 (24)	0.090
C-reactive protein (CRP) (mg/dL)	540	0.30 (1)	254	0.45 (1)	286	0.20 (0)	<0.001

* All hypothesis testing took place via use of the Mann–Whitney test. ** Variable follows the normal distribution and is presented as mean ± sd.

2.2. The STANISLAS Family Study Cohort

The STANISLAS (Suivi Temporaire Annuel Non Invasif de la Sante des Lorrains Assures Sociaux) Family Study constitutes a cross-sectional study conducted during the period 1993–1995 in the region of Vosges and the South of Meurthe and Moselle (East part of France) [28,29]. The study was approved by the advisory committee for the protection of people in biomedical research in Nancy, France. The study consisted of a sample of nuclear families with parents aged up to 65 years old and children older than 6 years at the time of recruitment, residing in the aforementioned region. The study only included families with healthy family members, reporting no comorbidities and/or chronic diseases, residing in the aforementioned regions at the time of recruitment. Willing participants residing in the region of Nancy further participated in 5-year follow-ups up to the period 2003–2005 [30].

All included families provided informed consent. The families enrolled participated in an in-person session with trained professionals, which included clinical examination, collection of blood samples and collection of anthropometric, dietary and lifestyle data. Collection of the food-related surveys was conducted by dietitians. Blood pressure, pulse rate, skinfold thickness and bone density were measured by nurses and pubertal development and family history of cardiovascular diseases was assessed by general practitioners. Data on alcohol and tobacco consumption, physical activity, education and socio-professional status were collected through questionnaires, under the supervision of trained nurses. Overall, data for an original sample of 1006 families were cross-sectionally collected.

Weight, height, waist-to-hip ratio and impedancemetry measurements were conducted by technical operators. BMI was, again, calculated as weight divided by height (kg/m^2). Assessment of dietary habits took place via collection of a 3 day food consumption diary, for two continuous days within the week and one day of the weekend. Analysis of the data took place via use of the GENI package, nutritional database program [31].

For the purposes of the present study, we used the available anthropometric, biochemical and dietary data of 287 adolescents at the time of the baseline recruitment (as shown in Table 2). Dietary pattern extraction was based on the mean consumption of food groups, deriving from the 3 day food consumption diary. Low-density cholesterol (LDL-C) for this cohort was calculated based on the available data for total cholesterol (TC), high-density lipoprotein cholesterol (HDL- C) and triglyceride (TG) levels, using the Friedeweld Equation, as follows [32]:

$$\mathrm{LDL} - \mathrm{C} = (\mathrm{TC}) - (\mathrm{HDL} - \mathrm{C}) - (\mathrm{TG}/5)$$

2.3. Statistical Analysis

The entirety of the data handling and data analyses was carried out using the SPSS Software [33]. Body Mass Index (BMI) was calculated as weight divided by height (kg/m^2). Assessment of the variables' distribution was conducted via use of the Shapiro–Wilk test, demonstrating the mean and standard deviation for all normally distributed variables and the median and interquartile range for all variables not following the normal distribution (Shapiro–Wilk p-value > 0.05). We used the Student's t-test and Mann–Whitney test for all hypotheses testing for continuous variables.

We performed Principal Component Analyses (PCA) in order to extract all dietary patterns for both populations [34]. PCA constitutes an epidemiological tool, largely used in the assessment of dietary data and the subsequent extraction of dietary patterns [35], having been previously tested in large young populations [36]. PCA was conducted on 15 food groups for the TEENAGE study population and 15 food groups for the STANISLAS Family study population, based on the available data for the cohorts.

The Kaiser–Meyer–Olkin (KMO) test was calculated at 0.545 and 0.576 for the TEENAGE and the STANISLAS teenagers, respectively, indicating mediocre to sufficient data adequacy. The varimax orthogonal rotation was used for the extraction of the patterns and the Kaiser criterion was set at retaining 5 components with Eigen values bigger than 1.

Table 2. Anthropometric, biochemical and dietary characteristics of the STANISLAS Family Study population.

	STANISLAS Family Study						
	All		Boys		Girls		*p*-Value *
	n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	
Age (years)	287	13.08 (2.92)	137	13.08 (2.92)	150	13.08 (2.85)	0.416
Weight (kg)	263	46.59 (18.10)	129	47.20 (21.90)	134	46.05 (14.84)	0.136
Body Mass Index (BMI) (kg/m^2)	263	18.44 (3.61)	129	18.30 (3.20)	134	18.52 (4.18)	0.853
WHR	221	0.77 (0.04) **	110	0.81 (0.03) **	111	0.75 (0.06)	<0.001
Systolic Blood Pressure (SBP) (mmHg)	263	112.00 (14.50)	129	115.60 (11.53) **	134	110.46 (8.76) **	<0.001
Diastolic Blood Pressure (DBP) (mmHg)	263	57.00 (15.50)	129	56.69 (16.00) **	134	57.02 (10.23) **	0.829
Energy Intake (kcal/d)	287	2056.03 (662.24)	137	2070.99 (495.20) **	150	2094.92 (681.16)	0.469
Glucose (mg/dL),	263	88.28 (6.12) **	129	89.18 (6.48) **	134	87.38 (5.76) **	0.018 ***
Total Cholesterol, (mg/dL)	263	179.15 (40.93)	129	173.36 (30.89) **	134	183.01 (36.29)	0.002
Low density lipoprotein cholesterol (LDL-C) (mg/dL)	263	116.99 (33.98)	129	113.13 (28.19) **	134	120.85 (32.05)	0.030
High density lipoprotein cholesterol (HDL-C)(mg/dL)	263	54.05 (20.08)	129	54.44 (15.44) **	134	156.37 (16.99)	0.222
Triglycerides (mg/dL)	263	51.33 (33.63)	129	52.21 (38.05)	134	46.56 (30.09)	0.930
C-reactive protein (CRP) (mg/L)	243	0.30 (0.53)	118	0.32 (0.54)	125	0.26 (0.55)	0.765

* Hypothesis testing took place via use of the Mann–Whitney test wherever at least one variable did not follow the normal distribution. ** Variable follows the normal distribution and is presented as mean ± sd. *** Hypothesis testing took place via the Student's Independent Samples *t*-test.

We further tested for potential associations between the extracted dietary patterns, blood pressure and biomarkers of glycemic and lipidemic control, as well as levels of CRP, via use of multiple linear regressions in the TEENAGE cohort and linear mixed models in the STANISLAS cohort. Given that the STANISLAS Family Study consisted of a cohort of families, we used the latter in order to correct for the potential familial bias of siblings included in the analyses [37,38]. We classified the different siblings of each family as the repeated measures, compound symmetry as the repeated covariance type and all adjusting factors and dietary patterns as the fixed effects. Potential associations were investigated, adjusting for 3 different models of confounding factors. Model 1 included adjustment solely for the age and sex of the participants; Model 2 included adjustment for sex, age and level of physical activity; Model 3 consisted of adjustment for their age, sex, level of physical activity and BMI; and, finally, Model 4 included adjustment for age, sex, physical activity, BMI and energy intake. All tested variables were log-transformed. Multiple linear regression results are presented as beta coefficients (β) and standard error (SE). Linear mixed model results are presented as estimates and standard error (SE). All statistical analyses included the level of nominal significance set at $\alpha = 0.05$. The adjusted threshold after multiple testing was set to (0.05/5 components examined, i.e., dietary patterns = 0.01).

3. Results

3.1. Descriptive Characteristics

The anthropometric and biochemical characteristics of the two populations are depicted in Tables 1 and 2. Concerning the TEENAGE cohort, a total of 766 teenagers (45.56% boys, 54.43% girls), with a median age of 13.30 years, were included in the analyses. The STANISLAS cohort provided data for 287 teenagers (47.73% boys, 525.26% girls), with a median age of 13.08 years.

The daily energy intake for the two populations by sex, is depicted in Figure 1. As shown in Tables 1 and 2, the Greek teenagers reported a median energy intake of 1741.00 kcal/d (IQR = 760), significantly different between the two genders, with boys reporting a higher intake. The French teenagers reported a median energy intake of 2056.03 kcal/d (IQR = 662.24), without presenting significant differences between sexes.

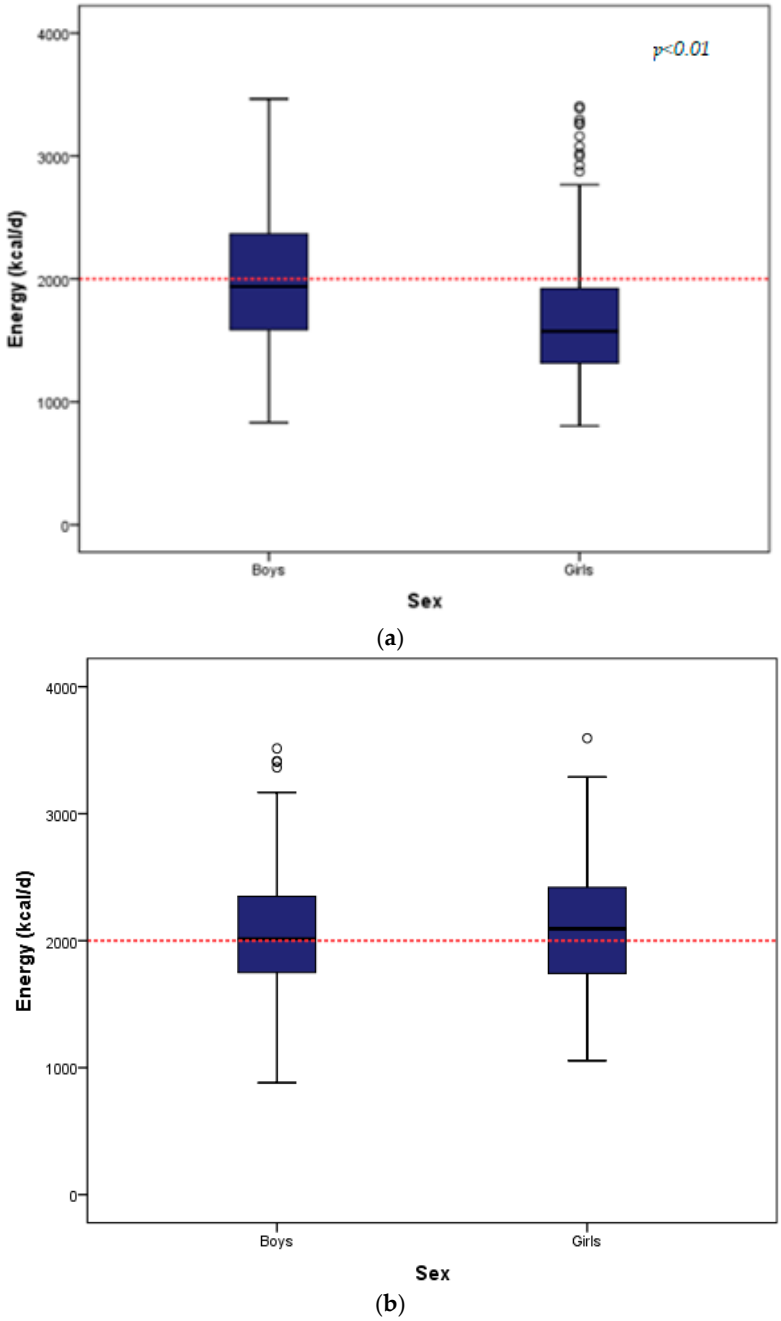

Figure 1. (**a**) Boxplot of daily caloric intake in the TEENAGE study. (**b**) Boxplot of daily caloric intake in the STANISLAS Family Study.

3.2. Extraction of Dietary Patterns

PCA for the TEENAGE cohort resulted in the identification of 5 dietary patterns, accounting for 49.35% of the sample's total variance. Food groups' factor loadings in the respective patterns are presented in Table 3.

The presented factor loadings depict each food group's highest contribution and subsequent inclusion in one out of the five patterns (components) highlighted. Therefore, the dietary patterns formed are the following: (a) a "western breakfast" dietary pattern, consisting of cheese, dairy and processed meat, accounting for the highest percentage of the individual variance explained (15.61%); (b) a "legumes and good fat" pattern, including high consumption of legumes, olives, olive oil and nuts and accounting for 10.32% of the variance explained; (c) a "homemade meal" pattern, referring to high consumption of

red meat and potatoes, associated with lower fish consumption and explaining 8.33% of the total variance; (d) a "chicken and sugars" pattern, including high consumption of chicken and sweets, associated with lower the consumption of fruits and juices, with a 7.60% of the variance explained; and (e) a "eggs and fibers" pattern, comprising of high consumption of non-refined cereals, vegetables and eggs, associated with lower refined cereals' consumption and explaining 7.47% of the total variance.

Table 3. Principal Components Analysis' factor loadings for the 15 food groups in the TEENAGE study (n = 766).

	Component				
Food Groups	**1**	**2**	**3**	**4**	**5**
Cheese	0.897	-	-	-	-
Dairy	0.863	-	-	-	-
Processed Meat	0.635	-	-	-	-
Legumes	-	0.739	-	-	-
Olives, Olive Oil, Nuts	-	0.668	-	-	-
Red Meat	-	-	0.712	−0.429	-
Potatoes	-	-	0.661	-	-
Fish	-	−0.358	−0.480	-	-
Chicken	-	-	-	0.649	-
Sweets	-	-	-	0.518	-
Fruit and Juices	-	-	-	−0.368	-
Non-refined cereals	-	-	-	-	0.674
Vegetables	-	-	-	-	0.342
Eggs	-	-	-	-	0.303
Refined Cereals	0.512	-	-	-	−0.595
Total Variance Explained (%)	15.61	10.32	8.33	7.60	7.47

Only loadings with an absolute values > 0.3 are presented in the table.

PCA for the STANISLAS cohort resulted in the identification of 5 dietary patterns accounting for 46.69% of the sample's total variance. Food groups' factor loadings in the respective patterns are presented in Table 4.

In a similar way to the aforementioned, the presented factor loadings depict each food group's highest contribution and subsequent inclusion in one out of the five patterns (components) highlighted. Therefore, the dietary patterns formed for this cohort are the following: (a) a "western breakfast" dietary pattern, consisting of cheese, breads and flours, processed meat and vegetables and accounting for the highest percentage of the individual variance explained (10.58%); (b) a "prudent snacking" pattern, including high consumption of eggs and vegetable fats, lower consumption of salty snacks and accounting for 10.44% of the variance explained; (c) a "high protein and animal fat" pattern, referring to consumption of red meat, animal fat and milk and yogurt, explaining 9.26% of the total variance; (d) a "fish and seafood" pattern, including high consumption of fish and seafood and lower consumption of poultry, with a 8.19% of the variance explained; and (e) a "sugary snacks" pattern, comprising of consumption of soft drinks, sugars, sweets and cereal bars and explaining 8.19% of the total variance.

3.3. Multiple Linear Regressions in the TEENAGE Study

The multiple linear regressions adjusted for the three models of confounding factors, as described above, are shown in Table 5. Based on the available data, we examined associations between the patterns and the log-transformed values for BMI, WHR, SBP, DBP, glucose, insulin, HOMA-IR, TC, HDL- C, LDL- C, TG and CRP levels. The "legumes and good fat" pattern was associated with lower values of logBMI ($\beta = -0.006$, p-value = 0.017) and logInsulin ($\beta = -0.020$, p-value = 0.030), after the adjustments of Model 1. The "homemade meal" pattern was associated with lower values of logBMI ($\beta = -0.005$, p-value = 0.042), adjusting for Model 1. The "chicken and sugars" pattern was slightly associated with log-

Glucose Model 1 (β = 0.015, *p*-value = 0.017). The same pattern was associated with lower values of logInsulin after adjusting for Model 1 (β = −0.020, *p*-value = 0.030), Model 3 (β = 0.018, *p*-value = 0.049) and Model 4 (β = 0.018, *p*-value = 0.041). Moreover, the latter was further associated with lower values of logCRP in all models (Model 1: β = −0.051, *p*-value = 0.006, Model 2: β = −0.057, *p*-value = 0.004, Model 3: β = −0.050, *p*-value = 0.008, Model 4: β = −0.051, *p*-value = 0.008). No associations were found between the "eggs and fibers" pattern and the variables in all models. Statistically significant associations after assessment of the adjusted threshold were only maintained for the "legumes and good fat" pattern and the "chicken and sugars" pattern and logCRP in all models.

Table 4. Principal Components Analysis' factor loadings for the 15 food groups in the STANISLAS Family study. (*n* = 287).

	Component				
Food Groups	**1**	**2**	**3**	**4**	**5**
Cheese	0.664	-	-	-	-
Breads and Flours	0.605	-	-	-	-
Processed Meat	0.523	-	-	-	-
Vegetables	0.483	-	-	-	-
Eggs	-	0.630	-	-	-
Salty Snacks	-	−0.580	-	-	-
Vegetable Fat	-	0.576	-	-	-
Red Meat	-	-	0.703	-	-
Animal Fat	-	-	0.610	-	-
Milk and Yogurt	-	-	0.473	−0.338	-
Fish	-	-	-	0.666	-
Seafood	-	-	-	0.628	-
Poultry	-	-	-	−0.380	-
Soft Drinks	-	-	-	-	0.777
Sugars, Sweets and Cereal Bars	-	-	-	-	0.746
Total Variance Explained (%)	10.58	10.44	9.26	8.19	8.19

Only loadings with an absolute values > 0.3 are presented in the table.

3.4. Linear Mixed Models in the STANISLAS Family Study

The linear mixed models adjusted for the three models of confounding factors, as described above, are shown in Table 6. Based on the available data, we examined associations between the patterns and the log-transformed values for BMI, WHR, SBP, DBP, glucose, TC, HDL-C, LDL-C, TG and CRP levels. The "western breakfast" pattern was associated with lower values of logCRP, in Model 4 (est = −0.076, *p*-value = 0.024). The "high protein and animal fat" pattern was associated with higher values of logBMI after adjustment for Models 1 and 2 (est = 0.011, *p*-value = 0.002, est = 0.009, *p*-value = 0.020), lower values of logDBP adjusting for Models 3 and 4 (est = −0.010, *p*-value = 0.045, est = −0.012, *p*-value=0.028, respectively) and higher values of logTriglycerides in all models (Model 1: est = 0.054, *p*-value < 0.001; Model 2: est = 0.049, p-value = 0.001; Model 3: est = 0.045, *p*-value = 0.002, Model 4:est = 0.041, *p*-value = 0.009) The "fish and seafood" pattern was associated with lower logDBP values (est = 0.009, *p*-value = 0.039), in Model 1. The "sugary snacks" pattern was associated with lower values of logHDL-C (est = −0.014, *p*-value = 0.049) in Model 3. No associations were found between the "prudent snacking" pattern and the variables in all models. Statistically significant associations after assessment of the adjusted threshold were only maintained for the maintained for the "high protein and animal fat" pattern and logBMI, in Model 1, as well as logTriglycerides in all models. Table 5. Linear Regression Analyses on the association between the dietary patterns, anthropometric indices and biomarkers of glycemic and lipidemic control in the TEENAGE study.

Table 5. Linear Regression Analyses on the association between the dietary patterns, anthropometric indices and biomarkers of glycemic and lipidemic control in the TEENAGE study.

	Model 1			Model 2			Model 3			Model 4		
	β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*
LogBMI												
Western Breakfast	−0.004	0.003	0.150	−0.003	0.003	0.308	-	-	-	-	-	-
Legumes and Good Fat	−0.006	0.003	0.017	−0.004	0.003	0.194	-	-	-	-	-	-
Homemade Meal	−0.005	0.003	0.042	−0.003	0.003	0.242	-	-	-	-	-	-
Chicken and Sugars	−0.005	0.003	0.069	−0.004	0.003	0.128	-	-	-	-	-	-
Eggs and Fibers	0.004	0.003	0.111	0.004	0.003	0.115	-	-	-	-	-	-
LogWHR												
Western Breakfast	0.013	0.012	0.270	0.016	0.13	0.247	0.017	0.013	0.198	0.017	0.014	0.250
Legumes and Good Fat	−0.006	0.011	0.622	−0.008	0.013	0.527	−0.007	0.013	0.608	−0.007	0.013	0.597
Homemade Meal	−0.009	0.011	0.445	−0.008	0.013	0.517	−0.007	0.013	0.599	−0.008	0.013	0.562
Chicken and Sugars	−0.003	0.011	0.760	−0.005	0.013	0.696	−0.003	0.013	0.828	−0.003	0.013	0.800
Eggs and Fibers	−0.011	0.011	0.320	−0.0013	0.013	0.339	−0.015	0.013	0.268	−0.015	0.013	0.267
LogSBP												
Western Breakfast	−0.003	0.002	0.085	−0.002	0.002	0.174	−0.002	0.002	0.295	−0.001	0.002	0.646
Legumes and Good Fat	0.000	0.002	0.838	0.001	0.002	0.729	0.001	0.002	0.499	0.001	0.002	0.472
Homemade Meal	0.000	0.002	0.937	0.000	0.002	0.819	0.001	0.002	0.579	0.001	0.002	0.481
Chicken and Sugars	0.002	0.002	0.169	0.002	0.002	0.246	0.003	0.002	0.090	0.003	0.002	0.071
Eggs and Fibers	2.294×10^{-5}	0.002	0.988	−0.001	0.002	0.680	−0.001	0.002	0.409	−0.001	0.002	0.411
LogDBP												
Western Breakfast	−0.003	0.002	0.224	−0.003	0.002	0.256	−0.002	0.002	0.361	0.000	0.003	0.894
Legumes and Good Fat	−0.002	0.002	0.482	−0.001	0.002	0.786	0.000	0.002	0.948	-3.047×10^{-5}	0.002	0.990
Homemade Meal	0.001	0.002	0.551	0.003	0.002	0.155	0.004	0.002	0.097	0.004	0.002	0.063
Chicken and Sugars	0.001	0.002	0.609	0.001	0.002	0.528	0.002	0.002	0.333	0.003	0.002	0.271
Eggs and Fibers	0.001	0.002	0.802	0.000	0.002	0.878	0.000	0.002	0.914	0.000	0.002	0.919
LogGlucose												
Western Breakfast	−0.003	0.007	0.655	−0.003	0.007	0.632	−0.003	0.007	0.631	−0.004	0.008	0.615
Legumes and Good Fat	0.010	0.006	0.120	0.011	0.007	0.111	0.011	0.007	0.110	0.011	0.007	0.111
Homemade Meal	−0.002	0.006	0.740	−0.004	0.007	0.531	−0.004	0.007	0.531	−0.004	0.007	0.532
Chicken and Sugars	0.015	0.006	0.017	0.013	0.007	0.051	0.013	0.007	0.051	0.013	0.007	0.051
Eggs and Fibers	0.003	0.006	0.588	0.003	0.007	0.659	0.003	0.007	0.659	0.003	0.007	0.660
LogInsulin												
Western Breakfast	−0.015	0.010	0.119	−0.015	0.010	0.139	−0.009	0.010	0.356	−0.007	0.010	0.521
Legumes and Good Fat	−0.020	0.009	0.030	−0.019	0.010	0.066	−0.017	0.009	0.066	−0.017	0.009	0.064
Homemade Meal	0.011	0.010	0.247	0.011	0.010	0.250	0.013	0.009	0.167	0.014	0.009	0.142
Chicken and Sugars	0.012	0.009	0.191	0.013	0.010	0.173	0.018	0.009	0.049	0.018	0.009	0.041
Eggs and Fibers	−0.015	0.009	0.113	−0.011	0.010	0.281	−0.014	0.010	0.133	−0.014	0.010	0.132

Table 5. *Cont.*

	Model 1			Model 2			Model 3			Model 4		
	β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*
LogHOMA-IR												
Western Breakfast	−0.016	0.011	0.158	−0.016	0.012	0.180	−0.035	0.011	0.422	−0.004	0.012	0.728
Legumes and Good Fat	−0.020	0.010	0.054	−0.020	0.011	0.074	−0.019	0.011	0.075	−0.019	0.011	0.072
Homemade Meal	0.014	0.011	0.205	0.013	0.011	0.231	0.015	0.010	0.157	0.016	0.010	0.124
Chicken and Sugars	0.010	0.010	0.349	0.010	0.011	0.345	0.015	0.010	0.139	0.016	0.010	0.114
Eggs and Fibers	−0.018	0.010	0.089	−0.017	0.012	0.157	−0.020	0.011	0.067	−0.020	0.011	0.066
LogTotalCholesterol												
Western Breakfast	−0.005	0.003	0.066	−0.006	0.003	0.060	−0.006	0.003	0.054	−0.003	0.003	0.422
Legumes and Good Fat	0.001	0.003	0.721	0.001	0.003	0.863	0.000	0.003	0.883	0.000	0.003	0.908
Homemade Meal	0.002	0.003	0.402	0.002	0.003	0.538	0.002	0.003	0.549	0.003	0.003	0.353
Chicken and Sugars	0.000	0.003	0.917	2.502×10^{-5}	0.003	0.993	-5.600×10^{-5}	0.003	0.985	0.000	0.003	0.868
Eggs and Fibers	0.003	0.003	0.269	0.002	0.003	0.521	0.002	0.003	0.511	0.002	0.003	0.511
LogHDL-C												
Western Breakfast	−0.002	0.004	0.553	−0.002	0.004	0.692	−0.004	0.004	0.313	−0.002	0.005	0.643
Legumes and Good Fat	0.006	0.004	0.160	0.005	0.004	0.210	0.004	0.004	0.343	0.004	0.004	0.351
Homemade Meal	0.001	0.004	0.832	0.001	0.004	0.900	0.000	0.004	0.919	0.000	0.004	0.958
Chicken and Sugars	0.009	0.004	0.022	0.007	0.004	0.080	0.006	0.004	0.153	0.006	0.004	0.128
Eggs and Fibers	−0.001	0.004	0.885	−0.002	0.004	0.600	−0.001	0.004	0.761	−0.001	0.004	0.759
LogLDL-C												
Western Breakfast	−0.008	0.005	0.099	−0.009	0.005	0.053	−0.009	0.005	0.073	−0.004	0.005	0.460
Legumes and Good Fat	−0.001	0.004	0.761	−0.003	0.005	0.547	−0.002	0.005	0.610	−0.003	0.005	0.586
Homemade Meal	0.003	0.004	0.566	0.001	0.005	0.800	0.001	0.005	0.753	0.003	0.005	0.537
Chicken and Sugars	−0.005	0.004	0.246	−0.005	0.005	0.278	−0.004	0.005	0.324	−0.004	0.004	0.411
Eggs and Fibers	0.005	0.004	0.233	0.004	0.005	0.389	0.004	0.005	0.423	0.004	0.005	0.423
LogTriglycerides												
Western Breakfast	−0.003	0.006	0.632	0.002	0.007	0.747	0.001	0.006	0.831	0.004	0.007	0.573
Legumes and Good Fat	0.006	0.006	0.307	0.008	0.006	0.208	0.010	0.006	0.101	0.010	0.006	0.103
Homemade Meal	−0.005	0.006	0.441	−0.004	0.006	0.550	−0.002	0.006	0.686	−0.002	0.006	0.745
Chicken and Sugars	−0.006	0.006	0.329	−0.004	0.006	0.491	−0.002	0.006	0.728	−0.002	0.006	0.764
Eggs and Fibers	−0.002	0.006	0.776	−0.005	0.007	0.418	−0.007	0.006	0.288	−0.007	0.006	0.287
LogCRP												
Western Breakfast	0.002	0.020	0.939	0.006	0.021	0.775	0.018	0.020	0.383	0.021	0.022	0.349
Legumes and Good Fat	0.006	0.019	0.759	0.019	0.021	0.369	0.022	0.020	0.275	0.022	0.020	0.276
Homemade Meal	0.015	0.020	0.444	0.005	0.021	0.795	0.007	0.019	0.714	0.007	0.020	0.714
Chicken and Sugars	−0.051	0.019	0.006	−0.057	0.020	0.004	−0.050	0.019	0.008	−0.051	0.019	0.008
Eggs and Fibers	0.016	0.019	0.418	0.029	0.021	0.175	0.023	0.020	0.266	0.023	0.020	0.266

Model 1: Adjusted for age and sex; Model 2: Adjusted for age, sex, physical activity; Model 3: Adjusted for age, sex, physical activity, BMI; Model 4: Adjusted for age, sex, physical activity, BMI, energy intake.

Table 6. Linear mixed model analyses on the association between the dietary patterns, anthropometric indices and biomarkers of glycemic and lipidemic control in the STANISLAS Family study.

	Model 1			Model 2			Model 3			Model 4		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***
LogBMI												
Western Breakfast	0.000	0.003	0.878	0.000	0.005	0.459	-	-	-	-	-	-
Prudent Snacking	0.000	0.003	0.950	0.001	0.003	0.738	-	-	-	-	-	-
High Protein and Animal Fat	0.011	0.003	0.002	0.009	0.003	0.018	-	-	-	-	-	-
Fish and Seafood	−0.002	0.003	0.430	−0.001	0.003	0.700	-	-	-	-	-	-
Sugary Snacks	−0.001	0.003	0.701	−0.002	0.003	0.437	-	-	-	-	-	-
LogWHR												
Western Breakfast	−0.000	0.001	0.800	−0.000	0.001	0.539	−0.000	0.001	0.540	−0.000	0.001	0.840
Prudent Snacking	3.965729×10^{-5}	0.001	0.976	0.000	0.001	0.809	0.000	0.001	0.797	0.000	0.001	0.722
High protein and animal Fat	0.000	0.001	0.723	0.000	0.001	0.616	0.000	0.001	0.757	0.001	0.001	0.486
Fish and Seafood	0.001	0.001	0.134	0.002	0.001	0.146	0.002	0.001	0.126	0.002	0.001	0.130
Sugary Snacks	−0.001	0.001	0.392	−0.001	0.001	0.363	−0.001	0.001	0.409	−0.000	0.001	0.691
LogSBP												
Western Breakfast	-2.288744×10^{-5}	0.002	0.991	0.000	0.002	0.892	0.000	0.002	0.837	−0.000	0.002	0.792
Prudent Snacking	0.003	0.002	0.114	0.003	0.002	0.181	0.002	0.002	0.189	0.002	0.002	0.215
High protein and Animal Fat	0.000	0.002	0.733	0.000	0.002	0.822	−0.000	0.002	0.802	−0.001	0.002	0.504
Fish and Seafood	−0.000	0.002	0.751	−0.000	0.002	0.766	−0.000	0.002	0.801	−0.000	0.002	0.794
Sugary Snacks	0.000	0.002	0.640	0.000	0.002	0.787	0.000	0.002	0.673	−0.000	0.002	0.894
LogDBP												
Western Breakfast	−0.000	0.004	0.948	0.003	0.004	0.510	0.003	0.004	0.483	0.003	0.005	0.464
Prudent Snacking	0.002	0.004	0.593	0.001	0.004	0.833	0.000	0.004	0.841	0.000	0.004	0.845
High Protein and Animal Fat	−0.008	0.004	0.089	−0.008	0.005	0.099	−0.010	0.005	0.045	−0.012	0.005	0.028
Fish and Seafood	0.009	0.004	0.039	0.008	0.004	0.077	0.008	0.004	0.069	0.008	0.004	0.070
Sugary Snacks	−0.000	0.004	0.936	−0.002	0.005	0.651	−0.001	0.005	0.718	−0.002	0.006	0.632
LogGlucose												
Western Breakfast	0.000	0.001	0.604	0.001	0.002	0.448	0.001	0.002	0.462	0.000	0.002	0.868
Prudent Snacking	−0.000	0.001	0.917	−0.000	0.002	0.793	−0.000	0.002	0.805	−0.000	0.002	0.727
High Protein and Animal Fat	−0.001	0.002	0.428	−0.001	0.002	0.632	−0.000	0.002	0.708	−0.002	0.002	0.365
Fish and Seafood	−0.002	0.001	0.202	−0.001	0.001	0.331	−0.001	0.001	0.323	−0.001	0.001	0.323
Sugary Snacks	0.001	0.001	0.568	0.000	0.002	0.906	0.000	0.002	0.928	−0.001	0.002	0.502

Table 6. *Cont.*

	Model 1			Model 2			Model 3			Model 4		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***
LogTotalCholesterol												
Western Breakfast	−0.001	0.004	0.728	−0.002	0.004	0.644	−0.002	0.004	0.66	−0.002	0.005	0.703
Prudent Snacking	0.002	0.004	0.599	0.004	0.004	0.347	0.004	0.004	0.369	0.004	0.004	0.358
High Protein and Animal Fat	−0.003	0.005	0.490	−0.006	0.005	0.236	−0.007	0.005	0.157	−0.008	0.005	0.151
Fish and Seafood	0.005	0.004	0.224	0.006	0.004	0.173	0.006	0.004	0.171	0.006	0.004	0.172
Sugary Snacks	−0.001	0.004	0.712	6.925668×10^{-7}	0.005	1.000	0.000	0.005	0.940	0.001	0.006	0.833
LogHDL-C												
Western Breakfast	0.006	0.006	0.303	0.005	0.007	0.426	0.005	0.007	0.443	0.011	0.007	0.139
Prudent Snacking	−0.005	0.006	0.419	−0.004	0.007	0.547	−0.003	0.007	0.584	−0.003	0.007	0.657
High Protein and Animal Fat	−0.003	0.007	0.621	−0.002	0.008	0.762	0.000	0.008	0.983	0.004	0.008	0.622
Fish and Seafood	0.004	0.006	0.462	0.002	0.006	0.710	0.002	0.006	0.728	0.002	0.006	0.746
Sugary Snacks	−0.007	0.006	0.237	−0.014	0.007	0.065	−0.014	0.007	0.049	−0.013	0.008	0.114
LogLDL-C												
Western Breakfast	−0.006	0.006	0.333	−0.007	0.006	0.275	−0.007	0.006	0.292	−0.060	0.053	0.254
Prudent Snacking	0.004	0.006	0.493	0.007	0.006	0.293	0.006	0.006	0.332	0.041	0.047	0.391
High Protein and Animal Fat	−0.005	0.007	0.472	−0.010	0.007	0.168	−0.013	0.007	0.073	−0.112	0.057	0.050
Fish and Seafood	0.004	0.006	0.475	0.007	0.006	0.292	0.006	0.006	0.288	0.035	0.045	0.435
Sugary Snacks	−0.001	0.006	0.810	0.005	0.007	0.492	0.005	0.007	0.410	0.042	0.059	0.473
LogTriglycerides												
Western Breakfast	0.011	0.012	0.338	0.009	0.013	0.467	0.010	0.013	0.444	−0.001	0.014	0.911
Prudent Snacking	0.003	0.012	0.237	0.000	0.013	0.990	-6.768397×10^{-5}	0.013	0.996	−0.001	0.013	0.893
High Protein and Animal Fat	0.054	0.013	<0.001	0.049	0.014	0.001	0.045	0.014	0.002	0.041	0.015	0.009
Fish and Seafood	0.014	0.012	0.252	0.019	0.012	0.133	0.020	0.012	0.114	0.021	0.012	0.093
Sugary Snacks	0.009	0.012	0.428	0.010	0.013	0.462	0.011	0.013	0.399	−0.002	0.016	0.855
LogCRP												
Western Breakfast	−0.045	0.029	0.125	−0.053	0.031	0.085	−0.050	0.030	0.096	−0.076	0.033	0.024
Prudent Snacking	0.031	0.028	0.274	0.037	0.030	0.217	0.037	0.029	0.201	0.036	0.029	0.222
High Protein and Animal Fat	0.009	0.031	0.757	−0.005	0.033	0.873	−0.019	0.032	0.558	−0.033	0.034	0.334
Fish and Seafood	0.018	0.029	0.516	0.009	0.030	0.745	0.010	0.029	0.733	0.008	0.030	0.774
Sugary Snacks	0.010	0.031	0.743	0.011	0.032	0.729	0.016	0.032	0.603	0.004	0.036	0.905

Model 1: Adjusted for age and sex; Model 2: Adjusted for age, sex, physical exercise; Model 3, Adjusted for age, sex, physical activity, BMI. Original data values in mmol/l were used for creation of the logGlucose, logTotalCholesterol, logHDL-C, logLDL-C, LogTriglycerides variables.

4. Discussion

The present study sought to investigate the dietary patterns of two adolescent, European populations, based on data from the Greek TEENAGE and the French STANISLAS Family studies, as well as their potential relations with blood pressure, biomarkers of glycemic and lipidemic control and levels of CRP. The study includes healthy teenagers from the two European populations, with a median BMI of 20.88 kg/m^2 (IQR = 5.88 kg/m^2) and 18.44 kg/m^2 (IQR = 3.61 kg/m^2). For the Greek teenagers, weight, waist-to-hip ratio (WHR), systolic blood pressure (SBP), levels for glucose, HOMA-IR, insulin, HDL-C and CRP significantly differed between boys and girls. Boys presented slightly higher values for weight, WHR, SP and glucose levels, while girls reported slightly higher levels of HOMA-IR, insulin and HDL-C. In the French teenagers group, WHR, SBP, glucose and total cholesterol levels presented statistically significant differences between the two sexes, with boys reporting slightly higher values for WHR, SBP and glucose levels and girls for total cholesterol levels. The teenagers of the study were mostly normal weighted. Both populations reported a mediocre energy intake (TEENAGE: 1741.00 kcal/d and STANISLAS: 2056.03 kcal/d), based on the present dietary guidelines for adolescents [39]. This could explain the fact that teenagers of both populations mostly reported BMI values within the normal range (18.5–25 kg/m^2).

Five dietary patterns were identified in each population. The Greek "eggs and fibers" and the French "prudent snacking" patterns, explaining 7.47% and 10.44% of the respective total variance, included consumption of Mediterranean diet-related food groups, such as non-refined cereals, vegetables and eggs in the Greek teenagers and consumption of eggs and vegetable fats in French adolescents. The Greek teenagers showed a preference for healthy and traditional food combinations, such as consumption of legumes, olives, olive oil and nuts in the "legumes and good fat" pattern and consumption of red meat and potatoes in the "homemade meal" pattern, respectively. The French teenagers opted for consumption of more energy-dense food groups, such as red meat, animal fat and milk and yogurt in the "high protein and animal fat" pattern and soft drinks and sugary snacks in the "sugary snacks" pattern. A number of significant associations were found between the respective dietary patterns and the populations' glycemic and lipidemic profile. However, after adjusting for the overall adjusted threshold, a smaller number of significant associations remained observed.

The predominant pattern in both populations (the "western breakfast" pattern) appears to relate to food groups whose consumption is primarily found in the basis of a western-type diet [40], such as cheese, processed meat and food items high in carbohydrates (breads and flours for the French). The "western breakfast" pattern reflects a higher percentage (15.61%) of the variance explained in the Greek population, in comparison to the French one (10.58%). This could be explained by the increasing influences of the westernized world trends in the Greek socio-economic scene during the late 2000s. Moreover, breakfast habits were also highlighted in the first 5-year follow-up in the STANISLAS Cohort, which underlined the importance of the household environment in dietary habits by finding a household variance of 42.5 to 52.9% in the energy intake observed in breakfast [29]. The importance of breakfast consumption and its contribution to daily energy intake of French children and families, is also supported by another, recent cross-sectional survey [41].

Although the western diet has been associated with elevated inflammation biomarkers [42], the cohort of the Greek teenagers reported no comorbidities and we found no associations between adherence to the "western breakfast" pattern and respective CRP levels. Interestingly enough, the "chicken and sugars" pattern identified in the Greek cohort was significantly associated with lower levels of logCRP (Model 1: $\beta = -0.051$, p-value = 0.006, Model 2: $\beta = -0.057$, p-value = 0.004 and Model 3: $\beta = -0.050$, p-value = 0.008). An inverse association between the consumption of poultry and CRP levels in teenagers has previously been reported, in the general context of adherence to the Dietary Approach to Stop Hypertension (DASH) diet regime [43], although a recent umbrella review showed

no association between the DASH diet and CRP levels in adults [44]. On the contrary, an inverse association between consumption of sweets and CRP levels is not supported by other studies. In fact, consumption of sugars and especially sugar-sweetened beverages has previously been associated with higher CRP levels in adults [45,46]. In adolescents, a different review has shown a positive association between sugar consumption and CRP [47], whereas another review found greater consumption of sugars by normal weight adolescents in comparison with overweight ones, but did not find any association between sugar consumption and CRP [48]. A cross-sectional study investigating the relation between food intake and CRP levels in children also found that consumption of milk, citrus, melons and berries displayed associations with lower levels of CRP, potentially due to the general high content of fruits and vegetables in antioxidants and the association of dairy consumption with greater satiety and potential adherence to a generally healthier diet [49].

Furthermore, our study found that the "high protein and animal fat" pattern displayed significant associations with higher logtriglyceride and logBMI levels ($p < 0.01$), for French teenagers. The latter is in accordance with various cross-sectional studies that have researched the dietary habits of adolescents and their potential associations to BMI. A study by Gutiérrez-Pliego et al. unveiled three major dietary patterns in a population of 373 Mexican teenagers including a pattern high in refined "unhealthy" products, such as snacks, sugars and sweets, a pattern with high protein/high fat content and a pattern including high consumption of fruits, vegetables, nuts and whole grains. The study found a strong relationship ($p < 0.01$) between higher consumptions of the first two dietary patterns and higher BMI [50]. In the same context, a different study in Northeastern Brazil investigated data from 1247 adolescents. The study identified two dietary patterns, one referring to high consumption of sugars, sweets and cakes, amongst others, and one correlated with high consumption of fruits and vegetables. Higher adherence to the dietary pattern including "unhealthy" products, was, again, positively correlated with higher values of BMI ($p = 0.018$) [51]. Furthermore, a different study on the dietary habits of female adolescents showed that higher adherence to a "Western" pattern referring to increased consumption of fat and mediocre consumption of protein, among others, was associated with higher levels of BMI, waist circumference, as well as total cholesterol levels [52].

Although dietary patterns with a higher consumption of fat have generally been positively associated with cardiometabolic risk factors in teenagers [53,54], certain diets, including consumption of specific food groups, such as the DASH diet [55], have been related with a better metabolic profile [56]. Indeed, higher adherence to the DASH diet has been shown to relate to a reduced prevalence of metabolic syndrome and increased blood pressure during adolescence [43], as well as lower levels of HbA1c and systolic blood pressure, in young adults with type 1 and type 2 diabetes, respectively [57]. Better adherence to the components of the DASH diet was even associated with a lower risk of being a metabolic unhealthy obese, in children and adolescents with increased body weight [58]. Additionally, other high protein diets, such as the ketogenic diet and the Modified Atkins diet, have been associated with better effects on adolescents with epilepsy [59,60], with the ketogenic diet to have been related to reduced weight and fasting insulin and HOMA-IR levels in obese teenagers [61]. However, the aforementioned diets also usually include consumption of vegetables fats and fats derived from nuts, seeds, white meat (such as poultry and fish), as well as food groups like grains, vegetable fats, fruits and vegetables, which are not met when referring to dietary patterns centred on high protein or animal fat consumption. Furthermore, the aforementioned beneficial associations have been primarily observed in adults or obese adolescent populations, who could potentially benefit from the adherence to a structured diet with the above food groups. This could potentially explain why our study demonstrated positive associations between the high consumption of protein and animal fats with BMI and triglyceride levels in adolescents mostly displaying BMI of a normal range. Moreover, the present study evaluates the adherence to each dietary pattern, without comparing them with the respective adherence to the rest of the patterns extracted.

The identification of dietary patterns of adolescents has generally been a subject of interest in recent literature. Gonzalez-Gil et al. investigated the dietary patterns of 5328 European adolescents in the context of the cross-sectional HELENA study [62]. The latter consisted of adolescent cohorts of 10 different European countries, including Greek teenagers from the cities of Athens and Heraklion, Crete. The study identified four dietary patterns in teenage boys and six dietary patterns in teenage girls. Patterns explaining greater total variance in boys referred to consumption of vegetables, pasta, rice, cheese and sweets among others, at the same time as dominant patterns in girls referred to consumption of Mediterranean-type food items, dairy and consumption of a healthy breakfast [62].

Additionally, when investigating the dietary habits of adolescents based on data collected in the 1995 Australian National Nutrition Survey, McNaughton et al. showed that a dietary pattern rich in fruit, salads, cereals and fish was found to be negatively associated with levels of diastolic blood pressure in teenagers older than 16 years of age [63]. Our study found no associations between the patterns containing fruit, vegetable and fish consumption and the levels of diastolic pressure in adolescents younger than 16 years of age.

Furthermore, the I. Family Study investigated the association between the dietary patterns of 2451 pairs of European children and their parents, with regards to the existing food environment conditions. The study showed the role of food availability in the children's dietary choices, highlighting that the consumption of soft drinks was greatly dependent on their availability in the immediate food environment [64]. Moreno LA et al. also showed that increased consumption of sweet beverages was also associated with increased risk of adolescent obesity [65]. In our study, the "sugary snacks" pattern of the French population, which included consumption of sweetened beverages, was not related to logBMI values, but was associated with lower values of logHDL-C. However the effect disappeared when taking into account the adjusted threshold of statistical significance ($0.04 > 0.01$). A different study of German adolescents demonstrated that higher consumption of dietary patterns containing high-fat and high-carbohydrate, energy-dense foods was associated with lower socioeconomic levels and a lower intake of various vitamins and minerals [66].

A previous publication on the Greek adolescents of the TEENAGE study investigated a spectrum of factors potentially contributing to the development of overweight, leading to the creation of an Overweight Preventive Score, which included breakfast intake, family meals and consumption of sugar-sweetened beverages, among other factors, and further supports the aforementioned findings. The score was found to be significantly associated with a lower likelihood of overweight presence and better levels of glycemic control [67].

The limitations of the present study are summarized in the following: (a) data for both populations were collected in a cross-sectional manner, limiting the potential for generalized cause and effect conclusions to be drawn; (b) use of the PCA for the dietary patterns' extraction, including subjective choices regarding the amount of food groups that are included in the analysis, as well as the number of components to be drawn; (c) comparisons between the two populations' dietary habits might be affected by the different socio-economic conditions existing in the two countries during the mid-1990s for the STANISLAS and late 2000s for the TEENAGE study. This prolonged gap between the two baseline data collections might manifest itself in the Greek teenagers' dietary habits, which could have potentially been affected by social changes and changes in food availability and accessibility, mediated by the growing social and technological advancements taking place throughout the 15-year gap.

5. Conclusions

Our study focused on the dietary habits of European adolescents and their potential influence on blood pressure, glycemic and lipidemic profile and inflammation levels. The patterns identified demonstrated associations with indices, such as BMI, and biomarkers, such as triglycerides and CRP. The relations highlighted in the present study display great interest and enhance the need for further research on the pivotal role of diet in the

essential-for-development period of adolescence, as a modifying factor for cardiometabolic risk factor-related disorders, such as obesity, hypertension and type 2 diabetes.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available on request due to restrictions eg privacy or ethical. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy/ethical restrictions on the data provided by the volunteers.

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nutrients

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Associations of VEGF-A-Related Variants with Adolescent Cardiometabolic and Dietary Parameters

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Abstract: Previous research has allowed the identification of variants related to the vascular endothelial growth factor-A (VEGF-A) and their association with anthropometric, lipidemic and glycemic indices. The present study examined potential relations between key VEGF-A-related single-nucleotide polymorphisms (SNPs), cardiometabolic parameters and dietary habits in an adolescent cohort. Cross-sectional analyses were conducted using baseline data from 766 participants of the Greek TEENAGE study. Eleven VEGF-A-related SNPs were examined for associations with cardiometabolic indices through multivariate linear regressions after adjusting for confounding factors. A 9-SNP unweighted genetic risk score (uGRS) for increased VEGF-A levels was constructed to examine associations and the effect of its interactions with previously extracted dietary patterns for the cohort. Two variants (rs4416670, rs7043199) displayed significant associations (p-values < 0.005) with the logarithms of systolic and diastolic blood pressure (logSBP and logDBP). The uGRS was significantly associated with higher values of the logarithm of Body Mass Index (logBMI) and logSBP (p-values < 0.05). Interactions between the uGRS and specific dietary patterns were related to higher logDBP and logGlucose (p-values < 0.01). The present analyses constitute the first-ever attempt to investigate the influence of VEGF-A-related variants on teenage cardiometabolic determinants, unveiling several associations and the modifying effect of diet.

Keywords: vascular endothelial growth factor A (VEGF-A); cardiometabolic profile; genetic risk score; adolescents; dietary patterns; genetic risk score

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1. Introduction

Vascular endothelial growth factor A (VEGF-A) is involved in various biological functions, primarily as a major contributor to angiogenesis induction which extends its activities to cell proliferation, migration and even differentiation [1–3]. Due to its versatile roles in endothelial function [4], its involvement in activating the cortisol–adrenocorticotrophic hormone (ACTH) stress axis, its promotion of aldosterone [5] production as well as its multifactorial influence on energy homeostasis [2,6,7], insulin resistance [2,8] and cardiac function [9], VEGF-A is involved in various reciprocal relationships influencing cardiovascular and cardiometabolic risk factors such as glucose sensitivity, lipidemic profile, obesity and blood pressure.

Altered VEGF-A expression is observed in the presence of disturbed cardiometabolic states, denoting a requited relationship between the biomarker's levels and disrupted

cardiometabolic profile. For example, VEGF-A is known to be involved in glucose homeostasis, where both its over- and under-expression can affect glucose tolerance [8], as well as lipid metabolism, through its regulation of lipases and the creation of chylomicrons [7]. In a similar manner, VEGF-A is highly expressed in the adipose tissue, where an increase in the number of adipocytes signifies increased VEGF-A and subsequent angiogenesis and further cell proliferation and differentiation [1].

Circulating VEGF-A levels have been conclusively demonstrated as greatly heritable [10]. The past decades have marked the conduct of large meta-analyses of multiple genome-wide association studies (GWAS), revealing key variants significantly associated with the marker's levels. More specifically, Debette and Visvikis-Siest et al. brought four key single-nucleotide polymorphisms (SNPs) to light, collectively explaining 48.7% of VEGF-A variation [10]. Subsequent studies have unveiled additional VEGF-A-related SNPs, which have, in turn, been further associated with adult cardiometabolic indices [11,12] and even the presence of neurodegenerative disorders such as Alzheimer's disease [13]. Selected VEGF-A-associated SNPs have even been directly linked to the presence of hypercholesterolemia and metabolic syndrome in adults [14,15]. In addition, the interplay between VEGF-A SNPs and dietary components has also been associated with multiple metabolic syndrome determinants [16,17]. An example of the importance of the interplay between VEGF-A, anthropometric indices and dietary compounds was recently highlighted in the finding that the effect of VEGF- A variants on circulating iron levels might depend on anthropometric indices [(i.e., Body Mass Index (BMI)] [18].

The present study constitutes the continuation of our team's previous research aiming at exploring the effect of the interplay between genetic makeup and lifestyle habits on adolescent anthropometric, lipidemic and glycemic indices. In this context, the present findings concern the first-ever attempt to investigate the role of key VEGF-A-related variants exclusively on the cardiometabolic profile of adolescents, using data from the Greek TEENAGE Study. We hereby present the results of the analyses on selected target variants, the subsequent examinations of their cumulative effect in the form of an unweighted genetic risk score (uGRS) and its respective interactions with previously extracted dietary patterns on the teenagers' cardiometabolic indices.

2. Materials and Methods

2.1. The TEENAGE Study

The present analyses constitute the next step in the research of our team's Gutenberg Chair 2018 project, where building on our previous findings [19], we hereby present the subsequent examinations between genetic makeup and teenage cardiometabolic profile in the TEENAGE Study. The latter (TEENs of Attica: Genes and Environment) refers to the cross-sectional collection of various data from adolescent students conducted during the years 2008–2010 in Attica, Greece. The project was approved by the Institutional Review Board of Harokopio University of Athens, as well as the Greek Ministry of Education and Religious Affairs. All nodes conducted within the study took place adhering to the guidelines of the Declaration of Helsinki.

Details of the study protocol and characteristics have been previously extensively described elsewhere [20–22]. The TEENAGE desired target population were children and adolescents of 13–15 years of age attending the primary three classes of public secondary schools in the Attica region, coming from all groups and backgrounds [22]. Schools and participants were invited to be involved in the study from the pool of the teenage population of the GENDAI study [23]. The latter constituted a previous study also conducted and approved by Harokopio University of Athens, including children attending fifth and sixth grade of 1440 schools from a wide range of neighborhoods of different socioeconomic status across the Attica region [23]. Overall, 857 out of 1440 teenagers attending the participating schools were recruited for the purposes of the TEENAGE study [20,21]. The volunteers were recruited to the study after undergoing a briefing session on the study aims, their voluntary inclusion and the confidentiality measures surrounding their data [20,21]. Verbal

consent by all adolescent participants and their respective guardians' written consent was collected prior to study enrollment.

After enrollment in the study, all children and adolescents participated in a baseline, in-person session with healthcare professionals, where anthropometric, dietary, biochemical and lifestyle data were collected. Measurements of body and height were conducted for each individual in a barefoot state and with light clothes on, and the BMI was calculated as weight (kg)/height^2 (m^2). Waist circumference was measured in centimeters using a non-extensible soft tape, and body fat was evaluated by measuring the triceps and subscapular skinfolds. Dietary intake was assessed via conduct of a 24 h recall for the day prior to recruitment and the completion of a questionnaire for meal patterns and eating behavior. A second recall was conducted via telephone in the 10 days after the baseline session. Physical activity habits were assessed via the completion of a relative checklist for two non-consecutive days [20–22].

Moreover, DNA samples were collected for each participant and were further genotyped via the use of the Illumina HumanOmniExpress BeadChips (Illumina, San Diego, CA, USA) at the Wellcome Trust Sanger Institute, Hinxton, UK [20]. The imputation of the genotyped data was conducted using the Haplotype Reference Consortium (HRC) panel [20,24].

For the purposes of the present study, we used anthropometric, biochemical and genetic data from an initial pool of 766 participants with available data. We investigated associations between 11 VEGF-A-associated SNPs and various cardiometabolic indices. Pulse pressure (PP) was calculated to allow for comparisons with the previous findings, based on the available data for systolic and diastolic blood pressure (SBP and DBP, respectively) and via using the following formula:

$$\begin{gathered} Pulse\ Pressure\ (PP) \\ = Systolic\ Blood\ Pressure\ (SBP, mmHg) \\ - Diastolic\ Blood\ Pressure\ (DBP, mmHg) \end{gathered}$$

Furthermore, we proceeded to construct an unweighted genetic risk score (uGRS) for VEGF-A using the target SNPs identified by Choi et al. For the purposes of the present analyses, we used the SNPs with the available data in the TEENAGE cohort (i.e., 9 out of 10 variants). The uGRS was constructed by scoring the risk alleles positively associated with the VEGF-A levels. We subsequently examined its respective relations with the cardiometabolic indices and further split the uGRS into two groups of high and low genetic risk for higher levels of VEGF based on the sample median value. Additionally, we proceeded to investigate the potential effect of interactions between the uGRS and the previously identified dietary patterns for the TEENAGE cohort [19] on the various indices.

2.2. Statistical Analyses

In the present analyses, we set out to investigate the potential impact of 11 VEGF-A-related target SNPs on cardiometabolic indices using available data from the Greek TEENAGE study (Table 1). Based on our team's previously published findings [10,11], we chose to examine the rs4416670, rs6921438, rs10738760 and rs6993770 variants, which have been shown to collectively explain 48.7% of VEGF-A variability and have been further associated with multiple cardiometabolic indices in healthy populations [10]. We additionally included 7 more SNPs identified by Choe et al. as strongly associated with circulating VEGF-A levels, with available data in the TEENAGE cohort [11].

Table 1. List of the VEGF-A-related single-nucleotide polymorphisms (SNPs) (n = 11) investigated for cardiometabolic associations in the TEENAGE cohort.

		Consortial Summary Statistics						TEENAGE Cohort	
SNP	**Gene**	**Chr**	**Position**	**Alleles**	**MAF**	**Effect Allele**	**Direction of Effect for VEGF**	**EAF**	**Ref.**
rs114694170	MEF2C, MEF2C-AS1	5	5:88884379	T/C	0.02 (C)	T	Negative (beta = −0.15)	0.96	[6]
rs6921438	SCIRT, LOC100132354	6	6:43957870	G/A/C	0.44 (A)	A	Negative (beta = −0.72)	0.39	[6,7]
rs1740073	LINC02537, SCIRT, C6orf223	6	6:43979661	T/A/C	0.20 (T)	T	Positive (beta = 0.09)	0.35	[6]
rs4416670	SCIRT	6	6:43982716	T/A/C	0.47 (C)	C	Negative (beta = −0.13)	0.44	[7]
rs6993770	ZFPM2-AS1,ZFPM2	8	8:105569300	A/T	0.36 (T)	T	Negative (beta = 0.17)	0.31	[6,7]
rs7043199	VLDLR-AS1	9	9:2621145	T/A	0.11 (A)	A	Negative (beta = −0.10)	0.19	[6]
rs10738760	VLDLR, KCNV2	9	9:2691186	A/G	0.41 (G)	G	Negative (beta = −0.28)	0.46	[7]
rs2375981	VLDLR, KCNV2	9	9:2692583	C/A/G/T	0.41 (G)	C	Positive (beta = 0.21)	0.44	[6]
rs74506613/proxy rs10761741 used	JMJD1C	10	10:63306426	G/T	0.37 (T)	T	Positive (beta = 0.08)	0.47	[6]
rs4782371	ZFPM1	16	16:88502423	T/A/C/G	0.41 (G)	T	Negative (beta = −0.07)	0.36	[6]
rs2639990	ZADH2	18	18:75203596	T/C	0.10 (C)	T	Positive (beta = 0.11)	0.10	[6]

SNP: Single-Nucleotide Polymorphism, Chr: Chromosome, bp: base pairs, MAF: Minor Allele Frequency (as shown in GWAS Catalog), Ref.: Reference.

We used a threshold of 0.7 for the imputation INFO score for all SNPs included in the analyses. Quality control for sample and SNP exclusion criteria consisted of: (i) sample call rate at 95%; (ii) Hardy–Weinberg Equilibrium (HWE) exact $p < 0.0001$; and (iii) genotyping call rate at 99%. Before testing for associations, an assessment of the cardiometabolic variables' distribution was carried out via the use of the Shapiro–Wilk and Kolmogorov–Smirnov tests. All variables not presenting a normal distribution were log-transformed. Hypothesis testing between cohort subgroups took place using the Mann–Whitney test. We investigated potential relations between the 11 target SNPs and the cardiometabolic parameters using linear regression analyses. Associations were examined after adjusting for 3 different models of confounding factors, namely: (i) Model 1, which consisted of adjustment for age and sex; (ii) Model 2, which further included exercise level; and (iii) Model 3, additionally incorporating the adjustment for the five previously extracted dietary patterns [19]. Multiple linear regression results for each SNP are presented as betas [regression coefficients (β)] and *p*-values. The threshold for statistical significance was set at 0.05. The adjusted threshold for multiple testing was set at 0.005 (0.05/11 components examined).

Following the associations explored for each SNP separately, we further used multiple linear regressions to examine the associations between the uGRS and the metabolic indices, as well as the potential effect of the interactions between the uGRS and the formerly extracted dietary patterns. Multiple linear regression results are presented as estimates [beta coefficients (β)] and standard error (SE). In the case of examining the interactions, the adjusted threshold for statistical significance was set at 0.01 (i.e., 0.05/5 components examined). All phenotypic analyses were conducted using the R Statistical Package [25], and genetic analyses were carried out with the Plink whole-genome association analysis toolset, version 1.9 [26].

3. Results

3.1. Population Characteristics

The characteristics of the population used have been previously described elsewhere [19]. This overall healthy population of 349 boys and 417 girls presented a median age of 13.30 years old (Table 2). The girls displayed an overall better cardiometabolic profile compared to boys, with the latter showing statistically significantly higher levels of SBP, PP, glucose and C-reactive protein (CRP) (*p*-value < 0.001). Additionally, girls demonstrated statistically significantly higher levels of high-density cholesterol (HDL) (*p*-value < 0.001). BMI, triglycerides, total cholesterol, SBP, while low-density cholesterol did not display any statistically significant differences between the two groups.

Table 2. Descriptive characteristics of the TEENAGE Study.

	All		**Boys**		**Girls**		
	n	**Median (IQR)**	***n***	**Median (IQR)**	***n***	**Median (IQR)**	***p*-Value ***
Age (years)	766	13.30 (1.31)	349	13.36 (1.38)	417	13.26 (1.25)	<0.001
BMI (kg/m^2)	766	20.88 (4.38)	349	20.85 (4.45)	417	20.93 (4.37)	0.517
Triglycerides (mg/dL)	611	56.00 (24)	283	55.00 (25)	328	57.00 (24)	0.090
Total Cholesterol (mg/dL)	611	157.00 (33)	283	156.49 (25.18) **	328	157.50 (31)	0.210
SBP (mmHg)	743	119.00 (16)	335	120.67 (11.93) **	408	118.00 (15)	0.001
DBP (mmHg)	743	70.00 (12)	335	71.00 (12)	408	70.00 (12)	0.825
PP	743	47.00 (13)	335	49.23 (10.61) **	408	46 (12)	<0.001
LDL (mg/dL)	611	54.00 (16)	283	90.57 (21.78) **	328	88.40 (26)	0.651
HDL (mg/dL)	611	89.20 (27)	283	53.00 (16)	328	56.00 (17)	0.001
Glucose (mg/dL),	611	80.00 (12)	283	81.00 (11)	328	79.00 (12)	<0.05
CRP (mg/dL)	540	0.30 (1)	254	0.45 (1)	286	0.20 (0)	<0.001

BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, PP: Pulse Pressure, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, CRP: C-reactive protein. * Hypothesis testing took place via use of the Mann–Whitney test. ** The variable summary statistics are shown as mean ± standard deviation (SD).

3.2. Associations between the 11 VEGF-A-Related SNPs and the Cardiometabolic Indices

Cross-sectional associations between the 11 SNPs and the various indices were assessed in participants with available data. Table 3 shows the multivariate linear regressions conducted for each of the 11 SNPs after adjustment for age and sex (Model 1), age, sex and exercise (Model 2) and age, sex, exercise and dietary pattern (Model 3). Our analyses showed statistically significant associations for two out of the eleven examined SNPs, namely the rs7043199 and the rs4416670 variants, with the latter having been found to explain 1,5% of the variance of VEGF-A levels in adults [7]. More specifically, the presence of the C allele of the latter was related, with a lower log of systolic blood pressure (logSBP) across all models (Model 1: $\beta = -0.007$, p-value = 0.002, Model 2: $\beta = -0.007$, p-value = 0.002, Model 3: $\beta = -0.07$, p-value = 0.0035). Another statistically significant but positive relation for logSBP was demonstrated for the A allele of the rs7043199 variant after adjusting for Model 2 (Model 2: $\beta = 0.009$, p-value = 0.004). The same SNP also displayed a statistically significant and positive association with log diastolic blood pressure (logDBP) after adjustment for Model 3 (Model 3: $\beta = 0.0138$, p-value = 0.0046).

Table 3. Associations between the 11 VEGF-A-related SNPs and cardiometabolic indices in the TEENAGE cohort.

	Model 1		**Model 2**		**Model 3**	
	Beta	***p*-Value**	**Beta**	***p*-Value**	**Beta**	***p*-Value**
LogBMI						
rs114694170	0.01009	0.3424	0.01317	0.2385	0.01239	0.2707
rs6921438	−0.00631	0.1131	−0.0053	0.2038	−0.00475	0.2564
rs1740073	0.005531	0.1785	0.003664	0.3826	0.002784	0.5088
rs4416670	−0.00698	0.06125	−0.00389	0.3099	−0.00363	0.3452
rs6993770	−0.00649	0.1252	−0.00866	0.04606	−0.00858	0.0483
rs7043199	−0.01265	0.01352	−0.01202	0.02304	−0.01185	0.02551
rs10738760	0.003147	0.4208	0.002341	0.5588	0.00203	0.6125
rs2375981	0.003426	0.3883	0.002837	0.4846	0.002472	0.5432
rs10761741	0.003055	0.4467	0.003455	0.3978	0.003062	0.4544
rs4782371	0.00442	0.2833	0.003158	0.4576	0.002953	0.4892
rs2639990	−0.00297	0.6463	−0.00232	0.7241	−0.0021	0.7516
logTriglycerides						
rs114694170	0.008907	0.7274	0.02828	0.2978	0.029	0.292
rs6921438	0.001028	0.9184	0.01319	0.2007	0.01328	0.2003
rs1740073	0.006261	0.5473	0.002573	0.8058	0.00253	0.8107
rs4416670	1.83×10^{-5}	0.9984	0.00513	0.5827	0.004898	0.6018
rs6993770	0.006058	0.5595	−0.00307	0.7726	−0.00332	0.7567
rs7043199	−0.01681	0.1822	−0.01787	0.1588	−0.01938	0.1304
rs10738760	−0.02382	0.01482	−0.0201	0.04157	−0.0201	0.04306
rs2375981	−0.01995	0.04558	−0.01675	0.09515	−0.01696	0.09375
rs10761741	0.004158	0.6738	−0.00254	0.7989	−0.00198	0.844
rs4782371	−0.00071	0.9448	0.00189	0.8571	0.001944	0.8546
rs2639990	−0.01428	0.3776	−0.01309	0.4196	−0.0138	0.4033
logCholesterol						
rs114694170	−0.00314	0.7859	−0.00783	0.5438	−0.00896	0.4916
rs6921438	−0.00051	0.9111	0.000254	0.9586	-9.61×10^{-5}	0.9844
rs1740073	0.000767	0.8706	0.000225	0.9639	−0.00033	0.947
rs4416670	0.001849	0.6564	0.004052	0.3602	0.004303	0.3322
rs6993770	0.0042	0.3709	0.002885	0.567	0.002729	0.5901
rs7043199	−0.00066	0.908	-9.11×10^{-5}	0.9879	−0.00107	0.8596
rs10738760	−0.00256	0.5642	−0.00355	0.4489	−0.00351	0.4558
rs2375981	−0.00357	0.4299	−0.00446	0.3497	−0.00424	0.3768
rs10761741	−0.00642	0.1503	−0.00856	0.0695	−0.0087	0.06685
rs4782371	0.003328	0.4736	0.001601	0.7478	0.002173	0.6649
rs2639990	−0.00337	0.645	−0.00521	0.4986	−0.00315	0.6864

Table 3. *Cont.*

	Model 1		Model 2		Model 3	
	Beta	***p*-Value**	**Beta**	***p*-Value**	**Beta**	***p*-Value**
logSBP						
rs114694170	0.004856	0.4602	0.01095	0.1322	0.01002	0.1704
rs6921438	−0.00528	0.03273	−0.00571	0.03214	−0.00614	0.02126
rs1740073	0.006211	0.01456	0.007036	0.008435	0.007113	0.007929
rs4416670	−0.00707	0.002172	−0.00744	0.002407	−0.00716	0.003524
rs6993770	−0.005	0.05437	−0.00489	0.07711	−0.005	0.07093
rs7043199	0.007357	0.02104	0.009594	0.004338	0.009446	0.005093
rs10738760	−0.00105	0.6643	−0.00018	0.9445	−0.0002	0.9368
rs2375981	−0.00048	0.8464	0.000475	0.8549	0.000676	0.7948
rs10761741	0.004394	0.07559	0.003574	0.1711	0.003634	0.1643
rs4782371	−0.0017	0.5082	−0.00148	0.5885	−0.00099	0.7192
rs2639990	−0.00027	0.9467	−0.00181	0.6667	−0.00112	0.7913
logDBP						
rs114694170	−0.00538	0.5747	−0.00023	0.9829	−0.00073	0.945
rs6921438	−0.00617	0.08685	−0.00804	0.03627	−0.00845	0.0283
rs1740073	0.005599	0.1311	0.006755	0.07975	0.006983	0.07167
rs4416670	−0.00556	0.09872	−0.00686	0.05272	−0.00661	0.06318
rs6993770	−0.00621	0.101	−0.0043	0.281	−0.00443	0.2685
rs7043199	0.01191	0.01033	0.01359	0.005051	0.0138	0.004611
rs10738760	6.32×10^{-6}	0.9986	0.001639	0.6575	0.001642	0.6579
rs2375981	−0.00022	0.9508	0.001781	0.6339	0.002048	0.5851
rs10761741	0.005385	0.135	0.006435	0.08701	0.006501	0.0848
rs4782371	0.000505	0.8928	0.002055	0.6027	0.002789	0.4824
rs2639990	0.004213	0.4671	0.003025	0.6163	0.003598	0.5553
logPP						
rs114694170	0.02169	0.1799	0.03011	0.0877	0.02892	0.1044
rs6921438	−0.00429	0.4814	−0.00136	0.8342	−0.00166	0.7989
rs1740073	0.008354	0.1826	0.008206	0.2063	0.007979	0.223
rs4416670	−0.01232	0.03026	−0.01075	0.07144	−0.0104	0.08316
rs6993770	−0.0003	0.9623	−0.00313	0.6417	−0.0031	0.6466
rs7043199	−0.00119	0.8798	0.002393	0.77	0.001466	0.859
rs10738760	−0.0021	0.7244	−0.00156	0.8026	−0.00142	0.8201
rs2375981	−0.00033	0.9559	−0.00017	0.9786	9.90×10^{-5}	0.9875
rs10761741	0.005041	0.4081	0.000931	0.8832	0.000839	0.8954
rs4782371	−0.00663	0.2943	−0.00846	0.2027	−0.00844	0.2076
rs2639990	−0.00571	0.5596	−0.00865	0.3943	−0.00733	0.4765
logGlucose						
rs114694170	0.01915	0.4259	0.01844	0.488	0.01499	0.5762
rs6921438	−0.00684	0.4689	−0.01078	0.2855	−0.01227	0.2245
rs1740073	0.00942	0.3361	0.007099	0.4879	0.006708	0.5143
rs4416670	0.000832	0.9235	0.000346	0.9698	0.000223	0.9806
rs6993770	−0.01043	0.2856	−0.00569	0.5839	−0.00679	0.5148
rs7043199	0.008424	0.4782	0.008428	0.4973	0.006293	0.6144
rs10738760	0.006866	0.457	0.003822	0.6927	0.002642	0.7852
rs2375981	0.007188	0.445	0.004344	0.6588	0.003512	0.722
rs10761741	0.003465	0.7095	0.004664	0.6322	0.006317	0.5187
rs4782371	−0.01497	0.1213	−0.00968	0.3456	−0.00954	0.3557
rs2639990	−0.00127	0.9336	−0.0042	0.7913	−0.00359	0.8233
logLDL						
rs114694170	−0.0082	0.6443	−0.02002	0.3046	−0.02187	0.2661
rs6921438	−0.00502	0.4711	−0.00418	0.573	−0.00419	0.5718
rs1740073	0.000988	0.8914	−0.00091	0.9035	−0.0022	0.7704
rs4416670	0.001987	0.7558	0.006226	0.3529	0.006893	0.3039
rs6993770	−0.00281	0.6968	−0.00581	0.4461	−0.00551	0.4718
rs7043199	0.006725	0.4431	0.006013	0.5094	0.005337	0.5605
rs10738760	−0.01029	0.1306	−0.01186	0.09438	−0.01145	0.1071
rs2375981	−0.01274	0.06626	−0.01425	0.04787	−0.01372	0.05769
rs10761741	−0.00519	0.4493	−0.00794	0.2667	−0.0091	0.2047
rs4782371	0.01135	0.1115	0.007783	0.3015	0.008257	0.2758
rs2639990	−0.00388	0.7136	−0.00713	0.517	−0.00744	0.5042

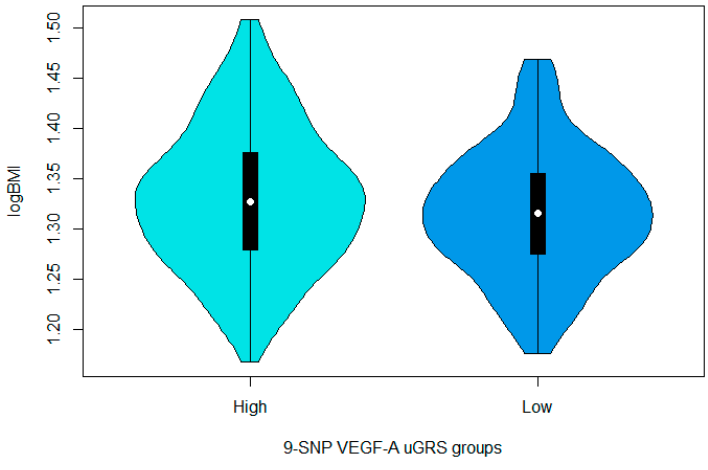
Table 3. *Cont.*

	Model 1		Model 2		Model 3	
	Beta	***p*-Value**	**Beta**	***p*-Value**	**Beta**	***p*-Value**
logHDL						
rs114694170	0.001151	0.9449	−0.00031	0.9867	−0.00111	0.9524
rs6921438	0.002231	0.7332	0.00056	0.9363	−0.00014	0.9837
rs1740073	0.002099	0.7572	0.005597	0.4303	0.00606	0.3951
rs4416670	0.002402	0.6887	0.000127	0.984	−0.00021	0.9737
rs6993770	0.01151	0.08893	0.0148	0.03953	0.01427	0.04781
rs7043199	−0.00711	0.3875	−0.00429	0.6186	−0.00585	0.4992
rs10738760	0.01409	0.02729	0.01249	0.06206	0.01223	0.06815
rs2375981	0.01261	0.05275	0.01139	0.09454	0.01129	0.09822
rs10761741	−0.01029	0.1098	−0.01098	0.1037	−0.00975	0.15
rs4782371	−0.00762	0.2552	−0.0072	0.3117	−0.0068	0.3417
rs2639990	−0.00388	0.7136	−0.00713	0.517	−0.00744	0.5042
logCRP						
rs114694170	−0.0379	0.6541	−0.04237	0.6554	−0.03521	0.711
rs6921438	−0.0418	0.1947	−0.04414	0.2017	−0.04039	0.241
rs1740073	−0.00433	0.8972	−0.0181	0.606	−0.02466	0.482
rs4416670	−0.0194	0.511	−0.01528	0.6242	−0.0162	0.6012
rs6993770	−0.01718	0.6107	−0.00339	0.9251	−0.0048	0.8941
rs7043199	0.02666	0.5029	0.003378	0.9353	0.000455	0.9913
rs10738760	0.02319	0.4658	0.02242	0.5016	0.02371	0.4762
rs2375981	0.02867	0.3747	0.02603	0.441	0.02572	0.4462
rs10761741	0.0237	0.4588	0.01415	0.6735	0.01207	0.7179
rs4782371	−0.04092	0.2165	−0.03658	0.3002	−0.03689	0.2958
rs2639990	−0.05523	0.2803	−0.05647	0.2884	−0.05193	0.3325

Model 1: Adjusted for age and sex, Model 2: Adjusted for age, sex and exercise, Model 3: Adjusted for age, sex, exercise and dietary patterns. BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, PP: Pulse Pressure, LDL: Low-density cholesterol, HDL: High-density cholesterol, CRP: C-reactive protein.

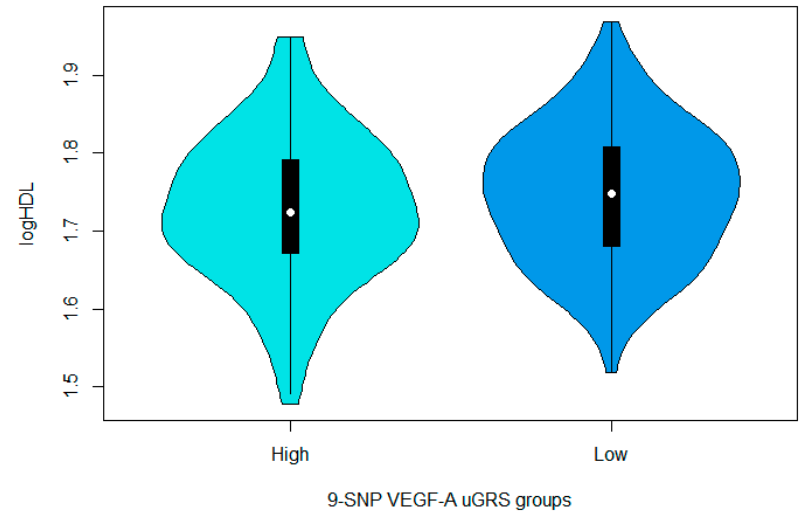
3.3. Associations between the 9-SNP uGRS and the Cardiometabolic Indices

In the effort to examine the potential effect of uGRS in the formation of the investigated indices, we separated the 9-SNP uGRS into the two categories of "low" and "high" risk based on the sample median, where logBMI displayed statistically significant differences between the two groups (Figure 1), with individuals in the higher category presenting greater logBMI (p-value < 0.05), indicating that higher risk for increased VEGF-A levels is also associated with elevated logBMI. People in the higher percentile of uGRS also presented statistically significantly higher values of logSBP compared to the ones in the lower group (p-value < 0.05), also denoting that elevated risk for increased VEGF-A levels is further associated with increased logSBP. To boot, individuals with higher versus lower uGRS did display statistically significantly lower levels of logHDL (p-value < 0.05), highlighting an inverse association between increased risk for VEGF-A and levels of logHDL.

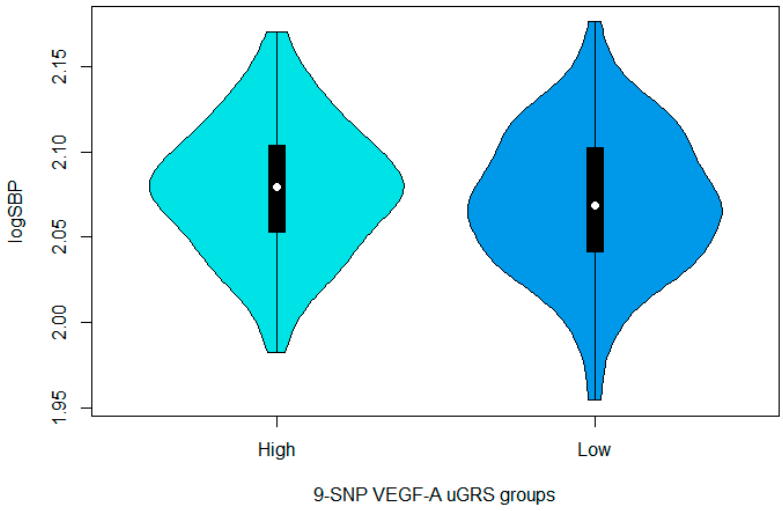


(**A**)

Figure 1. *Cont.*



(**B**)



(**C**)

Figure 1. Violin plots depicting the distribution of (**A**) logBMI, (**B**) logSBP and (**C**) logHDL between the two groups of the 9-SNP VEGF-A unweighted GRS (low versus high), separated by the sample median (p-values < 0.05).

Furthermore, the creation of the 9-SNP uGRS was followed by association testing for all cardiometabolic indices explored via linear regressions after adjusting for age and sex (Model 1), age, sex and exercise (Model 2) and age, sex, exercise and dietary patterns (Model 3). Similar to the results deriving from the within-group comparisons and as shown in Table 4, significant associations were observed between higher uGRS values and increased levels of logBMI across all models (Model 1: $\beta = 0.0044$, p-value = 0.003, Model 2: $\beta = 0.0043$, p-value = 0.005, Model 3: $\beta = 0.004$, p-value = 0.009). Additionally, a statistically significant, positive association was also observed between the uGRS and logSBP, again after adjusting for all models (Model 1: $\beta = 0.002$, p-value = 0.03, Model 2: $\beta = 0.019$, p-value = 0.047, Model 3: $\beta = 0.002$, p-value = 0.037). The score was further negatively associated with logHDL levels after adjustment for age and sex (Model 1: $\beta = -0.005$, p-value = 0.032), an association which was not maintained after correcting for the additional confounders (exercise and dietary patterns).

Table 4. Associations between the 9-SNP uGRS and selected cardiometabolic indices in the TEENAGE cohort.

	Model 1			**Model 2**			**Model 3**		
	Estimate	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**
logBMI 9-SNP uGRS for VEGF-A	0.004445	0.001494	0.00305	0.004349	0.001553	0.005277	0.0040937	0.0015678	0.009281
logTriglycerides 9-SNP uGRS for VEGF-A	0.005892	0.003854	0.127	0.004260	0.003915	0.2771	0.004650	0.003994	0.2450
logCholesterol 9-SNP uGRS for VEGF-A	−0.0001979	0.0017479	0.90992	−0.000716	0.001859	0.70024	−0.0007685	0.0018917	0.68474
logSBP 9-SNP uGRS for VEGF-A	0.002006	0.000924	0.0303	0.0019840	0.0009974	0.047203	0.0020983	0.0010045	0.037205
logDBP 9-SNP uGRS for VEGF-A	0.001891	0.001351	0.161963	0.002211	0.001441	0.12569	0.002365	0.001455	0.10458
LogPP 9-SNP uGRS for VEGF-A	0.002425	0.002268	0.2854	0.001599	0.002413	0.50779	0.0015523	0.0024439	0.52558
LogGlucose 9-SNP uGRS for VEGF-A	0.0009057	0.0036448	0.804	0.001952	0.003840	0.611	0.0028415	0.0038989	0.4665
logLDL 9-SNP uGRS for VEGF-A	0.003038	0.002688	0.2589	0.002300	0.002818	0.4148	0.001733	0.002863	0.5454
LogHDL 9-SNP uGRS for VEGF-A	−0.005336	0.002493	0.03279	−0.004999	0.002631	0.05812	−0.004455	0.002673	0.09630
LogCRP 9-SNP uGRS for VEGF-A	0.001437	0.012397	0.90778	−0.0001663	0.0131008	0.98988	−0.001631	0.013250	0.90207

Model 1: Adjusted for age and sex, Model 2: Adjusted for age, sex and exercise, Model 3: Adjusted for age, sex, exercise and dietary patterns. BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PP: Pulse Pressure; LDL: Low-density cholesterol; HDL: High-density cholesterol; CRP: C-reactive protein; SE: Standard Error.

3.4. Interactions between the uGRS and Dietary Patterns

After calculating the 9-SNP uGRS, we carried on to examine the potential associations between the cardiometabolic indices and their interactions with the five previously extracted patterns of food choices in the teenagers, namely the "Western Breakfast", the "Legumes and Good Fat", the "Homemade Meal", the "Chickens and Sugars", and the "Eggs and Fibers" patterns [19]. Table 5 shows the multivariate linear regressions carried out for each examined index and the interaction between the uGRS and each of the dietary patterns after adjusting for age, sex, uGRS and each dietary pattern (Model 1) and age, sex, and exercise. uGRS and each dietary pattern (Model 2).

Table 5. Associations between the 9-SNP uGRS for VEGF-A and dietary patterns in the TEENAGE cohort.

	Model 1 *			**Model 2 ***		
	Estimate	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**
logBMI						
uGRS*Western Breakfast	0.0006259	0.0016544	0.70532	0.0009623	0.0016699	0.564684
uGRS*Legumes and Good Fat	0.0004362	0.0014115	0.75742	−0.0002951	0.0015027	0.844375
uGRS*Homemade Meal	−0.001836	0.001302	0.15906	−0.001894	0.001326	0.153652
uGRS*Chicken and Sugars	−0.001955	0.001442	0.17566	−0.001508	0.001577	0.339236
uGRS*Eggs and Fibers	−0.000687	0.001204	0.56840	0.0004325	0.0014616	0.767393
logTriglycerides						
uGRS*Western Breakfast	−0.003976	0.004121	0.335	−0.003394	0.004147	0.4135
uGRS*Legumes and Good Fat	−0.003084	0.003643	0.398	−0.002993	0.003701	0.4192
uGRS*Homemade Meal	−0.0003673	0.0031521	0.907	−0.0004249	0.0031042	0.8912
uGRS*Chicken and Sugars	−0.000562	0.003527	0.873	0.000446	0.003723	0.9047
uGRS*Eggs and Fibers	0.0004714	0.0029163	0.872	-8.952×10^{-7}	3.645×10^{-3}	0.9998
logCholesterol						
uGRS*Western Breakfast	−0.0003120	0.0018673	0.86737	−0.0003595	0.0019652	0.85495
uGRS*Legumes and Good Fat	4.399×10^{-4}	1.654×10^{-3}	0.79038	0.0006190	0.0017604	0.72529
uGRS*Homemade Meal	0.0022544	0.0014247	0.11421	0.0024594	0.0014679	0.09455
uGRS*Chicken and Sugars	0.0005882	0.0015997	0.71324	0.0011419	0.0017668	0.51840
uGRS*Eggs and Fibers	−0.0024429	0.0013171	0.064231	−0.0035654	0.0017221	0.0390
logSBP						
uGRS*Western Breakfast	0.0019835	0.0010171	0.05164	0.0021791	0.0010716	0.042500
uGRS*Legumes and Good Fat	0.0009800	0.0008694	0.2601	0.001112	0.000966	0.250296
uGRS*Homemade Meal	−0.0004048	0.0008249	0.6238	−0.0006534	0.0008508	0.442827
uGRS*Chicken and Sugars	0.0003776	0.0008987	0.6745	0.0003459	0.0010081	0.731659
uGRS*Eggs and Fibers	−0.0011855	0.0007341	0.1068	−0.0018073	0.0009354	0.053889
logDBP						
uGRS*Western Breakfast	0.0060753	0.0014736	4.28×10^{-5}	0.005687	0.001537	0.000239
uGRS*Legumes and Good Fat	0.0009039	0.0012713	0.477344	0.001483	0.001396	0.28856
uGRS*Homemade Meal	−0.0008981	0.0012064	0.45691	−0.001097	0.001229	0.37234
uGRS*Chicken and Sugars	1.822×10^{-5}	1.316×10^{-3}	0.988960	0.001229	0.001457	0.39932
uGRS*Eggs and Fibers	0.0001876	0.0010752	0.86156	−0.0009524	0.0013559	0.48273
logPP						
uGRS*Western Breakfast	−0.004375	0.002501	0.08081	−0.003179	0.002602	0.22237
uGRS*Legumes and Good Fat	0.0006745	0.0021355	0.75221	0.0001765	0.0023393	0.93989
uGRS*Homemade Meal	0.0001585	0.0020281	0.93772	-5.986×10^{-5}	2.067×10^{-3}	0.97691
uGRS*Chicken and Sugars	0.0006736	0.0022094	0.76055	−0.001662	0.002442	0.49637
uGRS*Eggs and Fibers	−0.003235	0.001801	0.07296	−0.002587	0.002269	0.2548
logGlucose						
uGRS*Western Breakfast	−0.0002371	0.0038992	0.952	−0.0006882	0.0040671	0.866
uGRS*Legumes and Good Fat	−0.004075	0.003441	0.237	−0.002575	0.003628	0.478
uGRS*Homemade Meal	−0.0035228	0.0029773	0.237	−0.003946	0.003039	0.195
uGRS*Chicken and Sugars	0.003550	0.003317	0.285	0.003922	0.003634	0.281
uGRS*Eggs and Fibers	5.869×10^{-3}	2.745×10^{-3}	0.0330	0.008830	0.003550	0.0132
logLDL						
uGRS*Western Breakfast	−0.0003845	0.0028733	0.8936	−0.0008217	0.0029791	0.7828
uGRS*Legumes and Good Fat	0.001102	0.002545	0.6652	0.001857	0.002669	0.4870
uGRS*Homemade Meal	0.002229	0.002194	0.3103	0.002617	0.002230	0.2412
uGRS*Chicken and Sugars	0.0024563	0.0024563	0.9468	0.0008795	0.0026757	0.7425
uGRS*Eggs and Fibers	−0.004027	0.002024	0.0472	−0.005950	0.002606	0.0229

Table 5. *Cont.*

	Model 1 *			Model 2 *		
	Estimate	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**
logHDL						
uGRS*Western Breakfast	0.0007058	0.0026675	0.79145	0.001002	0.002789	0.71958
uGRS*Legumes and Good Fat	0.0004628	0.0023529	0.84413	-7.341×10^{-5}	2.485×10^{-3}	0.97644
uGRS*Homemade Meal	0.003719	0.002032	0.06787	0.003693	0.002080	0.07649
uGRS*Chicken and Sugars	0.001880	0.002275	0.40903	0.002321	0.002496	0.3529
uGRS*Eggs and Fibers	−0.0003372	0.0018861	0.85819	−0.0007087	0.0024472	0.77227
logCRP						
uGRS*Western Breakfast	−0.009797	0.013082	0.45430	−0.0072781	0.0136345	0.59379
uGRS*Legumes and Good Fat	0.002883	0.011393	0.80035	−0.0031947	0.0119986	0.79019
uGRS*Homemade Meal	0.010795	0.009823	0.27239	0.011024	0.010010	0.27144
uGRS*Chicken and Sugars	0.004140	0.010979	0.70632	−0.0006592	0.0120081	0.95625
uGRS*Eggs and Fibers	−0.006220	0.008995	0.48963	0.0010038	0.011644	0.93135

* Model 1: Adjusted for age, sex, uGRS and each dietary pattern, Model 2: Adjusted for age, sex, and exercise. uGRS and each dietary pattern. BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PP: Pulse Pressure; LDL: Low-density cholesterol; HDL: High-density cholesterol; CRP: C-reactive protein; SE: Standard Error.

As shown in the table, after evaluation based on the adjusted threshold ($p = 0.01$), the interaction between the uGRS and the "Western Breakfast" was associated with higher levels of logDBP (Model 1: β = 0.0060, *p*-value = 4.28×10^{-5}, Model 2: β = 0.00568, *p*-value = 0.000239), suggesting that increased risk for high VEGF-A and adherence to a western-diet-like pattern is associated with elevated logDBP. A different nominally statistically significant, positive association was found for the interaction between the uGRS and consumption of the "Eggs and Fibers" pattern and increased levels of logGlucose after adjusting for age, sex, and exercise (Model 2: β = 0.00883, *p*-value = 0.0132), potentially indicating that elevated risk for increased VEGF-A and increased consumption of fiber-rich foods or eggs is associated with increased levels of logGlucose.

4. Discussion

The present study sought to conduct the first-ever attempt to investigate the role of VEGF-A-related variants on adolescent cardiometabolic profile, as well as their potential interplay with dietary habits. In this population of Greek teenagers, two VEGF-A-related SNPs, namely the rs7043199 and the rs4416670 variants, presented significant relations with blood pressure indices. Moreover, the 9-SNP uGRS constructed out of risk variants for higher VEGF-A levels was associated with higher levels of logBMI and logSBP but lower levels of logHDL. Furthermore, the exploration of associations between the uGRS and the teenagers' dietary patterns revealed a significant relationship between the adherence to the "Western Breakfast" pattern and higher logDBP, as well as a nominal association for the "Eggs and Fibers" pattern and higher logGlucose.

In our sample, the negatively associated with VEGF-A levels C allele of the rs4416670 SNP was also negatively associated with logSBP levels. Debette et Visvikis-Siest et al. previously showed a positive relationship between the allele and increased pulse pressure in a healthy population [10]; this could potentially be attributed to the relationship between lower levels of SBP, which would subsequently signify greater values of pulse pressure. On the contrary, the A allele of the rs7043199 variant, which was previously negatively associated with VEGF-A [10,11], was hereby linked with higher levels of logSBP and logDBP. Although not as statistically strong (*p*-value = 0.004), this observed effect could possibly be attributed to the yet-to-be-fully elucidated pleiotropic influence of the variant, the role of which has been previously investigated for overall risk for other disorders related to cardiometabolic profile, namely ischemic heart disease [27] and osteoporosis [28].

To the best of our knowledge, VEGF-A has not been extensively and exclusively examined in adolescents, and the present constitutes the first attempt to construct a uGRS for teenagers using VEGF-A-associated variants. The present 9-SNP uGRS was linked to higher

levels of logSBP (Model 1: β = 0.002, *p*-value = 0.03, Model 2: β = 0.019, *p*-value = 0.047, Model 3: β = 0.002, *p*-value = 0.037) and individuals with high GRS presented greater values compared to the ones with low GRS (*p*-value = 0.027), showing that increased genetic predisposition to higher levels of VEGF-A is associated with higher blood pressure in adolescents. This finding is aligned with the well-known relationship between VEGF-A and hypertension, as the current literature has shown that the inhibition of VEGF-A receptors signifies higher levels of circulating VEGF-A, which have, in turn, been associated with a greater risk for hypertension [29–31]. In a similar manner and supporting the reciprocal relationship between the VEGF family and hypertension, Zorena et al. showed that adolescents with type 1 diabetes and hypertension displayed greater levels of VEGF compared to healthy individuals or patients with type 1 diabetes but without hypertension [32].

Although this is an overall healthy population with most adolescents presenting normal weight, the accumulating effect of the nine examined SNPs from Choi et al. displayed a statistically significant, positive association with higher logBMI values. In addition to the already underlined positive relationship between VEGF-B and VEGF-C levels and obesity presence [33,34], the current literature further highlights the role of VEGF-A in obesity control [2,35,36]. In the presence of obesity and fat cell proliferation, VEGF-A expression increases as it participates in angiogenesis, cell differentiation and thermogenesis in the white and brown adipose tissues. In this context, VEGF-A contributes to the subsequent increase in energy expenditure and attempts to suppress further diet-induced increase and ameliorate insulin resistance in a compensatory effect [2,35,36]. However, as the increase in adipocytes progresses, VEGF-A is produced more, and angiogenesis is further promoted in the white adipose tissue, thus allowing for further obesity establishment. This cascade of events creates a reciprocal circle where obesity presence induces VEGF-A expression and vice versa. For that reason, the effect of VEGF-A on increased weight can be described as reciprocal and context-dependent, being mainly influenced by the potential pre-existence of increased body weight [1,35]. Hereby, the positive association between the uGRS and logBMI was steadily maintained after adjustments for all three models of confounding factors (Model 1: β = 0.0044, *p*-value = 0.003, Model 2: β = 0.0043, *p*-value = 0.005, Model 3: β = 0.004, *p*-value = 0.009) and adolescents with high versus low genetic risk also presented higher values of logBMI, suggesting an aggravating effect in BMI as a genetic risk for higher VEGF-A increases. In a similar context to the present, Novikova et al. showed that compared to individuals of normal weight, adolescents with obesity presented a 12-fold increase in corresponding VEGF-A levels [37]. To boot, Loebig et al. showed a similar positive association in healthy young men (aged 18–30 years old) under normal blood sugar conditions, where higher levels of VEGF-A were consistently associated with increased weight [38]. VEGF-A was also related to abdominal obesity in a sample of young individuals, as demonstrated by Guzman-Guzman et al. when investigating relations with parameters of the metabolic syndrome [39]. Our present findings show that increased predisposition to higher levels of VEGF-A is related to higher BMI; however, according to the aforementioned, it should be noted that the reciprocity of the relationship remains significant, as increased VEGF-A levels can generally be observed due to increased BMI, thus potentially aggravating the positive predisposing genetic effect.

Another significant relation was observed between the uGRS and lower levels of logHDL (Model 1: β = 0.005, *p*-value = 0.032). Although this association was not maintained after correction for multiple confounding factors, when looking at individuals with higher versus lower genetic risk for increased VEGF-A, the former did present lower values of logHDL. When looking into potential associations between VEGF-A variants and HDL, both Debette et Visvikis-Siest and Stathopoulou et al. showed that the negatively associated with VEGF-A A allele of rs6921438 SNP was related to lower HDL levels in healthy populations [10,12]. The present finding denoting a positive association between increased VEGF-A and lower HDL levels can, thus, potentially be explained by the general overview of the role of elevated VEGF-A in worse lipidemic profile, rather than the direct effect of VEGF-A on HDL per se [40].

Furthermore, taking the biomarker's role in metabolism into account [2,6,7], we further attempted to unravel the meaning of the interplay between genetic predisposition for higher VEGF-A levels and multiple cardiometabolic indices by examining the potentially modifying role of dietary habits. In our sample, the interaction between the uGRS and the consumption of the "Western Breakfast" was associated with higher levels of logDBP (Model 1: $\beta = 0.0060$, p-value $= 4.28 \times 10^{-5}$, Model 2: $\beta = 0.00568$, p-value $= 0.000239$). This finding can be explained by the fact that the "Western Breakfast" pattern consists of food groups with high-fat content, namely cheese, dairy and processed meat [19], which have already been shown to associate with increased blood pressure in the literature [41]. Hojhabrimanesh et al. showed similar significant associations between a "Western" dietary pattern and overall and systolic blood pressure in Iranian adolescents, as well as a positive but not statistically significant association for diastolic pressure [42]. Although the pattern was not unilaterally associated with blood pressure measurements in our team's previous analyses [19], and an increased predisposition to higher VEGF-A appears to bring its aggravating effect to the forefront and vice versa. This could be partly attributed to the positive effect of the Western diet and red meat-derived protein, which has been previously shown to elevate VEGF-A expression among patients with breast cancer [43].

Furthermore, although the 9-SNP uGRS was not alone associated with glucose in our sample, it did present a nominally significant interaction with the protein-rich "Eggs and Fibers" dietary pattern (consisting of non-refined cereals, vegetables and eggs) in increasing logGlucose levels (Model 2: $\beta = 0.00883$, p-value $= 0.0132$). The involvement of VEGF-A in glucose homeostasis is well-known [8], as low levels of the biomarker are linked to insulin resistance, while its overexpression is associated with impaired insulin production and increased glucose levels [2,8]. Consequently, research in adolescent cohorts to date mainly surrounds diabetic individuals or related complications [30,44] and has yet to yield significant results in healthy populations. Although fiber intake is generally regarded as having protective effects in the production of inflammatory biomarkers [45], the present finding could possibly refer to the reciprocal effect of dietary carbohydrate and protein intake on aggravating the genetic risk for VEGF-A levels and subsequent influence the elevated glucose levels.

Moreover, similar gene–diet interactions have also been explored in individuals with metabolic syndrome in studies examining target SNPs for VEGF-A rather than using a holistic genetic risk score approach. Ghazizadeh et al. showed that individuals with the AA genotype for the rs10738760 variant, which was also included in the present uGRS, and higher adherence to foods with increased sugar and saturated fatty acids, among others, presented a greater risk for metabolic syndrome [16]. It was further demonstrated that the presence of the same A allele can significantly interact with even favorable dietary components (e.g., PUFAs) in ultimately elevating the risk for worse glycemic and lipidemic profile and, thus, metabolic syndrome [16]. Taking it one step further, Chedid et al. showed a significant association between BMI and the rs10738760 polymorphism in decreasing iron levels, an effect shown to be more prominent in individuals with obesity [18]. Finally, a different relation concerned the observed associations between the presence of the 9-SNP-uGRS rs6921438 and rs6993770 included SNPs and micronutrient contents, namely high manganese, low zinc, and low iron intakes in patients with metabolic syndrome [46–48].

The strengths of the present study concern its hypothesis of investigating demonstrated effects of known VEGF-A variants on the cardiometabolic profile of healthy adolescents for the first time. Various associations presented hereby underline the effect of the SNPs in this age group and further highlight the complementary and modifying effect of diet in this vulnerable and crucial for future development life stage. The limitations of the study are summarized as follows: (i) the limited but substantial number of participants compared to larger cohorts examining VEGF-A-related variants; (ii) the overall health status of the population used, which might not have promoted the identification of distinct associations with cardiometabolic risk factors, as for example in the case of patients with

obesity or disrupted glucose metabolism and; (iii) the restricted variance of the populations' habits explained by the previously extracted dietary patterns (46.69%) [19].

5. Conclusions

The results from the present study suggest that well-identified VEGF-A-related variants in adults affect the parameters of adolescent cardiometabolic profiles. Our findings highlight the complexity of the mechanisms in which VEGF-A-related variants affect cardiometabolic risk factors both directly but also potentially through pleiotropic effects. Assessment of the role of diet showed that interaction between genetic makeup and dietary habits could significantly influence the variation of glycemic and blood pressure indices in this age group. In this spectrum, our findings promote the enhancement of our understanding of VEGF-A influence and its individual interaction with dietary aspects. We hereby lay the ground for future GWAS studies to be held that include larger adolescent sample sizes, allowing for the establishment of corresponding effect sizes and the subsequent construction of weighted GRSs for VEGF-A in teenagers. The latter would broaden our abilities in evaluating this reciprocal relationship and even allow for the use of the risk scores as tools of individual and clinical utility in assessing the risk for adolescent cardiometabolic disorders.

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Institutional Review Board Statement: The TEENAGE study received approval from the Institutional Review Board of Harokopio University of Athens, as well as the Greek Ministry of Education, Lifelong Learning and Religious Affairs.

Informed Consent Statement: All participants in the TEENAGE study provided verbal consent in combination with their parents' written consent before enrolling in the study.

Data Availability Statement: Access to the study data is available upon request due to participants' privacy and ethical restrictions.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix E: Scientific Publication on Polygenic Risk Score for Body Mass Index

Journal of *Personalized Medicine*

MDPI

Article

Robust Bioinformatics Approaches Result in the First Polygenic Risk Score for BMI in Greek Adults

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Abstract: Quantifying the role of genetics via construction of polygenic risk scores (PRSs) is deemed a resourceful tool to enable and promote effective obesity prevention strategies. The present paper proposes a novel methodology for PRS extraction and presents the first PRS for body mass index (BMI) in a Greek population. A novel pipeline for PRS derivation was used to analyze genetic data from a unified database of three cohorts of Greek adults. The pipeline spans various steps of the process, from iterative dataset splitting to training and test partitions, calculation of summary statistics and PRS extraction, up to PRS aggregation and stabilization, achieving higher evaluation metrics. Using data from 2185 participants, implementation of the pipeline enabled consecutive repetitions in splitting training and testing samples and resulted in a 343-single nucleotide polymorphism PRS yielding an $R^2 = 0.3241$ (beta = 1.011, p-value = 4×10^{-193}) for BMI. PRS-included variants displayed a variety of associations with known traits (i.e., blood cell count, gut microbiome, lifestyle parameters). The proposed methodology led to creation of the first-ever PRS for BMI in Greek adults and aims at promoting a facilitating approach to reliable PRS development and integration in healthcare practice.

Keywords: polygenic risk score (PRS); bioinformatics; body mass index (BMI); Greek adults

1. Introduction

According to WHO estimates for 2016, a considerable 49% and 13% of the global adult population presented overweight or obesity, whereas worldwide obesity prevalence has tripled since 1975 [1]. In this context, respective linear predictions dictate that about 50% of the global population will suffer from obesity by 2030 should similar increasing trends continue uninterrupted [2]. Increased body weight and fat accumulation are evidently directly related to elevated cardiometabolic risk and, subsequently, augmented prevalence of chronic diseases related to glycemic and lipidemic profile, such as type 2 diabetes and

cancer [3]. Due to its preventable nature and demand for effective prevention strategies [4], current research focuses on deepening understanding of multifactorial obesity etiology by focusing on the quantified role of genetic predisposition and its reciprocal relation with lifestyle and environmental determinants in populations with various characteristics.

Indeed, aggregation of multiple single nucleotide polymorphisms (SNPs) in construction of polygenic risk scores (PRS) is increasingly gaining ground as a practical tool to enable quantification and interpretation of genetic information on phenotypic variance. From identification of the first 97 key BMI-related variants [5] up to creation of multiple BMI-specific PRSs presented in the PGS Catalog database [6], using polygenic predictions is increasingly viewed as a useful tool to assess and explain the relevant attributed obesity variance [7–11]. The advantages of the role of PRS use for disease prevention and augmented accuracy in precision medicine are discussed in the context of potentially increasing both personal and clinical utility [12]. Recent studies show that inclusion of PRS in prediction models for certain disease outcomes, such as cardiovascular disease or cancer, carries similar importance to other contributing factors, namely lipidemic biomarkers or smoking [13–15]. For that reason, future PRS integration in personalized medicine is deemed useful for disease diagnosis, risk prediction and forming contextualized lifestyle recommendations [13].

The current literature highlights the need for an efficient translational approach to integrating PRS use into daily practice, potentially via inclusion in tools predicting disease risk [13]. In an effort to increase validity and straightforward application, various methodologies for PRS creation have been suggested. In the case of examining BMI, such examples refer to conduct of large genome-wide association studies (GWAS) and subsequent inclusion of significant SNPs in the form of a score [11,16], a priori aggregation of literature-based SNPs [9] or even use of other techniques, such as functional data analysis [17]. However, most approaches suggested to date focus on the use of one methodology and do not display increased portability and applicability across populations [18]. The need of improving their constructive parameters is, therefore, deemed central in order to increase PRS validity and wider implementation [12].

Hereby, we introduce the use of a novel, automated and iterative approach for PRS construction using repetitive sample splitting processes, informed decision-making through real-time comparison of different summary statistics' methodologies and aggregation of PRS candidates based on a stabilizing iterative procedure. We present the results of its application in creating the first PRS for BMI in Greek adults using data from a unified database of three separate cohorts. The suggested outlined pipeline constitutes an innovative approach in facilitating PRS construction in a straightforward manner, applicable to cohorts of various sizes and characteristics.

2. Materials and Methods

2.1. Study Population

For the purpose of the present analyses, data from three cohorts of Greek adults were used, namely the case-control Greek Non-Alcoholic Fatty Liver Disease (NAFLD) study [19], the cross-sectional OSTEOS study [20] and the case-control THISEAS (The Hellenic Study of Interactions between Single Nucleotide Polymorphisms and Eating in Atherosclerosis Susceptibility) [21] study. All studies were approved by the Research Ethics Committee of Harokopio University of Athens and further required participants' written informed consent prior to enrolment (NALFD protocol number: 38074/13-07-2012, OSTEOS protocol number: 15/8-12-2005, 8/12/2005, THISEAS protocol number: 10/9-6-2004, 14/6/2004).

The detailed protocols of all three studies have been previously described elsewhere [19–23]. Briefly, the NAFLD study recruited adult participants without liver disease/injury and reporting absence of excess alcohol drinking at the time of induction to the study. Volunteers were recruited from the Outpatient Clinics of the First Department of Propaedeutic and Internal Medicine in Laiko General Hospital, during the period 2012 to 2015 [19]. Recruits were further screened for NAFLD through abdominal ultrasound and deemed as controls

in the absence of hepatic steatosis or in the presence of mild-stage, or cases in presence of moderate or severe hepatic steatosis [20]. Concerning the nodes of the OSTEOS study, 970 community-dwelling adults were recruited from rural and urban areas of Greece and assessed for quantitative ultrasound (QUS) parameters of bone health during the 2010–2012 period and in cooperation with the Hellenic Society for the Support of Patients with Osteoporosis and the Laboratory for the Research of Musculoskeletal System "Th. Garofalidis", School of Medicine, National and Kapodistrian University of Athens [21]. Last, within the THISEAS study, a total of 2565 participants were recruited from three Athenian hospitals, open protection centers and municipalities during the years 2006–2010. Recruits were mainly assessed using coronary angiography information and were categorized as controls if they presented negative coronary findings or a negative stress test or did not report any related clinical symptoms. Volunteers were categorized as cases in the presence of acute coronary syndrome or stable coronary artery disease (> 50% stenosis in $\geq 1/3$ main coronary vessels) [22,23].

2.2. Anthropometric Measurements

Anthropometric characteristics, including body weight and body height, were measured for all three studies. Body weight was measured using the TANITA Segmental Body Composition Analyzer BC-418 and a calibrated scale to the nearest 0.1 kg. Height was calculated to the nearest 0.5 cm using a mounted stadiometer. Participants were barefoot and maintained light clothing and measurements occurred twice and average values were kept as final in all projects. All measurements were conducted by trained professionals. BMI was calculated for all participants via use of the following formula:

$$\mathrm{BMI}\left(\frac{\mathrm{kg}}{\mathrm{m}^2}\right) = \mathrm{Body\ Weight(kg)} / \left(\mathrm{Body\ Height}\right)^2(\mathrm{m}^2)$$

Participants in all studies were classified based on BMI values in the categories of underweight (BMI < 18.5 $\mathrm{kg/m^2}$), normal weight (18 $\mathrm{kg/m^2} \leq$ BMI < 25 $\mathrm{kg/m^2}$), overweight (25 $\mathrm{kg/m^2} \leq$ BMI < 30 $\mathrm{kg/m^2}$) or obese (BMI $\geq$ 30 $\mathrm{kg/m^2}$). Within-study group differences in BMI were calculated using Kruskal–Wallis tests.

2.3. Genotyping Analyses

For the NAFLD study, DNA samples were isolated using peripheral blood lymphocytes and genotyped via use of the Infinium CoreExome-24 BeadChip, Illumina genome-wide SNP array (with 567,218 fixed markers). OSTEOS' DNA samples were isolated from buffy coats and genotyped using the Axiom Precision Medicine Diversity Research Array [with over 850,000 SNPs, insertions, deletions and copy number variations (CNVs)]. DNA samples from the THISEAS study were extracted from whole blood and genotyped using the Illumina Metabochip (with about 200.000 SNPs).

2.4. Preprocessing and Statistical Analysis

2.4.1. Dataset Merging and Genotype Imputation

Prior to joint statistical analysis and PRS derivation, the phenotypic and genotypic data of the three populations were merged. While the phenotypic integration was straightforward and comprised the simple join of the common phenotypes across the three datasets, the following steps were followed for the genotypic data which were converted to PLINK [24] 1.9 BED+BIM+FAM filesets. First, the PLINK filesets from NAFLD and THISEAS were imported into R version 4.2.0. using facilities from the package snpStats, version 1.46.0. Then, the process of merged dataset creation started with identifying the identical SNPs between the two datasets in terms of accession numbers, position and alleles. For the common but non-identical SNPs in terms of alleles, it was checked whether they could be resolved with strand-flipping. Those SNPs that could not be resolved with strand-flipping were not pointing to the same risk allele. This was resolved by querying online resources (Ensembl with the R package biomaRt, version 2.52.0 and dbSNP with

the R package rsnps, version 0.5.0). After the resolution, samples where the risk allele was changed based on online search were subjected to allele switching to maintain proper risk allele copies in the merged dataset. SNPs for which alleles could not be resolved by any means were dropped from the merged dataset. Finally, the SNPs and genotypes unique to each dataset were appended to the common ones to form the final SNP set. The same appending was applied to the samples of each dataset.

As expected, the aforementioned process created many missing genotypes, especially regarding non-common SNPs between the two datasets. To impute them, an iterative imputation approach was followed using facilities from package snpStats. The package includes genotype imputation functions based on linear regression of neighboring SNPs. This process was repeated until no further genotype imputation was possible. For the remaining missing genotypes of the merged dataset, a k-nearest-neighbors-based imputation technique was applied, implemented in the R package scrime, version 1.3.5.

The merging and the imputation process resulted in a merged NAFLD–THISEAS dataset. The OSTEOS dataset was merged with the latter by repeating all the aforementioned steps, resulting in a merged NAFLD–THISEAS-OSTEOS dataset. The final merged dataset was exported to PLINK format using functions from the snpStats package. Next, to enhance the pool of SNPs for PRS derivation, the merged dataset was extended using IMPUTE2 software [25] using the bundled 1000 Genomes Project reference panel. The imputed and extended dataset was re-imported to R for further analysis.

2.4.2. Data Filtering and Summary Statistics

The first filter applied to genotypic data was to exclude poorly imputed genotypes; therefore, SNPs with an IMPUTE2 INFO score less than 0.9 were excluded. Additional genotype and sample filtering was performed using functionalities from the snpStats package. Specifically, SNPs with an SNP call rate < 95% and minor allele frequency (MAF) < 5% and samples with a sample call rate < 90% were excluded from further analysis. The resulting filtered dataset was further subjected to a second round of genotype filtering based on the Hardy–Weinberg (HWE) equilibrium, where SNPs with HWE p-value < 10^{-9} were also excluded from further analysis.

After dataset filtering, principal component analysis (PCA) was performed to capture any underlying population stratification not reflected by the confounders used in the subsequent association tests using R package SNPRelate, version 1.30.1. Subsequently, regression models were fitted for each SNP against BMI phenotype using sex, age, NAFLD case/control and cardiovascular disease status along with selected PCs as correction covariates with the purpose of deriving summary statistics for each SNP, namely effects and statistical significance for contribution of each single SNP to the phenotype. The number of PCs was automatically selected using the Tracy–Widom statistic for assessment of the most significant PCs based on their eigen values [26]. Four different algorithms were used for derivation of summary statistics, namely simple General Linear Models (GLM, R version 4.2.0), statgenGWAS version 1.0.8. [27], SNPTEST version 2.5.4 [28] and PLINK.

2.4.3. Derivation of PRS

Several PRS candidates were derived using PRSice2 [29] combined with an iterative process for PRS derivation and validation and based on the merged dataset from the three populations. The PRS was calculated with the default PRSice2 option, which is:

$$\mathrm{PRS} = \sum_{i=1}^{k} \frac{\beta_i G_i}{N}$$

where βi represents the effect of PRS SNP i, Gi is the genotype coding (0, 1, 2 following PLINK notation, for the number of copies of risk alleles) and N the number of samples in the population. The PRS is reported in the figures of the present articles after applying min–max normalization to scale it to values between 0 and 1.

In each iteration, the following actions were performed: first, the total dataset was split to a training set (source set, 80% of samples) and a testing set (target set, 20% of samples). Then, the source set was used to perform de novo association tests for each SNP with four different methods (GLM, statgenGWAS, SNPTEST, PLINK) against the BMI phenotype. Sex, age, NAFLD status and several automatically selected PCs (varying between 5–12 across multiple iterations), using the Tracy–Widom test, were used as confounders in the regression models underlying each of the four methods, resulting in sets of summary statistics derived with each method. Then, these summary statistics were used along with the target dataset as inputs to PRSice2 for extraction of the optimal number of SNPs that would comprise a candidate PRS for the specific iteration. The aforementioned steps, from data splitting up to PRS synthesis with PRSice2, were repeated 100 times. At each iteration, several performance metrics were collected, among which the statistical significance of the PRS and the percentage of additional variance explained by the PRS (R^2) as returned by PRSice2. At this point, it should be noted that the PRSice2 PRS R^2 is the difference between the R2 of the "full" model, i.e., a regression model including all the covariates/confounders and the PRS, and the "null" or "reduced" model, i.e., a regression model only with the other covariates without the PRS. The PRS R^2 values were collected for each iteration, resulting in a baseline distribution that would be used later for assessing the statistical significance of the final PRS.

After completion of PRS derivation iterations, SNPs comprising PRS candidates for each summary statistics method were aggregated and number of appearances (frequency) of each SNP in the 100 iterations was counted considering an SNP to be appearing at least 5 times in order to further proceed to the downstream procedures. Then, for each frequency, a PRS comprising the SNPs appearing equally or above this frequency was assembled with effects averaged over iterations where each SNP appears and evaluated using previously described source/target dataset splits and linear regression, resulting in a series of evaluation metrics, among which also the PRS R2 as described above. This was repeated for all observed frequencies and a distribution of PRS R2 values was created. The PRS R^2 values were further penalized based on number of SNPs in PRS according to the following formula:

$$R_P^2 = \sqrt{\frac{R_{PRS}^2}{\log(N)}}$$

where R_P^2 is the PRS R^2 and N is the number of SNPs in the PRS. Then, a set of pre-final PRS candidates was defined by detecting local maxima in the R_P^2 distribution, reflecting PRSs with high values of R_P^2. The final PRS was selected based on the highest R_P^2 value. The statistical significance of the aggregated PRS R^2 as well as the R_P^2 was assessed using an empirical bootstrap defined as number of times where the baseline PRS R^2 was greater than the aggregated PRS R^2 divided by number of iterations.

3. Results

3.1. Population Characteristics

The anthropometric characteristics of the unified sample are described in Table 1. Overall, we used available data from 2083 participants, namely 342 participants from the NAFLD study, as well as 791 and 950 participants from the OSTEOS and THISEAS studies, respectively. A total of 841 men and 1242 women were included, with a median age of 53 years (calculated at 2075 participants) and a median BMI of 27.38 kg/m^2. Within the respective databases, participants presented median BMIs in the spectrum of overweight for all three studies (NAFLD median BMI = 26.5 kg/m^2, OSTEOS median BMI = 26.91 kg/m^2 and THISEAS median BMI = 27.81 kg/m^2). BMI was not statistically significantly different between the NAFLD and OSTEOS studies but did present a statistically significant difference between the NAFLD and THISEAS as well as the OSTEOS and THISEAS studies ($p < 0.001$ for both pairs). Differences in age were also statistically significant between all studies ($p < 0.001$ for the Kruskal–Wallis test).

Table 1. Descriptive characteristics of the NAFLD, OSTEOS and THISEAS study populations.

	All			NAFLD			OSTEOS			THISEAS		
	All (n = 2075 for age, n = 2083 for BMI)	Men (n = 841)	Women (n = 1234 for age, n = 1242 for BMI)	All (n = 342)	Men (n = 140)	Women (n = 202)	All (n = 783 for age, n = 791 for BMI)	Men (n = 101)	Women (n = 682 for age, n = 690 for BMI)	All (n = 950)	Men (n = 600)	Women (n = 350)
	Med (IQR)											
Age	53 (18)	54 (19)	52 (19)	47 (18)	44 (17)	50 (16)	50 (18)	47 (28.5)	51 (16.25)	59 (19)	58 (18.75)	60 (21)
BMI (kg/m^2)	27.38 (6.18)	27.68 (5.34)	27.02 (7.10)	26.5 (6.23)	26.8 (4.54)	25.9 (6.98)	26.91 (6.81)	26.70 (5.13)	26.94 7.01)	27.81 (5.80)	27.88 (5.43)	27.77 (6.51)

BMI: body mass index, Med: median, IQR: interquartile range.

Differences in BMI levels across the two sexes were statistically significant in the overall sample (p-value $< 2.2 \times 10^{-16}$), with men presenting higher values. Among the overall sample, 614 participants presented BMI in the range of 18.5–24.99 kg/m^2 (31.43% men, 68.56% women), whereas 875 and 579 participants presented overweight and obesity, respectively (Table 2). Most participants presenting overweight or obesity were in the THISEAS study (n = 730).

Table 2. Frequencies of BMI categories across the three studies.

	BMI < 18.5 kg/m^2			18.5 kg/m^2 ≤ BMI < 25 kg/m^2			25 kg/m^2 ≤ BMI < 30 kg/m^2			BMI ≥ 30 kg/m^2		
	All	Men	Women	All	Men	Women	All	Men	Women	All	Men	Women
All	15	0	15	614	193	421	875	405	470	579	243	336
NAFLD	3	0	3	117	36	81	141	74	67	81	30	51
OSTEOS	10	0	10	279	34	245	300	43	257	202	24	178
THISEAS	2	0	2	218	123	95	434	288	146	296	189	107

BMI: body mass index.

Regarding genotypic data, after imputation of IMPUTE2 with data from 1000 genomes project as a reference panel, a total of 24,307,245 variations were made available. Subsequently, variants with imputation confidence (INFO score returned by IMPUTE2) less than 0.9, structural and copy-number variations were excluded from further analysis. All downstream analyses were based only on known variants (i.e., variants recorded in dbSNP). This process led to 1,454,104 variants interrogated for PRS candidates. With respect to samples, 1970 (94.6%) had complete phenotypic records for covariates interrogated in regression models and included in further analyses.

3.2. Summary Statistics for PRS Derivation

Summary statistics for the merged dataset were calculated with BMI phenotype as a response variable and using the extended (imputed based on the 1000 genomes external reference panel) and further filtered genotypic dataset. In order to properly estimate the effects of individual SNPs that potentially contributed to the BMI phenotype in the unified dataset, we applied four different frameworks for summary statistics estimation, namely a simple generalized linear model (GLM) as implemented in the R statistical language, the regression algorithm implemented in the R package statgen GWAS as well as the SNPTEST software and the more generalized PLINK framework. In all cases, the sex, age, NAFLD status and cardiovascular disease status of individuals were incorporated in the regression models as confounders, along with several automatically selected principal components to capture potential underlying population stratifications not reflected by the other confounders. The four sets of summary statistics were used as input to PRSice2 along with the target samples in an iterative PRS derivation procedure, as described in Materials and Methods. To evaluate the performance of each summary statistics estimation method, we used the PRS R^2 metric returned by PRSice2, which measures percentage of BMI variability explained by the PRS in the regression models. The PRS R^2 values for each method were averaged over 100 PRS derivation iterations (Supplementary Figure S1) and the method that yielded the highest PRS R2 was selected to provide the summary statistics for final PRS derivation. In our case, SNPTEST yielded the highest average

PRS R^2 (0.012 ± 0.006, pmin = 0.0002, pmedian = 0.0375, pmax = 0.3194), followed by GLM (0.011 ± 0.006, pmin = 0.0003, pmedian = 0.0697, pmax = 0.4251) and statgenGWAS (0.010 ± 0.006, pmin = 0.0005, pmedian = 0.0718, pmax = 0.3579). PLINK yielded the lowest average PRS R^2 values but with the smallest variability across 100 iterations (0.009 ± 0.004, pmin = 0.0002, pmedian = 0.0802, pmax = 0.5282).

3.3. Selection of a PRS

After completion of 100 PRS derivation iterations, we assessed the stability of the extracted PRSs (Supplementary Figure S2). We observed that, in our case, PRS extraction process was highly dependent on source (training) dataset summary statistics. As a result, the SNP content of each PRS greatly varied between iterations, therefore affecting the performance of the latter and its contribution in explaining BMI. In order to mitigate the observed PRS instability, the 100 different SNP sets comprising the 100 different PRSs returned by PRSice2 with SNPTEST summary statistics were aggregated (Supplementary Table S1) as described in Materials and Methods, requiring that an SNP considered for inclusion in a PRS candidate should appear at least five times in the end of the iterative procedure.

Subsequently, several PRS candidates were assembled with SNP content based on frequency of appearance of the latter across the aggregated SNP set, new regression models were created based on the initial target dataset splits used by PRSice2 and PRS R^2 values were assembled (Figure 1A) along with their respective significance when compared with the baseline PRSice2 PRS R^2 distribution. As our goals included derivation of a PRS with a less extended number of SNPs but of high predictive value as a PRS with a larger number of SNPs, the new PRS R^2 values were further penalized based on the number of SNPs that each PRS candidate included (Figure 1B). Then, using the resulting distribution of penalized PRS R^2 values, we detected local maxima, denoting both high predictive value and lower SNP content. The number of SNPs yielding an adequately high penalized PRS R^2 while maintaining significance when compared to the baseline PRS R^2 distribution was found to be 343 (PRS R^2 = 0.1156 ± 0.0277). Notably, our iterative and aggregative PRS derivation process resulted in a PRS with ~10 times improved explanatory power (bootstrap p-value = 0, Figure 1A) than using PRSice2 alone.

3.4. PRS Evaluation

Next, we further evaluated the final 343-SNPs-selected PRS for BMI using the total merged dataset coupled with an iterative 10-fold cross-validation process, where, in each iteration of the process, we left out 5–50% of the total dataset samples, each time increasing the left-out samples by 5% and creating regression models including (full) and excluding (reduced) the PRS while maintaining the other covariates (Supplementary Table S2). Overall, the PRS increased the predictive power of the models by 31–33%, with the minimum PRS R^2 value observed at 0.3159 ± 0.0190 (p-value = 4×10^{-87}) when leaving out 50 of samples, with the maximum value at 0.3279 ± 0.0114 (p-value = 9×10^{-130}). A final regression model using the 343-SNP PRS for BMI with the total merged dataset yielded a PRS R^2 = 0.3241 (beta = 1.011, p-value = 4×10^{-193}). Finally, to evaluate the ability of the 343-SNP PRS to characterize close phenotypes, we created a regression model with the same covariates but using population weight instead of BMI. The model yielded PRS R^2 = 0.2313 (beta = 2.702, p-value = 4.15×10^{-158}, Supplementary Figure S3).

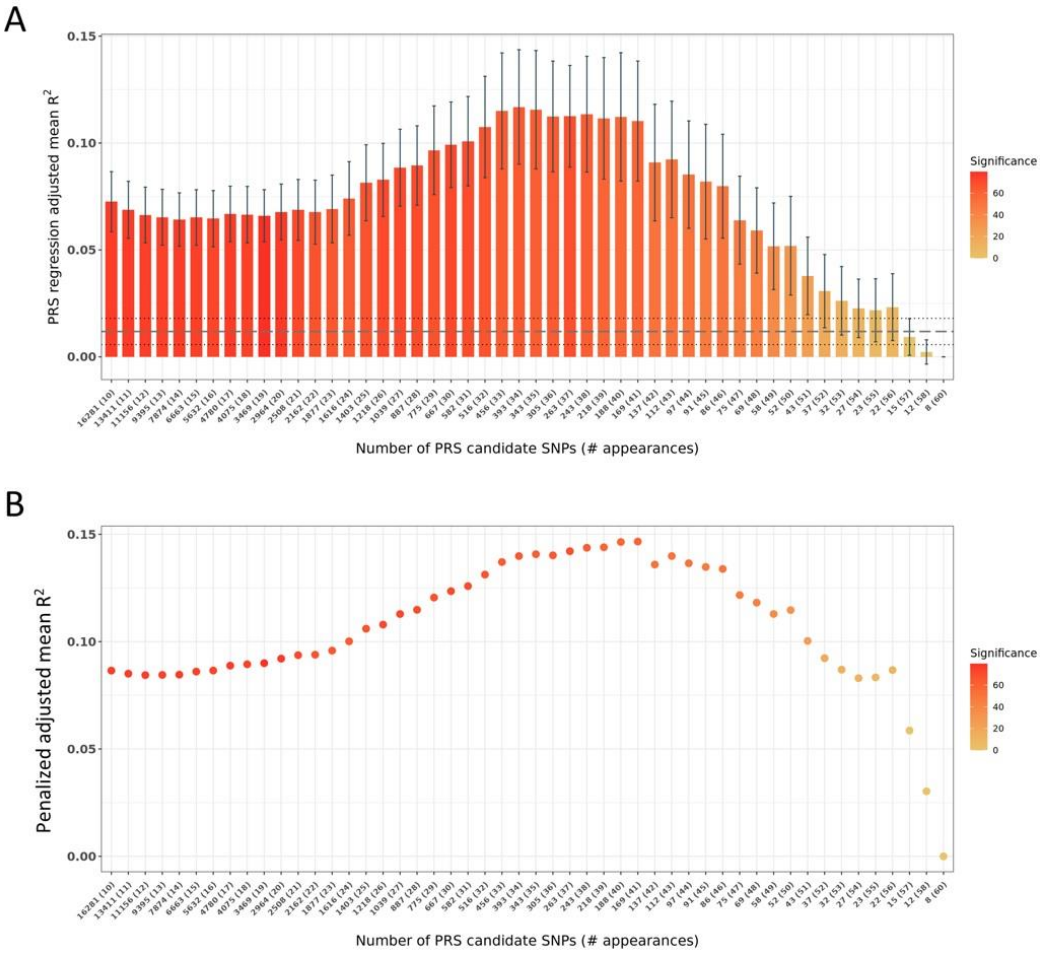

Figure 1. Mean PRS and penalized PRS R^2 for the assembled PRS candidates based on their frequency of appearance over 10 iterations as described in Materials and Methods. (**A**). Mean PRS R2 +/− standard deviation for PRS candidates assembled from SNPs at different frequencies of appearance in the PRS candidates across 100 PRS extraction iterations. The vertical axis depicts the mean adjusted PRS R^2, while the horizontal axis depicts the number of SNPs in each PRS candidate. The number inside the parentheses next to the number of SNPs in the horizontal axis depicts the SNP frequency of appearance in the PRS. For example, 393 (34) means that the PRS at that particular R^2 consists of 393 SNPs that appear at least 34 times over 100 iterations. The color scale denotes the statistical significance (Student's *t*-test *p*-value in −log10 scale) of the adjusted R^2 distribution over 100 de novo PRS extraction iterations (baseline R^2) as compared to the adjusted R2 distribution of each assembled PRS candidate in the horizontal axis. The mean baseline (derived directly from PRSice2 outcomes for each iteration) R2 is depicted with the dashed grey horizontal line, and the dotted grey horizontal lines depict the standard deviation of the former. (**B**). Mean penalized according to the number of SNPs PRS R^2.

3.5. PRS for BMI

The aforementioned 343-SNP PRS deriving from using SNPTEST displayed a statistically significant association for BMI (beta = 1.011, *p*-value = 4×10^{-193}) and a positive correlation, where increased PRS values were associated with increased BMI levels. As shown in Figure 2, the examined population presented an overall median risk, with most observations met in the 0.25–0.50 range. Out of the 343 SNPs identified in the PRS (see Supplementary Table S3), automatically identified known associations included in the GWAS Catalog were displayed for 16 SNPs, namely rs2710804 (27 associations) and rs2955742 (five associations) (see Table 3).

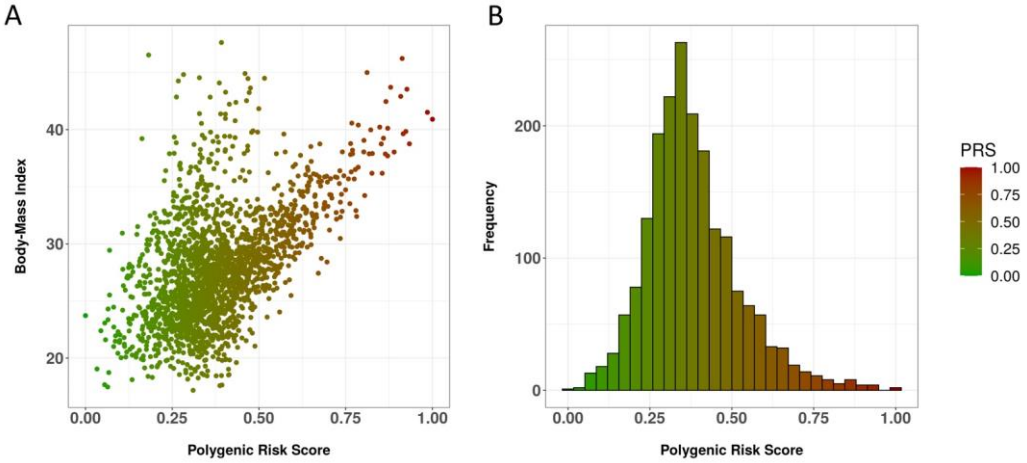

Figure 2. Correlation of the 343-SNP PRS for BMI with the phenotype and PRS distribution. (**A**). The BMI phenotype across the merged dataset is plotted against the min–max-normalized PRS value for each individual. (**B**). Histogram depicting the min–max-normalized PRS distribution for all individuals in the merged dataset.

Table 3. List of PRS SNPs with known associated traits in GWAS Catalog.

Consortial Summary Statistics (GWAS Catalog)						Known Associated Traits	Unified Cohort Summary Statistics	
SNP	Nearest gene	Position (Chr:bp)	Alleles	MAF	Effect Allele	Associated Traits	Effect allele	Beta [1]
rs11668205	IZUMO4	19:2096429-2099593	G/A	0.09 (A)	N/A	Abnormality of chromosome segregation	G	−0.32575
rs488248	LOC728192	13:105944370	C/A/T	0.23 (C)	T	Response to docetaxel, antineoplastic agent	C	−0.17048
rs480039	SLC35F3	1:234290732	G/A/C/T	0.37 (A)	N/A	Gut microbiome measurement	G	−0.17361
rs2288061	RPL18P13	16:76135833	G/A/C	0.34 (A)	G	Delta-5 desaturase measurement	G	−0.17776
rs2807854	HLX-AS1	1:220856499	T/C/G	0.25 (T)	T	LDL, apoB measurements	T	−0.13816
rs2955742	TMEM266	15:76153791	G/A	0.10 (A)	A	Serum urea, cystatin c, creatinine, urate, glomerular filtration measurement	G	−0.19108
Rs2710804	SEPT7,EEPD1	7:36044919	T/C	0.23 (C)	#N/A	Fibrinogen measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Serum alanine aminotransferase measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	KIAA1706	7:36044919	T/C	0.23 (C)	C	C-reactive protein measurement	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Leukocyte count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Myeloid white cell count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	N/A	Leukocyte count	T	−0.1356
rs2710804	SEPT7, EEPD1	7:36044919	T/C	0.23 (C)	N/A	Fibrinogen measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	T	Platelet count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Leukocyte count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Serum albumin measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	C-reactive protein measurement	T	−0.1356
rs2710804	EEPD1	7:36044919	T/C	0.23 (C)	C	Fibrinogen measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2710804	LOC101928618	7:36044919	T/C	0.23 (C)	T	Serum alanine aminotransferase measurement	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Myeloid white cell count	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Lymphocyte count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Platelet count	T	−0.1356
rs2710804	AC083864.3	7:36044919	T/C	0.23 (C)	C	Platelet crit	T	−0.1356
rs2710804	N/A	7:36044919	T/C	0.23 (C)	C	Neutrophil count	T	−0.1356
rs2251188	ZNF12, ZNF316	7:6664701	A/C/G/T	0.16 (A)	G	Basophil count, neutrophil count	A	0.13807
rs7589592	ENSG00000237720	2:2709171	T/A/C	0.41 (C)	N/A	Diffuse plaque measurement	T	0.11391
rs1010304	CHD6, EMILIN3	20:41473007	A/G	0.30 (G)	A	Memory performance, word list delayed recall measurement	A	−0.28657
rs12673506	CHN2	7:29382170	G/A	0.24 (A)	A	Gut microbiome measurement	G	−0.185
rs17662327	HNRNPA1P41,JAK2	9:4967587	T/C/G	0.16 (C)	T	Wellbeing measurement	T	0.14714
rs2485662	MEX3A/LMNA	1:156113677	T/C	0.31 (T)	N/A	Triacylglycerol 48:1, triacylglycerol 50:2 measurements	T	0.11601
rs4718965	AUTS2	7:70575462	C/A/T	0.08 (C)	C	Cortical surface area measurement	C	0.19049
rs9847987	intergenic/CFAP20DC-DT	3:59432807	C/T	0.20 (T)	T	Neuritic plaque measurement	C	0.26274
rs10252228	DPY19L1, NPSR1	7:34900427	A/G	0.29 (G)	G	Exercise	A	0.12063

SNP: single nucleotide polymorphism, Chr: chromosome, bp: base pairs, MAF: minor allele frequency, beta: effect size for BMI. [1] Results were derived via linear regressions after adjusting for sex, age, NAFLD status and number automatically selected PCs for population stratifications. Effect sizes (betas) and ORs shown for the corresponding SNP and effect sizes (betas) are reported for the respective effect allele.

4. Discussion

The present study sought to investigate application of an automated pipeline for PRS extraction using data from the three Greek studies of NAFLD, OSTEOS and THISEAS. In this population of Greek adults, the constructed PRS displayed a statistically significant association for BMI, with an R^2 of 0.3241 (beta = 1.011, p-value = 4×10^{-193}). The iterative pipeline presented here attempts to address various matters on PRS extraction, namely

selection of an appropriate threshold for SNP inclusion and prediction accuracy [18] as well as stability of the SNP content of PRS candidates across different training and test dataset splits.

In attempting to strengthen PRS construction methodology [30], this pipeline proposes implementation of iterative processes through repetitive steps of sample splitting, aggregating SNP frequency and effect size as well as comparative use of summary statistic metrics and consideration of lifestyle and genetic covariates. As a result, the suggested PRS includes a less extended number of variants but of high explanatory power. In this spectrum, this effort aims at facilitating construction of high-validity PRSs and subsequently promoting their use as a diagnostic tool accounting for various individual characteristics in daily practice. Use of the information of increased or reduced genetic risk for elevated BMI values, as demonstrated by the PRS, can potentially be translated in clinical practice to intensify (in the case of increased risk) or modify and personalize recommendations on lifestyle parameters to combat overweight and obesity.

To the best of our knowledge, the present study constitutes the first attempt to develop a PRS for BMI using data from a Greek population and a previous attempt for construction of a PRS has only been referred to once before in the current literature, exploring Parkinson's disease in older Greek adults [31]. Implementation of the suggested aggregated methodology refers, among others, to (a) repetitive splitting of the overall sample; (b) comparative use of different summary statistics in an attempt to reduce population size and SNP selection bias, respectively. Thus, future work will concern attempts in replicating the proposed PRS in wider populations of different ancestry.

Other attempts to create PRSs for BMI in populations of European ancestry are extensively described in the current literature, with an overall number of 56 BMI-related entries in the PGS Catalog [6]. All referred entries include parts of populations of European ancestry but present a wide range in the numbers of PRS-included variants, from a few tens up to several thousand or millions, with these numbers possibly limiting their effective usage in research or clinical settings. Although the PRS proposed here includes only 343 SNPs, the yielded R^2 of 0.3241 is substantially comparable, and, in some cases, higher, than the ones presented in other PRSs from BMI, which include thousands of SNPs [6]. An overall advantage is also observed when comparing the present results to other attempts in European populations, which have a priori calculated the effect of literature-based PRSs using a limited amount of SNPs. Use of our proposed pipeline is an advanced tool due to the notion that the aggregated approach of splitting processes strengthens identification of appropriate and sometimes novel SNPs increases the validity of the results and makes up for the need to have a very large sample size.

In the current study, we observe links for various indices related to cardiovascular profile for twelve out of the sixteen variants with GWAS-Catalog-identified associations. The latter could be explained by inclusion of data for THISEAS participants with diagnosed cardiovascular disease (19.58% of the participants). Although the mediating effect of BMI is usually accounted for when investigating the effect of genetic or polygenic risk scores on indices of cardiovascular disease, the reciprocal relation between variation in cardiometabolic indices levels and BMI levels has not been extensively demonstrated through BMI-PRS-included, CVD-related variants. Out of the associated SNPs, the C allele of the rs2710804-included variant presents the majority of reported associations, namely with cell count types (platelets, leukocytes, lymphocytes) and even measurements of C-reactive protein. In this context, the negative effect of the T allele observed in our study ($\beta = -0.1356$) could denote a positive relation of the C allele with metabolic pathways of inflammation and disturbed immunological responses in the subsequent increasing effect of BMI values.

Interestingly and among this PRS's novel associations, we find two variants previously linked to gut microbiome measurements in populations of European ancestry. More specifically, Rühlemann MC et al. previously associated the rs480039 SNP with a 0.082571946 unit increase in P_Bacteroidetes abundance among German individuals [32].

Similarly, a 0.1019 unit increase in the abundance of parabacteroides in stools of individuals of Finnish ancestry for the A allele of the rs12673506 SNP was shown by Qin et al. [33]. Comparably, our study showed that the G allele of the rs480039 and rs12673506 variants was negatively related to BMI levels ($\beta = -0.1736$ and $\beta = -0.1850$, respectively). This is not the first time that the *Parabacteroides* genus has been linked to body weight. The majority of studies denote a higher *Firmicutes:Bacteroidetes* ratio and a generalized reduction in species variation in individuals with increased body weight or obesity [34], and different studies have found positive associations between genus and normal weight or weight loss in mice, as well as fat loss in humans [35–39]. It is plausible that the corresponding SNPs are further linked to BMI through the genus's role in gut production of bile acids and succinate, which have, in turn, been associated with reduction in body weight [38].

When referring to SNPs related to lifestyle, our suggested PRS included one variant related to well-being (variant rs17662327) and one variant associated with exercise (rs10252228). More specifically, in our sample, presence of the T allele of the former SNP was linked to a 0.1471 change in BMI levels. Previously, Okbay et al. demonstrated a 0.0182 unit increase in sentiment of life satisfaction or emotional well-being of adults for the T allele [39]. Our study further showed that presence of the A allele of the rs10252228 SNP was related to higher BMI values ($\beta = 0.1206$). This finding could be in accordance with the 0.027 unit increase in exercise associated with leisure time shown for the SNP's G allele in Japanese adults [40], meaning that the positive effect of the A allele on BMI could be mediated by individuals' low exercise levels.

One of the great strengths of the present study entails implementation of our novel methodology for extraction of PRS, which enables effective management and analysis of the vast amounts of genetic data required for such analyses. The automated pipeline enables practical application of our suggested holistic approach for extensive examination of thousands of SNPs, leading to identification of various novel associations. Through the methodological approach of applying a repetitive process of continuous adjustment of the R^2 measure for the number of each-time-associated SNPs, the pipeline aims to facilitate integration of PRS use in daily healthcare practice, for example as part of widely distributed consumer reports. It should be stressed that, as this methodology is based on the highest R^2 values of the aggregate PRS candidates, it ensures high explanatory power of the reduced signature. At the same time, it mitigates any computational and data management burden imposed by PRSs with large (up to millions) numbers of SNPs.

Limitations of the present study mainly concern power given the restrained participant sample size available for conducting analyses. Another limitation refers to use of a unified database of participants from three different studies. It is possible that variation in participant characteristics and bias accompanying use of a large analogic sample size of participants with cardiovascular disease played a considerable part in identifying associations between BMI and SNPs related to regulation of cardiovascular indices. However, we determined that much of the potential variability introduced by the fact of joining three databases was successfully captured by one of the PCs incorporated in the model. In addition, although the hypothesized pathways through which the identified SNPs potentially affect BMI levels provide insight for novel relations, there is little evidence to establish direct causal relationships. However, the present analysis sets a foundation for the suggested causal SNPs, and further research is also needed to explore the possibility of relations through their role as proxies for different associated variants.

5. Conclusions

The present paper describes creation of the first PRS for BMI in Greek adults by introducing use of a novel, automated pipeline for PRS extraction. The findings of this study lead to identification of several novel SNPs associated with BMI, potentially through their implication in various metabolic pathways related to traits of cardiometabolic profile and gut microbiome. Our data provide novel insights into interactions of various biological pathways implicated in formation of BMI levels and subsequently affecting its

individual variation across different populations. The suggested pipeline aims at promoting maximization of PRS integration in daily healthcare practice by enabling rapid and straightforward development of risk scores. In this regard, this first-ever PRS of a Greek population highlights the need for further development of PRSs for anthropometric traits in larger databases of Greek adults and sets a foundation for wider use of the described iterative PRS methodology.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm13020327/s1, Figure S1: Mean PRSice2 PRS R^2 +/− standard deviation for each performed summary statistics derivation method across 100 PRS extraction iterations; Figure S2: Stability of the PRS candidates over 100 PRS extraction iterations as described in the main text; Figure S3: Correlation of the 343-SNP PRS for BMI with the weight phenotype and PRS distribution; Table S1: Number of iterations and effect of all SNPs examined; Table S2: PRS cross-validation statistics; Table S3: List of all single nucleotide polymorphisms (SNPs) (n = 343) included in the PRS for BMI, sorted by number of times they appeared in the split datasets (largest to smallest).

Author Contributions: Conceptualization, P.M.; methodology, P.M.; validation, P.M.; formal analysis, P.M.; investigation, P.M.; resources, G.V.D. and P.D.; data curation, I.P.K., M.D., E.G., A.K., L.R., G.K., G.T. and E.M.; writing—original draft preparation, M.K. and P.M.; writing—review and editing, M.K. and P.M.; visualization, P.M.; supervision, P.M. and G.V.D.; project administration, G.V.D.; funding acquisition, G.V.D. and P.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All studies contributing data in the analyses of the present paper were approved by the Research Ethics Committee of Harokopio, University of Athens (NALFD protocol number: 38074/13-07-2012, OSTEOS protocol number: 15/8-12-2005, 8/12/2005, THISEAS protocol number: 10/9-6-2004, 14/6/2004).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Summary statistics and data used for the purposes of the present study are available upon request from the corresponding author. Participant data are not publicly available due to participants' privacy and ethical restrictions.

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Appendix F: Supplementary Material

Appendix F. Supplementary Material

F1. iMPROVE Study

Supplementary Table S1. List of all BMI-related Single Nucleotide Polymorphisms (SNPs) (n=84) identified by Locke et al. and included in the uGRS and wGRS for BMI.

Consortial Summary Statistics							
SNP	**Gene**	**Chr**	**Position**	**Alleles**	**Effect Allele**	**Direction of Effect**	**beta**
rs977747_T	TAL1	1	1:47219005	T/C/G	T	Positive	0.017
rs657452_?	AGBL4	1	1:49124175	A/G	A	Positive	0.023
rs11583200_C	ELAVL4	1	1:50094148	C/T	C	Positive	0.018
rs3101336_C	RPL31P12, NEGR1	1	1:72285502	T/C	C	Positive	0.033
rs1514174_C (proxy_for_rs12566985)	TNNI3K,FPGT-TNNI3K	1	1:74527379	C/T	C	Positive	0.024
rs12401738_A	GIPC2,DNAJB4	1	1:77981077	G/A	A	Positive	0.021
rs11165643_T	EEF1A1P11, RN7SL831P	1	1:96458541	C/G/T	T	Positive	0.022
rs17024393_C	GNAT2	1	1:109612066	T/C	C	Positive	0.066
rs543874_G	LINC01741, SEC16B	1	1:177920345	A/G	G	Positive	0.048
rs2820292_C	IPO9-AS1,NAV1	1	1:201815159	A/C/T	C	Positive	0.02
rs7570232(proxy_for_rs13021737)_C	TMEM18,LINC01875	2	2:632348	A/C/G/T/	C	Positive	0.06
rs10182181_G	DNAJC27,ADCY3	2	2:24927427	A/G	G	Positive	0.031
rs11126666_A	KCNK3	2	2:26705943	G/A/C	A	Positive	0.021
rs1016287_T	LINC01122	2	2:59078490	T/A/C/G	T	Positive	0.023
rs11688816_G	EHBP1	2	2:62825913	G/A	G	Positive	0.017
rs2121279_T	TMEM163	2	2:142285716	C/T	T	Positive	0.025
rs1460676_C	FIGN	2	2:163711179	T/C	C	Positive	0.02
rs1528435_T	SCHLAP1	2	2:180686235	C/G/T	T	Positive	0.018
rs17203016_G	LINC01802,CREB1	2	2:207390794	A/G	G	Positive	0.021
rs7599312_G	LINC01878,ERBB4	2	2:212548507	G/A	G	Positive	0.022
rs492400_C	USP37	2	2:218485029	C/T	C	Positive	0.016
rs2176040_A	NYAP2,MIR5702	2	2:226228086	A/G/T	A	Positive	0.014
rs6804842_G	RARB	3	3:25064946	A/C/G/T	G	Positive	0.019
rs2365389_C	FHIT	3	3:61250788	C/A/G/T	C	Positive	0.02
rs3849570_A	GBE1	3	3:81742961	C/A	A	Positive	0.019

rs13078960_G	CADM2	3	3:85758440	T/G	G	Positive	0.03
rs16851483_T	RASA2	3	3:141556594	G/T	T	Positive	0.048
rs1516725_C	ETV5,DGKG	3	3:186106215	T/A/C	C	Positive	0.045
rs17001654_G	SCARB2	4	4:76208415	C/G	G	Positive	0.031
rs13107325_T	SLC29A8	4	4:102267552	C/A/T	T	Positive	0.048
rs11727676_T	HHIP	4	4:144737912	T/C	T	Positive	0.036
rs2112347_T	SLC25A5P9,POC5	5	5:75719417	T/G	T	Positive	0.026
rs7715256_G	MFAP3	5	5:154158333	G/T	G	Positive	0.016
rs205262_G	ILRUN	6	6:34595387	A/G	G	Positive	0.022
rs2207139_G	RPS17P5,FTH1P5	6	6:50877777	A/G	G	Positive	0.045
rs9400239_C	FOXO3	6	6:108656460	T/C/G	C	Positive	0.019
rs6931818_A(proxy_for_rs9374842)	Intergenic	6	6:119864519	C/G/T	A	Positive	0.019
rs13201877_G	OLIG3,IFNGR1	6	6:137354404	A/G	G	Positive	0.023
rs13191362_A	PRKN	6	6:162612318	A/G	A	Positive	0.028
rs1167827_G	HIP1	7	7:75533848	G/A	G	Positive	0.02
rs2245368_C	DTX2P1-UPK3BP1-PMS2P11,DTX2P1	7	7:76978826	C/G/T	C	Positive	0.032
rs17405819_T	RNU2-54P,HNF4G	8	8:75894349	T/A/C	T	Positive	0.022
rs2033732_C	TPM3P3,RALYL	8	8:84167474	T/C	C	Positive	0.019
rs4740619_T	CCDC171	9	9:15634328	T/A/C	T	Positive	0.018
rs10968576_G	LINGO2	9	9:28414341	A/G	G	Positive	0.025
rs6477694_C	EPB41L4B,FRRS1L	9	9:109170062	C/T	C	Positive	0.017
rs1928295_T	RPL35AP22,ASTN2	9	9:117616205	T/C/G	T	Positive	0.019
rs10733682_A	LMX1B	9	9:126698635	A/G/T	A	Positive	0.017
rs76223526_T(proxy_for_rs7899106)	GRID1	10	10:85651147	A/G	T	Positive	0.04
rs17094222_C	HIF1AN	10	10:100635683	T/C	C	Positive	0.025
rs11191560_C	NT5C2	10	10:103109281	T/C	C	Positive	0.031
rs7903146_C	TCF7L2	10	10:112998590	C/G/T	C	Positive	0.023
rs4256980_G	TRIM66	11	11:8652392	C/A/G	G	Positive	0.021
rs11030104_A	BDNF-AS,BDNF	11	11:27662970	A/G	A	Positive	0.041
rs3817334_T	MTCH2	11	11:47629441	C/T	T	Positive	0.026
rs12286929_G	CADM1	11	11:115151684	A/C/G	G	Positive	0.022

rs11057405_G	CLIP1	12	12:122297350	G/A/C	G	Positive	0.031
rs12429545_A	LINC00558, ZNF646P1	13	13:53528071	G/A/C/T	A	Positive	0.033
rs1441264_A	CCT5P2,NIPA2P5	13	13:79006784	G/A/T	A	Positive	0.018
rs10132280_C	OR7K1P,LINC02306	14	14:25458973	C/A	C	Positive	0.023
rs12885454_C	RNU11-5P,LINC02326	14	14:29267632	C/A/T	C	Positive	0.021
rs11847697_T	PRKD1	14	14:30045906	C/T	T	Positive	0.049
rs7141420_T	NRXN3	14	14:79433111	C/G/T	T	Positive	0.024
rs16951275_T	MAP2K5	15	15:67784830	T/C/G	T	Positive	0.031
rs7164727_T	ADPGK-AS1,NPM1P42	15	15:72801650	C/G/T	T	Positive	0.018
rs758747_T	NLRC3	16	16:3577357	C/T	T	Positive	0.023
rs12446632_G	GPRC5B,GPR139	16	16:19924067	G/A	G	Positive	0.04
rs2726034_C(proxy_for_rs2650492)	NPIPB6,SBK1	16	16:28325561	T/C	C	Positive	0.021
rs3888190_A	ATP2A1,SH2B1	16	16:28878165	C/A/T	A	Positive	0.031
rs9925964_A	KAT8	16	16:31118574	A/C/G/T	A	Positive	0.019
rs2080454_C	RNU6-257P,MTND4LP25	16	16:49028679	C/A/G	C	Positive	0.017
rs99401289_A(proxy_for_rs1558902)	FTO	16	16:53769662	T/A	A	Positive	0.082
rs9914578_G	SMG6	17	17:2101842	C/G	G	Positive	0.02
rs1000940_G	NUP88,RABEP1	17	17:5379957	A/C/G/T	G	Positive	0.019
rs12940622_G	RPTOR	17	17:80641771	G/A	G	Positive	0.018
rs1808579_C	RMC1,NPC1	18	18:23524924	C/T	C	Positive	0.017
rs7243357_T	SEC11C,GRP	18	18:59216087	T/G	T	Positive	0.022
rs10871777_G(proxy(for_rs6567160)	MC4R,RNU4-17P	18	18:60184530	A/G/T	G	Positive	0.056
rs17724992_A	PGPEP1	19	19:18344015	A/G	A	Positive	0.019
rs29941_G	KCTD15,SUNO1	19	19:33818627	A/G/T	G	Positive	0.018
rs2075650_A	TOMM40	19	19:44892362	A/G	A	Positive	0.026
rs2287019_C	QPCTL	19	19:45698914	C/T	C	Positive	0.036
rs3810291_A	ZC3H4	19	19:47065746	G/A	A	Positive	0.028
rs6091540_C	LINC01524	20	20:52471323	C/T	C	Positive	0.019

F2. Polygenic Risk Score for Body Mass Index on the NAFLD, THISEAS and OSTEOS studies

Supplementary Table S2. List of all Single Nucleotide Polymorphisms (SNPs) (n=343) included in the PRS for BMI, sorted by the N° of times they appeared in the split datasets (largest to smallest).

SNP	chromosome	position	Nearest Gene	Risk Allele	Reference Allele	beta	OR	Frequency
rs11668205	19	2099250	IZUMO4	G	A	-0.325754	1.385075	96
rs11994887	8	4391719	CSMD1	T	C	-0.262923	1.300727	64
rs9295609	6	23979222	#N/A	G	A	-0.221097	1.247444	62
rs4279903	1	205439769	#N/A	G	A	0.198356	0.820078	61
rs16883347	5	9667798	LINC02112	A	C	0.232483	0.792563	60
rs1332010	6	38996875	DNAH8	T	C	0.197648	0.820659	60
rs2108929	7	13146222	#N/A	C	T	-0.281552	1.325184	60
rs12588521	14	30475398	#N/A	A	C	-0.290499	1.337095	60
rs1499951	3	115195109	#N/A	C	T	-0.28408	1.32854	58
rs9848915	3	167160291	SERPINI2	G	A	-0.272895	1.313763	58
rs1213402	5	111112309	NREP	T	C	-0.277976	1.320455	58
rs7407266	18	6568765	#N/A	T	C	0.162551	0.849973	58
rs1461920	1	226794525	STUM	A	G	-0.144307	1.155238	57
rs2691505	6	71310131	#N/A	A	G	0.150908	0.859927	57
rs12941504	17	72478313	CD300A	T	C	-0.213346	1.237812	57
rs4861680	4	186721885	SORBS2	G	A	0.156324	0.855282	56
rs113990664	5	71376237	#N/A	G	A	-0.163085	1.177137	56
rs7122189	11	24407108	#N/A	C	T	-0.219925	1.245984	56
rs7176478	15	66534231	MEGF11	G	A	0.21009	0.810511	56
rs962682	16	84846251	#N/A	A	G	-0.171701	1.187323	56
rs9303353	17	52728276	#N/A	T	G	0.140039	0.869324	56
rs9897526	17	42426940	GRN	G	A	0.191341	0.825851	56
rs1056896	4	185677363	ACSL1	T	C	0.185506	0.830684	55
rs12478622	2	204979611	#N/A	G	A	-0.234437	1.264197	54
rs4906842	15	26274261	LINC02346	A	G	-0.173873	1.189905	54

rs1604953	16	78942601	WWOX	G	A	-0.17161	1.187214	54
rs12959396	18	60039309	TNFRSF11A	T	G	0.147374	0.862971	54
rs12145632	1	247393142	#N/A	T	C	-0.182569	1.200297	53
rs6541160	1	220981358	MTARC1	T	C	-0.209923	1.233583	53
rs756325	1	110478064	#N/A	T	C	0.211103	0.809691	53
rs6827349	4	122980440	#N/A	A	G	0.14465	0.865325	53
rs983585	7	107656377	#N/A	T	C	-0.169588	1.184817	53
rs55906926	3	143159873	SLC9A9	A	G	0.283481	0.753158	52
rs1563556	5	174770776	#N/A	G	A	-0.150676	1.16262	52
rs10500800	11	13929427	#N/A	G	T	-0.230429	1.259141	52
rs56115087	17	71660771	#N/A	A	G	-0.222391	1.249059	52
rs13058401	22	29862247	#N/A	G	A	-0.236776	1.267157	52
rs6677968	1	65776702	DNAJC6	G	A	0.144549	0.865412	51
rs989632	5	7823382	ADCY2	A	G	-0.250872	1.285146	51
rs1128250	7	21956405	CDCA7L	G	A	0.164662	0.848181	51
rs7101190	10	71588349	COL13A1	C	T	0.170232	0.843469	51
rs8064152	16	5721431	#N/A	A	C	-0.224151	1.251259	51
rs1673000	19	35575767	HPN-AS1	T	C	-0.215948	1.241038	51
rs12139329	1	193463330	#N/A	T	C	-0.165837	1.180381	50
rs11904043	2	53644605	#N/A	G	A	-0.160222	1.173771	50
rs16838023	2	207053416	GPR1	G	T	0.16384	0.848878	50
rs1109771	6	32187605	#N/A	A	G	0.151916	0.859061	50
rs10901376	10	127085818	#N/A	G	A	0.15818	0.853696	50
rs7925657	11	44287292	ALX4	A	G	0.142327	0.867337	50
rs488248	13	106596719	#N/A	C	T	-0.170482	1.185876	50
rs12909478	15	33829250	RYR3	C	T	0.16306	0.84954	50
rs12052178	19	46114829	EML2	C	T	-0.136768	1.146562	50
rs17455693	1	165917820	#N/A	A	G	-0.168336	1.183334	49
rs6694023	1	62130167	#N/A	A	G	-0.161533	1.175312	49
rs12466395	2	190780698	#N/A	A	G	-0.143846	1.154706	49
rs8016755	14	25111113	#N/A	C	T	-0.132007	1.141116	49
rs78457560	17	13657119	#N/A	G	A	-0.25513	1.29063	49

rs2839644	21	44604272	#N/A	T	C	-0.17819	1.195052	49
rs480039	1	234426478	SLC35F3	G	A	-0.173613	1.189596	48
rs987174	3	5925865	#N/A	A	G	-0.265751	1.30441	48
rs9877544	3	97684754	RIOX2	G	A	-0.135372	1.144963	48
rs4346760	5	150493470	ANXA6	C	A	-0.127959	1.136506	48
rs1345934	7	136772275	LOC349160	A	G	-0.144756	1.155758	48
rs955975	9	85117420	#N/A	G	A	0.165892	0.847138	48
rs3912662	11	92595671	FAT3	G	A	0.158048	0.853808	48
rs4937166	11	126506712	KIRREL3	C	T	-0.170755	1.1862	48
rs9924586	16	59533731	#N/A	C	T	-0.151622	1.16372	48
rs6016500	20	39634488	#N/A	C	T	0.220392	0.802204	48
rs7264181	20	13231342	ISM1	G	A	0.197286	0.820956	48
rs6449263	4	16669406	LDB2	G	A	-0.123672	1.131645	47
rs6881547	5	158946495	#N/A	G	A	-0.174727	1.19092	47
rs6900225	6	9314807	#N/A	G	A	0.189038	0.827755	47
rs2856330	12	11942777	ETV6	C	T	-0.213801	1.238376	47
rs7989395	13	38010367	#N/A	A	C	-0.172584	1.188372	47
rs7279498	21	42253571	#N/A	G	A	-0.169416	1.184613	47
rs11121141	1	8294662	#N/A	G	A	-0.166324	1.180956	46
rs2807854	1	221029841	#N/A	T	C	-0.13816	1.148159	46
rs237108	6	79415015	#N/A	C	T	-0.161081	1.17478	46
rs2392445	7	36547667	#N/A	A	G	-0.211141	1.235086	46
rs7943949	11	40840931	LRRC4C	T	C	-0.200311	1.221783	46
rs11106815	12	93428691	LOC643339	G	A	-0.190596	1.209971	46
rs575478	12	114166291	#N/A	C	T	-0.257003	1.293049	46
rs2288061	16	76169731	#N/A	G	A	-0.177761	1.19454	46
rs9897341	17	9123894	NTN1	T	C	-0.174271	1.190378	46
rs4599028	19	53970581	#N/A	A	G	-0.18578	1.204157	46
rs6510364	19	33671172	#N/A	C	T	-0.120742	1.128334	46
rs1382425	4	189541446	#N/A	A	C	-0.209858	1.233502	45
rs284729	5	35457590	#N/A	A	G	0.197514	0.820768	45
rs4867856	5	176144471	#N/A	T	C	-0.148169	1.159709	45

rs540375	5	152878727	GRIA1	G	A	-0.138013	1.147991	45
rs233958	16	4340305	#N/A	C	T	-0.146388	1.157646	45
rs17008392	3	71291620	#N/A	A	G	0.240232	0.786446	44
rs13357315	5	56809263	#N/A	T	C	-0.193457	1.213437	44
rs901285	11	12160054	MICAL2	T	C	-0.238964	1.269933	44
rs7144237	14	80897656	DIO2-AS1	A	G	-0.137906	1.147868	44
rs17776995	16	49447822	#N/A	G	A	-0.209867	1.233514	44
rs5765681	22	46259060	#N/A	T	C	-0.167349	1.182166	44
rs11121937	1	12625844	#N/A	T	C	0.13541	0.873358	43
rs16846210	2	212322255	ERBB4	C	T	-0.165111	1.179524	43
rs1357134	3	186979768	MASP1	A	G	-0.136223	1.145937	43
rs2309942	4	184839829	STOX2	G	A	0.138368	0.870778	43
rs13182748	5	5915186	#N/A	C	A	0.156118	0.855458	43
rs697505	6	158816875	TULP4	T	G	-0.175773	1.192167	43
rs6981400	8	23104612	CHMP7	T	C	-0.16746	1.182298	43
rs13297661	9	132926080	#N/A	G	A	0.130994	0.877223	43
rs2852786	11	61514085	DAGLA	C	T	0.145421	0.864659	43
rs3808986	11	129910584	#N/A	C	A	-0.231172	1.260076	43
rs3741565	12	130557260	#N/A	A	G	-0.182105	1.19974	43
rs1959169	14	33473192	NPAS3	C	T	-0.212534	1.236808	43
rs1860304	16	5631166	#N/A	A	G	-0.170029	1.185339	43
rs4807505	19	3750869	APBA3	G	A	0.171137	0.842706	43
rs4925403	22	49070719	TAFA5	G	A	-0.203892	1.226165	43
rs2758615	1	156158367	#N/A	T	C	0.129133	0.878857	42
rs11689662	2	154003392	#N/A	G	A	-0.18104	1.198463	42
rs12614570	2	228651252	#N/A	G	T	0.133843	0.874727	42
rs4852508	2	79906739	CTNNA2	C	T	-0.197861	1.218793	42
rs6730873	2	216346048	#N/A	C	T	0.129299	0.878711	42
rs10935254	3	137254071	#N/A	A	G	-0.150471	1.162382	42
rs7430102	3	54537866	CACNA2D3	G	A	0.185439	0.830739	42
rs9882796	3	189330551	#N/A	G	A	0.147893	0.862523	42
rs6822346	4	190065197	#N/A	G	A	0.138813	0.870391	42

rs7694518	4	7459344	SORCS2	G	A	0.180304	0.835017	42
rs2281144	6	167091844	RPS6KA2	A	G	-0.190103	1.209374	42
rs1420123	7	29647662	#N/A	T	C	0.155306	0.856153	42
rs4979009	9	114347774	PTGR1	A	G	-0.142361	1.152993	42
rs7077407	10	8140574	#N/A	A	G	0.120968	0.886062	42
rs7104900	11	64303696	#N/A	G	A	0.153867	0.857386	42
rs11608342	12	67406881	#N/A	A	G	-0.176385	1.192898	42
rs59038615	12	82253603	#N/A	C	T	-0.17599	1.192427	42
rs78036224	12	39279534	CPNE8	G	T	0.248957	0.779613	42
rs586379	13	27029720	#N/A	T	C	0.179712	0.835511	42
rs11157000	14	38920795	#N/A	G	T	-0.135777	1.145426	42
rs2955742	15	76446132	TMEM266	G	A	-0.191082	1.210559	42
rs12931774	16	6965041	#N/A	G	A	-0.183571	1.2015	42
rs4806908	19	1004823	GRIN3B	G	A	0.134689	0.873987	42
rs7256086	19	327182	MIER2	C	T	-0.20064	1.222184	42
rs8111919	19	54128771	#N/A	G	A	-0.171	1.186491	42
rs11687151	2	87949635	#N/A	C	T	-0.18503	1.203254	41
rs12466549	2	192615315	#N/A	G	A	-0.135485	1.145091	41
rs4341972	2	69042142	ARHGAP25	G	A	0.138324	0.870816	41
rs483394	2	14137805	#N/A	C	T	0.156618	0.85503	41
rs62107643	2	3882074	#N/A	G	A	-0.244019	1.276368	41
rs9878545	3	171621021	#N/A	T	C	-0.120873	1.128481	41
rs1400871	5	124678629	#N/A	C	A	-0.144597	1.155573	41
rs3101186	5	71409569	MAP1B	A	C	0.198388	0.820052	41
rs440583	5	80322231	RASGRF2	T	C	-0.169856	1.185134	41
rs12192629	6	8426587	SLC35B3	G	T	-0.159928	1.173426	41
rs1556675	6	141737996	#N/A	T	C	0.162115	0.850343	41
rs2472802	6	14767419	#N/A	G	A	-0.169044	1.184172	41
rs6455019	6	65668333	EYS	T	C	-0.180085	1.197319	41
rs6906804	6	1490582	#N/A	G	A	0.150242	0.860499	41
rs6918155	6	167145388	RPS6KA2	G	A	0.16043	0.851778	41
rs12681882	8	103578377	#N/A	G	A	-0.178524	1.195452	41

rs36107033	8	37556987	ZNF703	C	T	-0.247376	1.28066	41
rs10959370	9	10923969	#N/A	G	T	0.249514	0.779179	41
rs10508741	10	29252309	#N/A	T	G	-0.146386	1.157642	41
rs11259736	10	15595361	ITGA8	G	A	-0.167693	1.182573	41
rs2813458	10	1574443	ADARB2-AS1	C	T	-0.175466	1.191802	41
rs11224017	11	134655690	#N/A	T	C	0.150652	0.860147	41
rs12581737	12	63125469	PPM1H	T	G	-0.196294	1.216885	41
rs879681	13	106800033	#N/A	G	A	0.197196	0.821029	41
rs8003359	14	57126170	#N/A	T	C	-0.181305	1.198781	41
rs8013199	14	23247486	SLC7A7	T	C	-0.181414	1.198912	41
rs1015310	15	102110442	#N/A	G	T	-0.132038	1.141152	41
rs11648289	16	7773250	#N/A	G	T	-0.129338	1.138075	41
rs1561699	18	47996600	#N/A	C	T	-0.162987	1.177021	41
rs9304414	18	49515554	#N/A	T	G	-0.152022	1.164185	41
rs2076408	20	2313059	TGM3	T	G	-0.161035	1.174727	41
rs817362	20	62559964	DNAJC5	G	A	-0.22142	1.247847	41
rs857138	1	57191924	FYB2	C	T	-0.156906	1.169885	40
rs16838452	2	202798759	#N/A	C	A	-0.181695	1.199249	40
rs1354350	3	113960449	#N/A	T	G	-0.130177	1.13903	40
rs1405406	3	73946124	#N/A	G	A	-0.180443	1.197747	40
rs2863384	3	166085645	#N/A	C	T	0.180731	0.83466	40
rs17237942	4	105425576	#N/A	A	G	-0.146612	1.157905	40
rs2503815	6	5391268	FARS2	G	A	-0.185625	1.203971	40
rs17171046	7	37477863	ELMO1	C	T	-0.177167	1.193831	40
rs6989157	8	96320757	C8orf37-AS1	G	A	-0.189935	1.209171	40
rs9325887	10	48461083	#N/A	A	G	0.168659	0.844797	40
rs342174	12	63065145	PPM1H	G	A	-0.182831	1.200611	40
rs10148212	14	67095613	GPHN	A	C	-0.192537	1.212322	40
rs1822809	15	95798343	#N/A	G	A	-0.16178	1.175601	40
rs11866097	16	55104462	#N/A	G	A	-0.197333	1.21815	40
rs2589141	17	78843139	RPTOR	C	T	-0.148067	1.15959	40
rs4790904	17	64779154	PRKCA	T	C	-0.138626	1.148695	40

rs55667536	17	39293446	#N/A	A	G	-0.331439	1.392971	40
rs1106978	20	41678428	PTPRT	G	A	-0.14801	1.159525	40
rs6007778	22	48392923	#N/A	G	A	-0.200854	1.222446	40
rs11811436	1	97940231	DPYD	T	C	0.174909	0.839533	39
rs147762374	1	106873477	#N/A	G	A	-0.160322	1.173889	39
rs2376100	2	197833530	#N/A	T	C	0.270528	0.762976	39
rs6712770	2	164106998	#N/A	A	C	-0.161215	1.174937	39
rs1163377	3	108937719	#N/A	G	A	-0.1455	1.156618	39
rs11922267	3	65401156	MAGI1	C	T	-0.176977	1.193604	39
rs2649188	3	65780102	MAGI1	A	C	0.122599	0.884619	39
rs7620686	3	26413729	#N/A	T	C	-0.116506	1.123565	39
rs10076573	5	2094148	#N/A	T	C	0.165889	0.847141	39
rs10477436	5	108421424	FER	A	C	-0.129262	1.137988	39
rs4388209	5	125758373	GRAMD2B	T	C	0.113824	0.892415	39
rs6925019	6	1588714	#N/A	G	A	-0.179622	1.196765	39
rs2710804	7	36084529	#N/A	T	C	-0.135608	1.145233	39
rs4724298	7	44320434	CAMK2B	C	T	-0.179291	1.196369	39
rs2173537	8	129047325	PVT1	G	A	-0.180237	1.197501	39
rs1556146	9	132022478	#N/A	T	C	-0.198734	1.219857	39
rs16907144	9	93852382	#N/A	G	A	0.158523	0.853403	39
rs824003	9	113972955	#N/A	T	C	-0.162957	1.176986	39
rs12772906	10	62804925	#N/A	A	G	-0.184202	1.202258	39
rs12417297	11	128185154	#N/A	C	T	-0.232895	1.262248	39
rs10492094	12	5478148	#N/A	G	T	-0.125703	1.133945	39
rs1544686	12	107579464	#N/A	G	T	-0.184251	1.202317	39
rs17091684	12	43076137	#N/A	C	A	-0.169665	1.184908	39
rs1170994	13	36581177	DCLK1	C	T	0.161757	0.850648	39
rs9533784	13	44761837	#N/A	G	A	-0.182495	1.200208	39
rs10146690	14	79890456	NRXN3	G	A	-0.145193	1.156263	39
rs11621403	14	53877638	#N/A	A	G	0.203013	0.816268	39
rs1791159	18	29136463	DSG2-AS1	T	C	-0.134932	1.144459	39
rs275339	20	57988701	#N/A	C	T	0.17152	0.842384	39

rs4816499	21	36293381	RUNX1	A	C	-0.256245	1.292069	39
rs10458443	1	242712951	#N/A	T	C	0.157657	0.854143	38
rs285461	1	165497692	LRRC52-AS1	T	C	-0.221145	1.247505	38
rs4559489	1	240698138	GREM2	C	T	-0.232214	1.26139	38
rs12473883	2	236559912	AGAP1	G	T	-0.163999	1.178213	38
rs13405786	2	70844394	#N/A	T	C	-0.190585	1.209958	38
rs4253427	4	187209225	F11-AS1	G	A	0.127626	0.880183	38
rs11759402	6	42723809	#N/A	C	T	-0.154175	1.166695	38
rs1248465	7	129179844	#N/A	C	T	-0.118936	1.126298	38
rs2251188	7	6704332	#N/A	A	G	0.138076	0.871032	38
rs6973044	7	107416602	SLC26A3	T	C	-0.228111	1.256225	38
rs11780206	8	59385164	#N/A	T	C	-0.204419	1.226812	38
rs11783343	8	62673021	#N/A	T	C	-0.178607	1.195551	38
rs12246329	10	17059462	CUBN	C	T	-0.148457	1.160043	38
rs4751284	10	132653619	#N/A	G	A	0.116341	0.890172	38
rs1032151	11	12219217	MICAL2	G	T	-0.1486	1.160209	38
rs11602776	11	15448704	#N/A	A	G	0.170025	0.843643	38
rs11106473	12	92627100	#N/A	T	C	-0.185816	1.2042	38
rs9562038	13	96802616	HS6ST3	A	G	-0.166981	1.181732	38
rs34361729	15	56387142	RFX7	G	A	-0.211004	1.234918	38
rs4794785	17	36810197	#N/A	A	G	0.226712	0.797151	38
rs8464	17	64806716	PRKCA	C	A	-0.175982	1.192417	38
rs2422840	20	3076384	#N/A	A	G	-0.170721	1.18616	38
rs6065084	20	59862269	CDH4	G	A	0.157302	0.854446	38
rs1786412	21	21849932	#N/A	T	C	-0.144162	1.155071	38
rs7275495	21	33533452	#N/A	T	C	-0.159611	1.173054	38
rs1287949	1	226703467	#N/A	C	T	-0.135919	1.145589	37
rs2297563	1	109241669	PRPF38B	G	A	-0.138652	1.148725	37
rs3849346	2	163502721	KCNH7	C	T	-0.191176	1.210672	37
rs7589592	2	2712943	#N/A	T	C	0.113915	0.892334	37
rs10939667	4	16531086	LDB2	G	A	0.143542	0.866285	37
rs72696876	4	171635905	#N/A	A	G	0.17133	0.842543	37

rs11752561	6	128187461	THEMIS	A	G	0.178228	0.836752	37
rs2382394	9	13227063	MPDZ	T	C	-0.295083	1.343238	37
rs10508561	10	18734235	CACNB2	G	A	0.155748	0.855775	37
rs4143630	10	5063944	#N/A	C	T	-0.125875	1.134141	37
rs4261213	10	58261332	#N/A	G	A	0.121382	0.885696	37
rs10765861	11	11525424	GALNT18	A	G	-0.123483	1.131431	37
rs2581925	11	57207414	#N/A	G	A	0.135819	0.873	37
rs1446427	12	118153788	KSR2	G	A	0.145306	0.864758	37
rs2274442	14	69787749	GALNT16	T	C	-0.222228	1.248856	37
rs7144194	14	103495963	CDC42BPB	A	G	-0.131499	1.140536	37
rs1476078	15	91736401	SV2B	G	A	-0.207155	1.230173	37
rs2880974	15	86415200	#N/A	G	T	-0.143929	1.154802	37
rs12928792	16	60321890	#N/A	G	A	-0.11811	1.125368	37
rs9807305	18	59634276	#N/A	C	T	0.176512	0.838188	37
rs12118086	1	101732914	#N/A	G	A	0.181836	0.833738	36
rs1570818	1	29804613	#N/A	A	C	0.161503	0.850864	36
rs17366341	1	163834280	#N/A	A	G	-0.162322	1.176239	36
rs2485662	1	156083468	#N/A	T	C	0.116015	0.890461	36
rs784612	1	40122939	#N/A	T	C	-0.173568	1.189541	36
rs4850836	2	199122020	#N/A	C	A	-0.146091	1.157302	36
rs17009383	3	21771769	ZNF385D	A	G	0.168139	0.845236	36
rs7623055	3	7510891	GRM7	G	T	0.123122	0.884156	36
rs9847987	3	59418533	#N/A	C	T	0.26275	0.768934	36
rs1491386	4	23663069	#N/A	A	C	0.208738	0.811608	36
rs7732443	5	166290093	#N/A	C	T	0.1964	0.821683	36
rs2459110	6	152450763	SYNE1	C	A	-0.123803	1.131793	36
rs9363725	6	67931025	#N/A	G	A	0.277937	0.757344	36
rs9377661	6	105091850	#N/A	C	T	0.189219	0.827605	36
rs12673506	7	29421786	CHN2	G	A	-0.185004	1.203223	36
rs4718965	7	70040448	AUTS2	C	T	-0.190496	1.20985	36
rs4729041	7	92063500	#N/A	G	T	-0.18693	1.205543	36
rs10101912	8	88218888	CNBD1	C	T	-0.217885	1.243444	36

rs3913641	8	140342525	#N/A	C	T	-0.127474	1.135955	36
rs12337816	9	117125236	AKNA	T	C	-0.222317	1.248967	36
rs17662327	9	4967587	#N/A	T	C	0.14715	0.863165	36
rs7861175	9	113857337	#N/A	T	C	0.140404	0.869007	36
rs17106110	10	125601307	CPXM2	C	T	-0.139741	1.149975	36
rs10899014	11	74325109	POLD3	A	G	-0.299626	1.349355	36
rs2302688	12	15673103	PTPRO	C	T	0.13391	0.874669	36
rs7295237	12	71016832	PTPRB	T	C	-0.252748	1.287558	36
rs10851193	13	107335929	#N/A	T	C	0.105358	0.900002	36
rs1617356	14	43194459	#N/A	C	T	-0.131898	1.140992	36
rs17126387	14	90301314	EFCAB11	A	C	0.223227	0.799933	36
rs7149564	14	23912361	#N/A	T	C	-0.261682	1.299113	36
rs8009629	14	33379559	#N/A	G	A	-0.130628	1.139543	36
rs13335201	16	50912221	#N/A	G	A	-0.171548	1.187141	36
rs1350888	16	82784943	CDH13	C	T	0.11881	0.887977	36
rs12948545	17	30867317	MYO1D	C	T	-0.139738	1.149973	36
rs218685	17	6623172	#N/A	A	G	0.142751	0.86697	36
rs1790692	18	28644751	DSC2	G	A	-0.16607	1.180656	36
rs7241127	18	6651210	#N/A	T	C	-0.140085	1.150371	36
rs8088662	18	60928341	BCL2	T	C	0.212307	0.808717	36
rs1010304	20	40101647	CHD6	A	G	-0.28657	1.331852	36
rs1016071	20	40931328	PTPRT	T	C	-0.123569	1.131528	36
rs6019784	20	48002575	KCNB1	C	T	0.137512	0.871524	36
rs6084802	20	511356	CSNK2A1	C	A	-0.13511	1.144663	36
rs1808973	1	112487834	KCND3	C	T	-0.119334	1.126746	35
rs3820383	1	202197551	LGR6	C	A	0.171723	0.842213	35
rs4658879	1	231742575	LINC00582	T	C	-0.174403	1.190535	35
rs6436132	2	220160644	PTPRN	G	T	0.137995	0.871103	35
rs12485826	3	71342132	#N/A	A	G	-0.16939	1.184582	35
rs75838234	3	7534370	GRM7	A	G	-0.184033	1.202056	35
rs11726275	4	185348916	IRF2	A	G	-0.132627	1.141824	35
rs13128106	4	25325264	ZCCHC4	C	T	-0.237812	1.26847	35

rs6810740	4	17294785	#N/A	T	C	-0.159123	1.172483	35
rs6821900	4	158574310	#N/A	A	C	-0.188469	1.2074	35
rs2567819	5	121064897	#N/A	C	A	0.195192	0.822676	35
rs3853241	5	166379772	#N/A	C	T	-0.170603	1.186019	35
rs10252228	7	34940039	#N/A	A	G	0.120631	0.886361	35
rs17158276	7	29648077	#N/A	T	C	-0.178448	1.195361	35
rs73371641	8	142864765	#N/A	A	G	-0.251769	1.286298	35
rs2384792	9	138324679	#N/A	C	T	-0.129733	1.138525	35
rs4295727	9	112578906	PALM2AKAP2	G	A	-0.141765	1.152306	35
rs9696313	9	136406725	ADAMTSL2	A	G	-0.123854	1.13185	35
rs10824306	10	77160682	ZNF503	C	T	-0.144774	1.155778	35
rs11013723	10	18632601	CACNB2	A	G	0.205084	0.814579	35
rs12221258	10	107582259	#N/A	T	C	-0.149856	1.161666	35
rs2265958	10	10141681	#N/A	C	T	-0.182243	1.199906	35
rs7894073	10	28648001	#N/A	C	T	-0.256118	1.291906	35
rs12290206	11	107761113	#N/A	A	G	-0.153478	1.165882	35
rs4938044	11	113584177	#N/A	G	A	-0.204375	1.226758	35
rs12584740	13	98315257	#N/A	A	G	-0.140091	1.150379	35
rs714668	13	105019361	#N/A	C	A	0.237791	0.788368	35
rs73168114	13	22716081	#N/A	A	C	-0.221493	1.247939	35
rs7323430	13	65285662	#N/A	G	A	0.150152	0.860577	35
rs10131354	14	25499521	STXBP6	T	G	-0.183645	1.201589	35
rs2842344	14	68976971	RAD51B	C	T	-0.199264	1.220504	35
rs71415931	14	30683170	#N/A	T	C	-0.203381	1.22554	35
rs7499658	16	14430442	#N/A	C	T	0.15436	0.856963	35
rs2332264	17	52830577	#N/A	T	C	0.132596	0.875819	35
rs2850929	18	75068081	#N/A	G	A	0.157779	0.854039	35
rs755719	18	56877489	#N/A	T	C	0.111622	0.894382	35
rs762418	21	45610893	#N/A	C	T	0.142348	0.867319	35
rs5761487	22	26738225	SEZ6L	A	G	0.141173	0.868339	35

***Freq:** Number of times the SNPs appeared on the split datasets.