HAROKOPIO UNIVERSITY

SCHOOL OF HEALTH SCIENCE AND EDUCATION

DEPARTMENT OF NUTRITION AND DIETETICS

Development of epidemiological model for the role of sex in primary and secondary prevention of cardiovascular disease, taking into account psychosocial, clinical and environmental determinants, & health policies.

Matina Kouvari

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Η έγκριση της διδακτορικής διατριβής από το τμήμα Διαιτολογίας – Διατροφής του Χαροκοπείου Πανεπιστημίου δεν υποδηλώνει και αποδοχή των απόψεων του συγγραφέα.

Η Ματίνα Κούβαρη δηλώνω υπεύθυνα ότι:

1) Είμαι ο κάτοχος των πνευματικών δικαιωμάτων της πρωτότυπης αυτής εργασίας και από όσο γνωρίζω η εργασία μου δε συκοφαντεί πρόσωπα, ούτε προσβάλει τα πνευματικά δικαιώματα τρίτων.

2) Αποδέχομαι ότι η ΒΚΠ μπορεί, χωρίς να αλλάξει το περιεχόμενο της εργασίας μου, να τη διαθέσει σε ηλεκτρονική μορφή μέσα από τη ψηφιακή Βιβλιοθήκη της, να την αντιγράψει σε οποιοδήποτε μέσο ή/και σε οποιοδήποτε μορφότυπο καθώς και να κρατά περισσότερα από ένα 087 αντίγραφα για λόγους συντήρησης και ασφάλειας.

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Curriculum Vitae

Matina KOUVARI (she/her/hers) is a Dietician – Nutritionist. She holds a BSc in Nutrition & Dietetics from Harokopio University in Athens (HUA), where she was graduated 1st in the class and an MSc (hons) in Applied Nutrition and Dietetics - Clinical Nutrition from the same University (HUA). During her postgraduate studies she was specialized in cardiovascular disease epidemiology participating in large national epidemiological studies. Since 2019, she works as Research Associate of HUA in the "Fact-based personalised nutrition for the young – NutriShield" project (H2020, 2018-2022). Additionally, since 2015, she works as Research Associate and Project Manager in the Institute of Preventive Medicine, Environmental and Community Health (PROLEPSIS) with an active role in research and educational activities related to public health issues. In particular, she has actively involved in six (6) international projects (one (1) H2020, four (4) Erasmus+, one (1) DG SANTE) and various national projects including the DIATROFI food aid and healthy nutrition promotion program and the dissemination of the National Dietary Guidelines. She has also participated in the development of many educational materials and the implementation of webinars related with nutritional counselling for children/adolescents, parents, health professionals etc. Ms Kouvari has published 39 original research papers in international journals, as well as has 96 scientific presentations and two invited lectures in international and national congresses, in the field of public health, nutrition and cardiovascular disease epidemiology and prevention. She is also Associatee Editor in one peer-reviewed international journal (Nutrition and Health) and Reviewer in many national and international journals in the field of nutrition and dietetics. Additionally, since 2019 she has been elected as a Board Member of the Working Group of Epidemiology and Prevention of Atherosclerosis of the Hellenic Society of Atherosclerosis. For her academic performance, she has received 3 scholarships and 8 awards.

Abbreviations

ALT	Alanine transaminase
AMI	Acute myocardial infarction
ApoA1	Apolipoprotein a1
ApoB100	Apolipoprotein B100
AST	Aspartate transaminase
ATP	Adult Treatment Panel
BMI	Body mass index
CDC	Centre of Disease Control and Prevention
CES-D	Center for Epidemiologic Studies Depression Scale
CETP	Cholesterol ester transfer protein
CRP	C-Reactive Protein
CrCl	Creatinine clearance
CVD	Cardiovascular disease
DALYs	Disability-adjusted life years
D-AII	Dietary Anti-Inflammation Index
ERFC	Emerging Risk Factor Collaboration
ESC	European Society of Cardiology
EU	European Union
FA	Factor analysis
FDA	Food Drug and Administration
FLI	Fatty liver index
FMI	Fat mass index
GWAS	Genome-wide association study
HR	Hazard Ratio
HDL-C	High density lipoprotein cholesterol
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HSI	Hepatic steatosis index
IDF	International Diabetes Federation
IL-6	Interleukin 6
IL-10	Interleukin 10
IOM	Institute of Medicine
IQR	Interquartile range
IHD	Ischemic heart disease

LDL-C	Low density lipoprotein cholesterol
LMI	Lean mass index
Lp(a)	Lipoprotein a
MetS	Metabolic Syndrome
MHN	Metabolically healthy non-obese
MHO	Metabolically healthy obese
MUN	Metabolically unhealthy non-obese
MUO	Metabolically unhealthy obese
NAFLD	Non-alcoholic fatty liver disease
FLS	Fatty liver steatosis
NCEP	National Cholesterol Education Program
NCD	Non-communicable disease
NCD-RisC	NCD Risk Factor Collaboration
OR	Odds Ratio
NIH	National Institute of Health
PAR	Population attributable risk
PCOS	Polycystic ovary syndrome
PYLLs	Potential years of life lost
ROC	Receiver operating curve
STAI	State-Trait Anxiety Inventory
SUA	Serum uric acid
TAG	Triglycerides
TC	Total cholesterol
TNF-a	Tumor necrosis factor a
TyG	Triglycerides-glucose index
UA	Unstable angina
UN	United Nations
WHO	World Health Organization
ZUNG	Zung depression rating scale
95%CI	95% Confidence Interval

Contents

Abstract

Aim: The aim of this thesis was to evaluate the existence of sex-specific associations of environmental, clinical and biochemical factors with long-term cardiovascular disease (CVD) onset (*primary prevention*) and recurrence (*secondary prevention*) using the samples of two cohorts from Greece.

Materials and Methods: Two prospective epidemiological studies, ATTICA [2002-2012, *n*=3,042 free-of-CVD individuals from the greater Athens area (single centre study), *n*=1,514 men (46±13 years) and *n*=1,528 women (45±14 years)] and GREECS [2004-2014, *n*=2,172 consecutive patients with acute coronary syndrome from 6 major Greek hospitals (multicentre study), *n*=1,649 men (65±13 years) and *n*=523 women (62±11 years)] were used. Baseline examination included an extended set of sociodemographic, lifestyle, psychological, anthropometric, clinical and biochemical factors. Follow-up was performed in *n*=2,020 participants of ATTICA and *n*=2,172 participants of GREECS.

Results: Ten-year first CVD event rate in ATTICA was 15.7% (*n*=317) [19.7% (*n*=198) in men and 11.7% (*n*=119) in women, $p<0.001$]; whereas 10-year secondary CVD event rate in GREECS was 37.3% (*n*=811) (38.8% in men and 32.9% in women, $p=0.016$). The man-to-woman age-adjusted ratio was consistently higher to 1; this was higher when a first CVD was studied compared with a recurrent event (1.66 vs. 1.18, $p<0.001$). Various sex-specific associations between CVD risk factors and long-term CVD onset or recurrence were revealed. Specifically, the magnitude of association of lifestyle factors on first and recurrent CVD events was sex-oriented. Focusing on the dietary part, various sex-specific associations were revealed regarding dairy products, meat, dietary vitamin D, and the anti-inflammatory potential of diet. Additionally, two individuals with similarly moderate to high level of adherence to Mediterranean diet were not equally protected against CVD; with high consumption of plant-based products being more important than low consumption of unhealthier options. This plant-based orientation was more frequent in women. Higher magnitude of association between depressive symptomatology and CVD onset or recurrence was revealed for women. Moreover, the association of predicted lean and fat mass with CVD incidence varied according to sex, as well as according to prevention stage. Metabolically healthy obese status was an independent CVD risk factor only in women while the stability of this condition ten years later was challenged principally in women. Non-alcoholic fatty liver disease was suggested as an intermediate path. Various biomarkers (e.g., lipoproteins, apolipoproteins, uric acid) were also examined suggesting sex-specific associations. Finally, a nutrition-related sex-oriented microsimulation scenario for primary and secondary CVD prevention was developed. This process revealed that achieving

even a small percentage of the population to comply with Mediterranean diet was of added value of population's health status, for both men and women, with women being more benefited.

Conclusion: To maximize cost-effectiveness of prevention strategies, health disparities need being addressed. In this context, "female" and "male" CVD pattern needs to be presented in a coherent manner. Building upon this need, the present work highlighted sex-based specifications in relation to lifestyle and psychological patterns, revealed differences in the magnitude of associations of cardiometabolic risk factors while suggested cardiometabolic paths specified to men and women for earlier or more valid risk assessment and prevention.

Key words: sex; heart disease; primary prevention; secondary prevention; public health

Περίληψη

Σκοπός: Σκοπός της διατριβής ήταν η διάκριση μεταξύ των φύλων περιβαλλοντικών, κλινικών και βιοχημικών παραγόντων ως προς την εμφάνιση καρδιαγγειακής νόσου (ΚΝ) σε επίπεδο πρωτογενούς και δευτερογενούς πρόληψης.

Υλικό και Μέθοδοι: Χρησιμοποιήθηκαν δύο προοπτικές επιδημιολογικές μελέτες, η μελέτη ΑΤΤΙΚΗ [2002-2012, *ν*=3.042 συμμετέχοντες ελεύθεροι ΚΝ από την ευρήτερη περιοχή της Αττικής (μονοκεντρική μελέτη), *ν*=1.514 άντρες (46±13 ετών) and *ν*=1.528 γυναίκες (45±14 ετών)] και η μελέτη GREECS [2004-2014, *ν*=2.172 διαδοχικοί ασθενείς με διάγνωση οξέος στεφανιαίου συνδρόμου από έξι καρδιολογικές κλινικές της Ελλάδας (πολυκεντρική μελέτη), *ν*=1.649 άντρες (65±13 ετών) and *ν*=523 γυναίκες (62±11 ετών)]. Κατά την έναρξη των μελετών, η αξιολόγηση περιελαμβάνε κοινονικοδημογραφικά, κλινικά, ψυχοκοινωνικά χαρακτηριστικά καθώς και χαρακτηριστικά τους τρόπου ζωής αλλά και βιοδείκτες. Ο επανέλεγχος πραγματοποιήθηκε σε *ν*=2.020 συμμετέχοντες από την μελέτη ΑΤΤΙΚΗ και *ν*=2.172 συμμετέχοντες από την μελέτη GREECS.

Αποτελέσματα: Η 10ετής εμφάνιση 1^{ου} επεισοδίου ΚΝ στην ΑΤΤΙΚΗ ήταν 15,7% (*ν*=317) [19,7% (*ν*=198) στους άντρες και 11,7% (*ν*=119) στις γυναίκες, *p<0,001*]. Η 10ετής εμφάνιση 2^{ου} ή πολλαπλού επεισοδίου ΚΝ στην GREECS ήταν 37,3% (*ν*=811) (38,8% στους άντρες και 32,9% στις γυναίκες, *p=0,016*). Ο λόγος επίπτωσης ΚΝ τόσο στους υγιείς συμμετέχοντες της ΑΤΤΙΚΗ όσο και στους ασθενείς της GREECS ήταν >1 (1,66 έναντι 1,18, *p*<0,001). Αναδείχθηκαν συσχετίσεις μεταξύ παραγόντων κινδύνου ΚΝ και μακροπρόθεσης πρωτογενούς και δευτερογενούς εμφάνισης της νόσου διαφοροποιημένες μεταξύ αντρών και γυναικών. Αρχικά, η ένταση της σχεσής μεταξύ παραγόντων του τρόπου ζωής και εμφάνισης της νόσου φάνηκε να καθορίζεται από το φύλο. Εστιάζοντας στις διατροφικές συνήθειες, τρόφιμα όπως γαλακτοκομικά και κρέας, συστατικά όπως βιταμίνη D αλλά και το αντιφλεγμονώδες φορτίο της διατροφής συνολικά επηρέαζαν διαφορετικά την εμφάνιση ΚΝ σε άντρες και γυναίκες. Επιπλέον, δύο άτομα με μέτριο έως υψηλό βαθμό συμμόρφωσης στην Μεσογειακή διατροφή φάνηκε να μην προστατεύονται το ίδιο έναντι της νόσου. Συγκεκριμένα, η υψηλή κατανάλωση τροφίμων φυτικής προέλευσης φάνηκε να είναι πιο ευεργετική σε σχέση με τον περιορισμό κατανάλωσης τροφίμων ζωικής προέλευσης. Αυτή η «χορτοφαγική» προσέγγιση φάνηκε να παρατηρείται πιο συχνά στις γυναίκες. Η καταθλιπτική συμπτωματολογία φάνηκε να επηρεάζει περισσότερο τις γυναίκες τόσο σε επίπεδο πρωτογενούς όσο και δευτερογενούς πρόληψης. Επίσης, η σχέση λιπώδους και άλιπης μάζας σώματος με την εμφάνιση ΚΝ φάνηκε να διαφοροποιείται ανάλογα με το φύλο και το επίπεδο πρόληψης. Η μεταβολικά υγιής παχυσαρκία, αναδείχθηκε ως ανεξάρτητος επιβαρυντικός παράγονταςστις γυναίκες οι οποίες και παρουσίασαν

μεγαλύτερη πιθανότητα μεταβολής αυτού του προτύπου σε μεταβολικά μη υγιές σε βάθος δεκαετίας. Η μη αλκοολική λιπώδης διήθηση ήπατος προτάθηκε ως ενδιάμεσος μηχανισμός. Κάποιοι βιοδείκτες (π.χ. λιποπρωτεΐνες, απολιποπρωτεΐνες, ουρικό οξύ) φάνηκε να προβλέπουν διαφορετικά τον καρδιαγγειακό κίνδυνο σε άντρες και γυναίκες. Τέλος, αναπτύχθηκε ένα μοντέλο προσομοίωσης για την πρωτογενή και δευτερογενή πρόληψη ΚΝ αναδεικνύοντας την προστατευτική δράση της Μεσογειακής διατροφής σε επίπεδο πληθυσμού ακόμη κι όταν ένα μικρό μέρος αυτού συμμορφωνόταν στο συγκεκριμένο πρότυπο, Τα οφέλη ήταν πιο έντονα στις γυναίκες.

Συμπεράσματα: Για την ενίσχυση της αποτελεσματικότητας των στατηγικών πρόληψης της ΚΝ σε πρωτογενές και δευτερογενές επίπεδο απαιτείται η αντιμετώπιση των διακρίσεων στον τομέα της υγείας και το φύλο είναι μία από αυτές. Η παρούσα εργασία προτείνει διαφοροποιήσεις μεταξύ των φύλων σε επίπεδο ψυχοκοινωνικών παραγόντων και χαρακτηριστικών του τρόπους ζωής, αναδεικνύει διαφορές στην προβλεπτική και προγνωστική ικανότητα διαφόρων παραγόντων κινδύνου ενώ παρουσιάζει σχετικούς υποκείμενους μηχανισμούς.

Λέξεις κλειδιά: φύλο; καρδιαγγειακή νόσος; πρωτογενής πρόληψη; δευτερογενής πρόληψη; δημόσια υγεία

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1 Introduction

1.1 Epidemiology of cardiovascular disease: global data

The increasing life expectancy of the global population without the respective increase in the healthy life expectancy remains a major concern in public health. Fourty million deaths occurred due to NCDs, accounting for 70% of the overall total of 56 million deaths (WHO 2014). The vast majority of such deaths were caused by the four main NCDs, namely: CVD, 17.7 million deaths (accounting for 48% of all NCD deaths); cancer, 8.8 million deaths (21%); respiratory diseases, 3.9 million deaths (12%); and diabetes, 1.6 million deaths (3%) (WHO 2014).

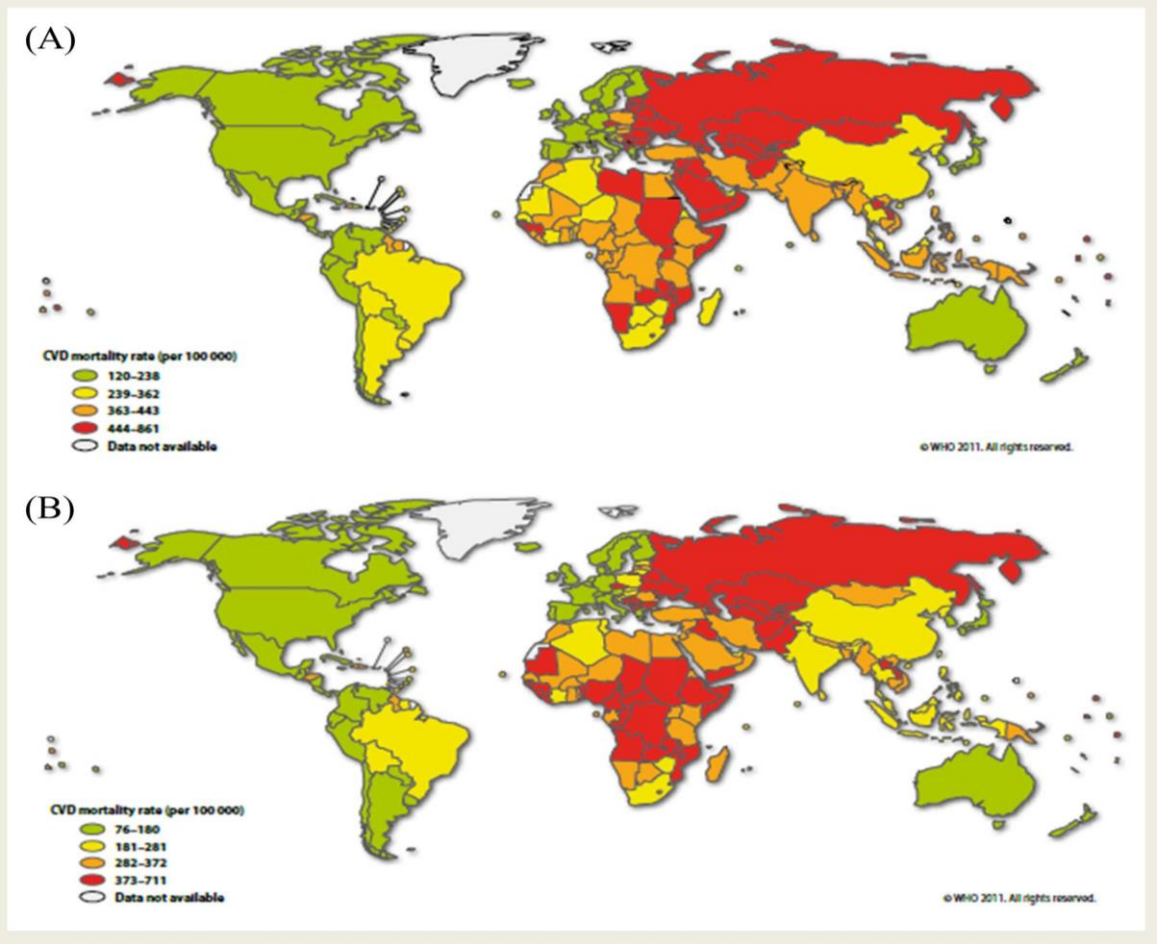

Figure 1.1 (A) World map showing the global distribution of CVD mortality rates in men (age standardized, per 100,000); (B) World map showing the global distribution of CVD mortality rates in men (age standardized, per 100,000)

According to more recent estimates of the WHO, 17.9 millions of people died of CVD in 2018 accounting for 46% of all NCD deaths (WHO 2018) (**Figure 1.1**). This rate is to increase by five units till 2030 (WHO 2018). Of these deaths, an estimated 7.4 million were due to CHD, 6.7 million due to stroke and the rest 3.4 million due to heart failure and arrhythmias as a consequence of hypertension, rheumatic heart disease, other cardiac valve disease and cardiomyopathies (WHO 2018). In most countries, the absolute number of deaths from CVD is

increasing due to increased longevity and associated population ageing and deaths of people older than 70 years of age (WHO 2018).

1.2 Epidemiology of cardiovascular disease: data from Europe

According to the most updated report by the European Heart Network on CVD epidemiology for Europe and EU, CVD mortality is now falling in most European countries, including Central and Eastern Europe which had seen considerable increases until the beginning of the 21st century (Wilkins et al 2017) (**Figure 1.2**). Much as this seems a promisingly positive scenario for the efficiency of CVD prevention strategies, it is, more or less contradicted, by the following metrics (Wilkins et al 2017): **a.** Age-specific death rates have actually fallen by 15.6% between 2005 and 2015 although recent data suggest that this rate of decline has been slowing; **b.** Over the past 25 years, the absolute number of CVD cases has increased in Europe and in the EU, with increases in the number of new CVD cases found in most countries; **c.** CVD is responsible for the loss of more than 64 million DALYs in Europe (23% of all DALYs lost) and 26 million DALYs in the EU (19%); **d.** CVD is the leading cause of premature death in Europe. In particular, deaths under 75 as well as 65 years old in Europe as a whole are primarily attributed to a major cardiac episode, accounting for more than 1.3 million deaths/year 667,000 deaths/year, respectively; **e.** The annual cost attributed to CVD in EU has reached the €210 billion.

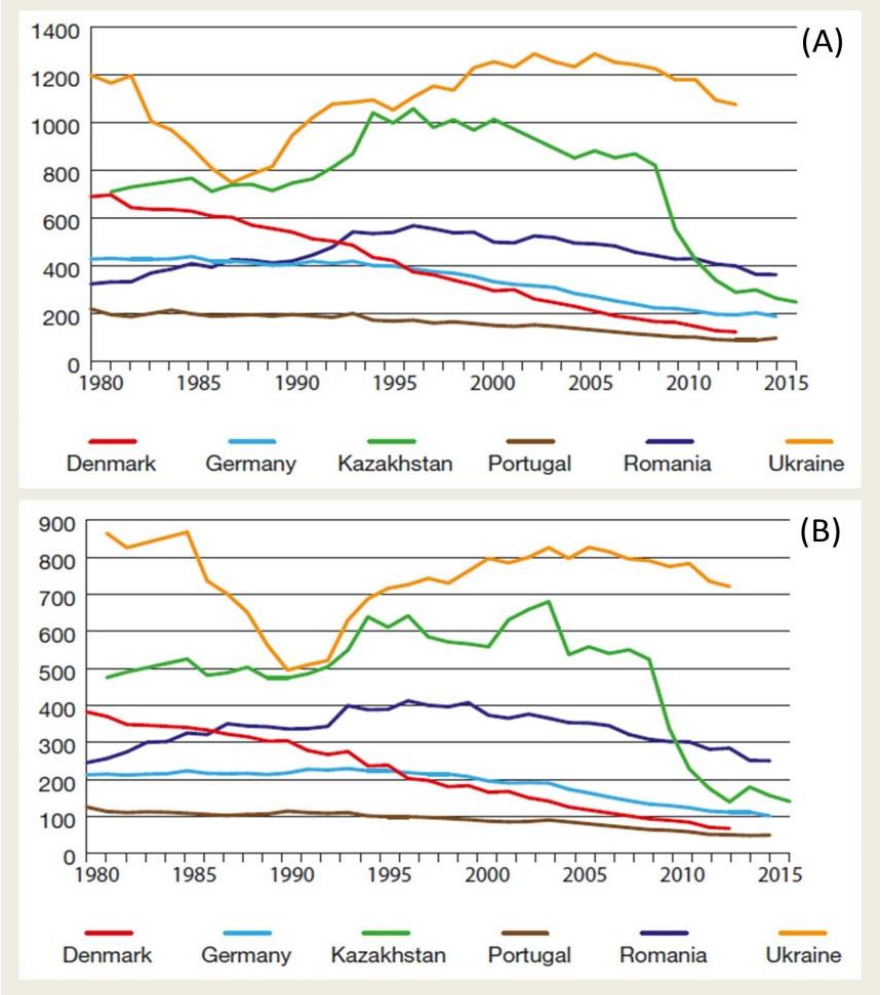

Figure 1.2 Age-standardised death rates/100,000 from IHD, (A) men (B) women, 1980 to 2015, selected.

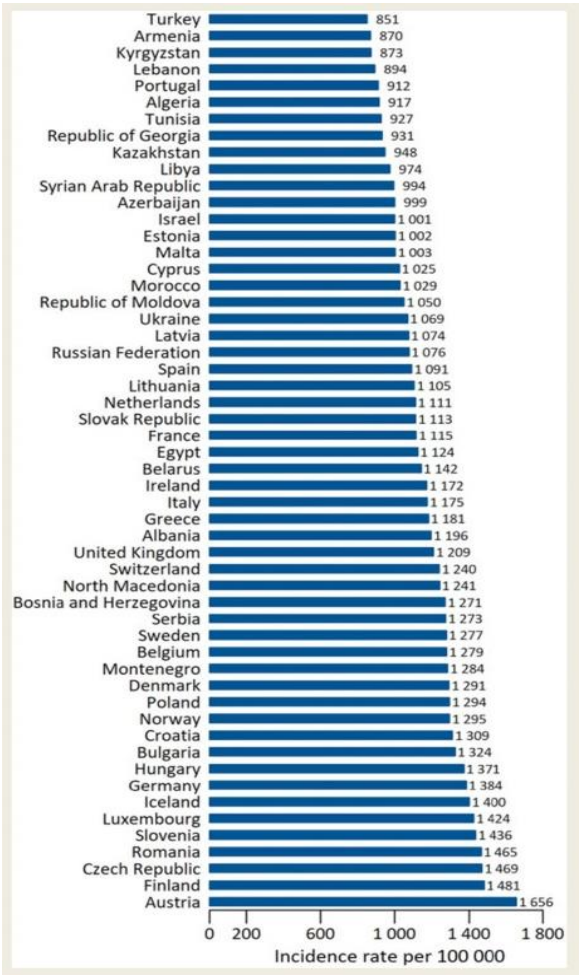

Figure 1.3 Age-standardized incidence of cardiovascular disease in ESC member countries (2017).

The ESC collects cardiovascular data from across its 57 member countries –including Greece– through the "Atlas in Cardiology". The latest analysis of Atlas data was published within the December 2019 where contemporary CVD statistics are summarized (Timmis et al 2019). In 2017, there were 19.9 million new cases of CVD in the 54 ESC member countries with data available. National contributions were in part determined by population size. The median, age-standardized incidence of CVD was 1,133 (IQR 1,002-1,289) per 100,000 inhabitants of each member country (**Figure 1.3**). What is more, the median age-standardized incidence of CVD per 100,000 inhabitants changed from 1,186 (IQR 1,078-1,340) in 1,990-1,133 (IQR 1,002-1,289) in 2017.

In high-income countries, age-standardized CVD mortality rates have declined rapidly in recent years. The median age-standardized incidence of CVD per 100,000 inhabitants was lower in middle-income countries compared with high-income countries [1,039 (IQR 930-1207) vs. 1,224 (IQR 1,106-1,356)] for both women and men (**Figure 1.4**).

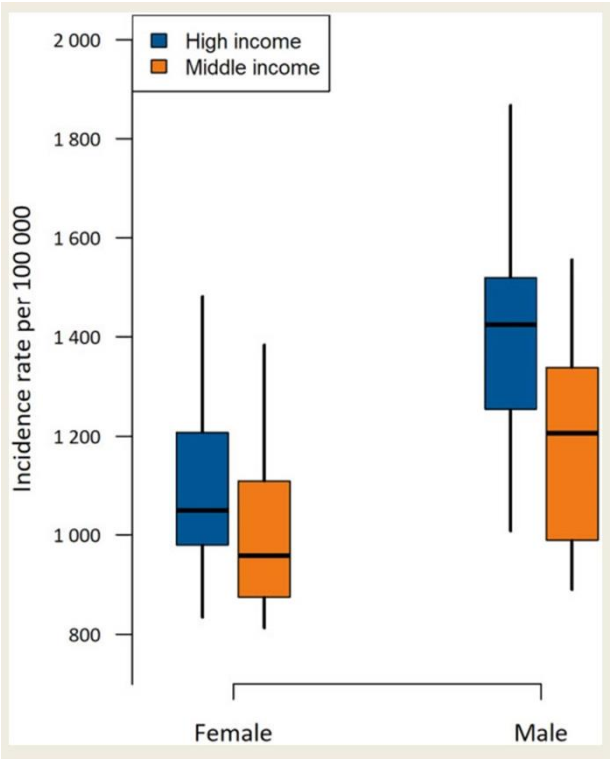

Figure 1.4 Age-standardised incidence of cardiovascular disease in ESC member countries by sex and national income

HID remains the most common manifestation of incident CVD. In 2017, 3.6 million new cases in the 54 ESC member countries were recorded with a median age-standardised rate per 100 000 inhabitants of each member country being at 176.3 (IQR 150.0-238.0). Between 1990 and 2017 a

significant decline was observed regarding IHD new cases with the age-standardised rate reduced by 35% (**Figure 1.5**).

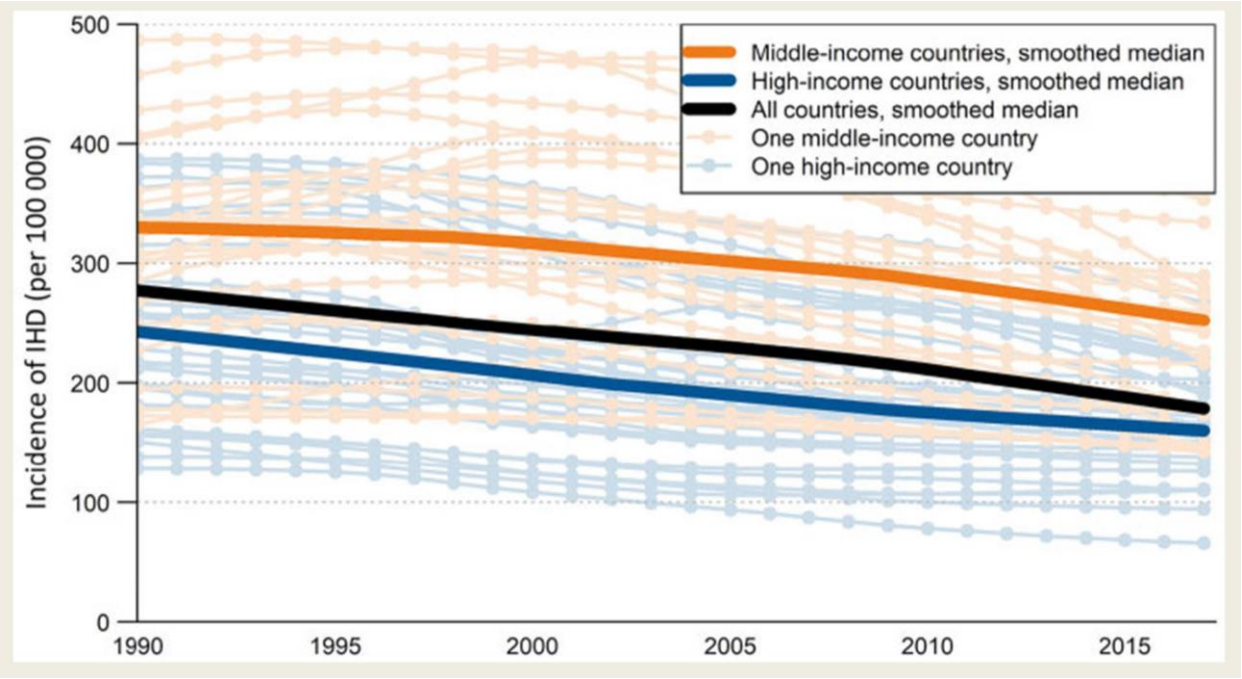

Figure 1.5 Age-standardized incidence of IHD in ESC member countries (1990-2017).

1.3 Epidemiology of cardiovascular disease in Europe: sex differences

For decades, the recognition of CVD as a "male privilege" was an unanimously propagated claim. Nonetheless, hitherto epidemiological data depict another reality. CVDs remain on the top of disability and mortality rank for both men and women (WHO 2018, Wilkins et al 2017, Timmis et al 2019). In Europe, almost half of deaths in women are attributed to a fatal cardiac episode surpassing the 40% men' CVD mortality rate: for IHD, 18% in women over 17% in men; for stroke, 12% in women over 8% in men; for other CVD causes, 17% in women over 14% in men (Timmis et al 2019). On the

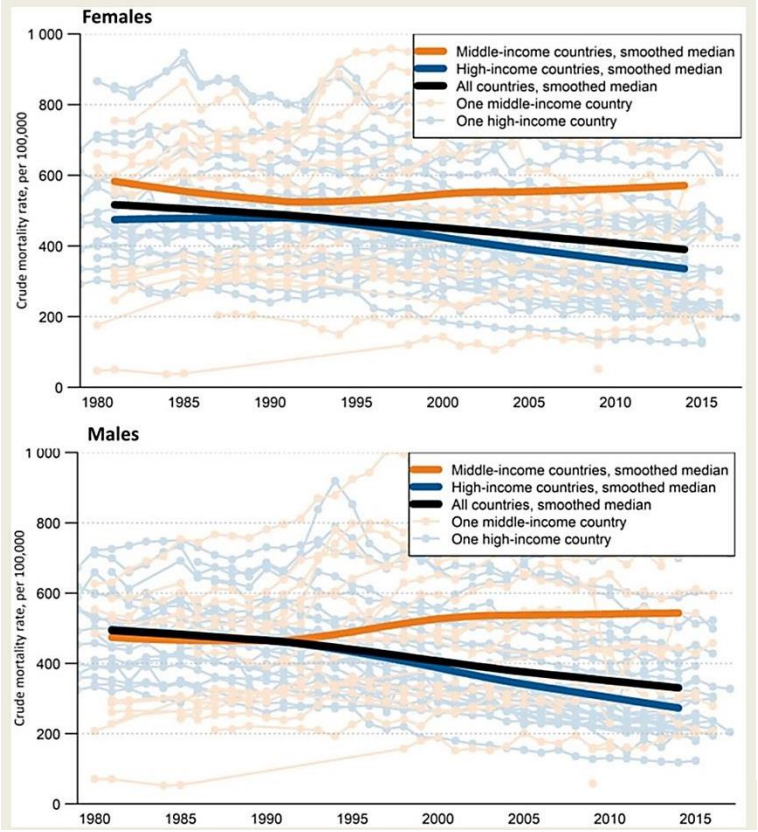

Figure 1.6 Crude cardiovascular disease mortality in women and men living in ESC member countries (1990-2017).

other side, much as median crude mortality rates in Europe declined in both sexes within the 1990-2017 period, the relative decrease was higher in case of men (18%) compared with women (12%) (Timmis et al 2019) (**Figure 1.6**).

Beyond the mortality rate, focusing on the principle CVD manifestations i.e. IHD and stroke, much as the age-standardized rates present women less likely to experience such episodes, differences in the crude incidence and prevalence of IHD and stroke are minor between sexes. In particular, in 2017 there were 34.9 million peopled in 54 ESC member countries with IHD. Women with IHD were fewer than men (16.2 million vs. 18.7 million). After age-standardization this apparently non-significant difference is modified to a twice as high IHD incidence in men compared with their female counterparts (**Figure 1.7**).

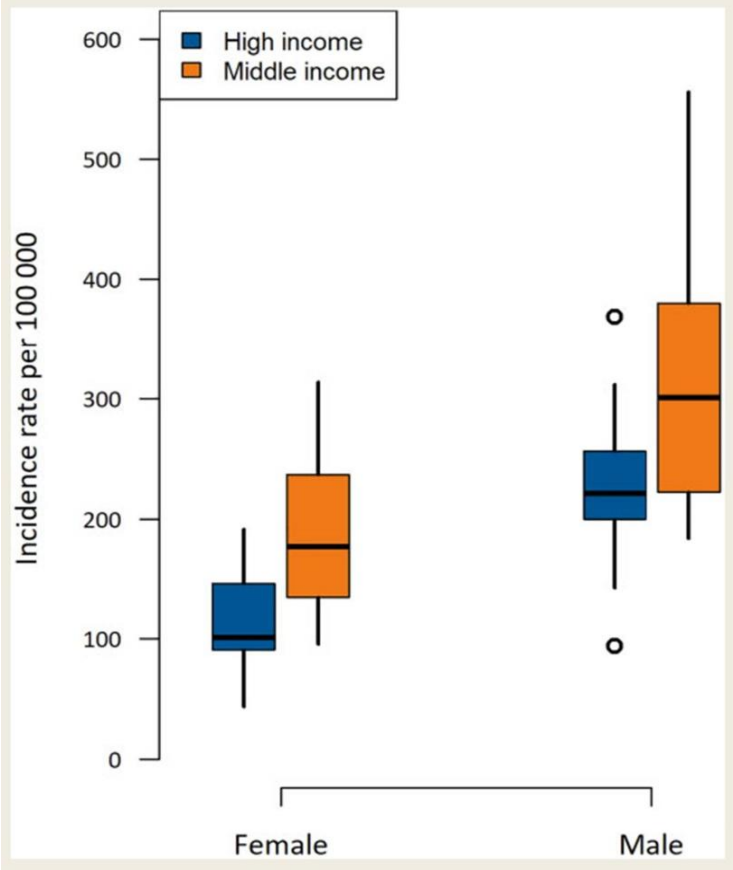

Figure 1.7 Age-standardized incidence of IHD in ESC member countries by sex and national income status (2017).

As for stroke, new cases across ESC member countries were shared almost equally between women and men (1.2 million vs. 1.1 million). However, the median age-standardized rate per 100,000 people was lower in women than men [130.3 (IQR 90.5-166.4) vs. 159.9 (IQR 111.0-190.7)].

CVD remains the most common cause of premature death for men in ESC member countries, but this is not the case for women in whom cancer now causes more premature deaths than any other disease. However, it is of interest that the trends related with premature death

attributed to heart disease do not present significant differences between sexes (in 2017, 33% in men vs. 30% in women, of total mortality rate) (Timmis et al 2019) (**Figure 1.8**). In addition to this, in case of IHD, women present a slightly lower rate of premature mortality attributed to this CVD manifestation (in 2017, 11% in women over 15% in men, of total mortality rate) while premature stroke mortality seems to be similar or slightly in favour of women (in 2017, 7% in women vs 6% in men, of total mortality rate).

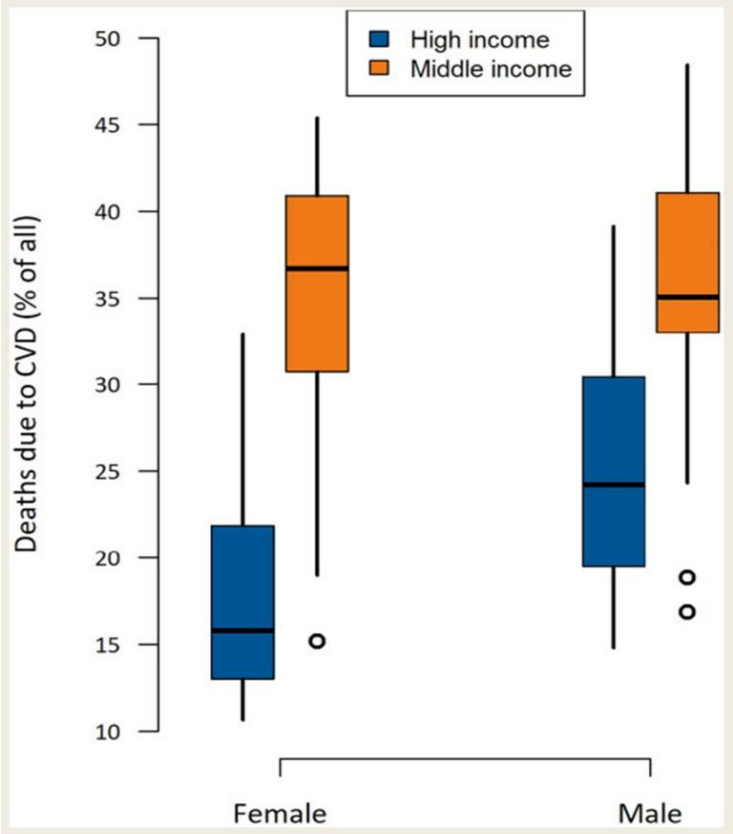

Figure 1.8 Proportion of all premature deaths (<70 years old) caused by cardiovascular diseases in ESC member countries by sex and national income status (latest year available).

PYLLs metric takes into account the age at which the death occurs, giving greater weight to deaths at a younger age and lower weight to deaths at older age, summarizing premature mortality. In the vast majority of European countries –including Greece–, more than 30% of total PYLLs are attributed to CVD (**Figure 1.9**). More impressively, this is the case for both men and women (Wilkins et al 2017; Timmis et al 2019). In particular, according to the most recent data, CVD accounted for 28 million and 38 million PYLLs within ESC member countries among women and men, respectively, making up 37% of cancer accounted for 25% of PYLLs in women and 22% of PYLLs in men, equivalent to 18.7 million and 25.5 million PYLLs, respectively.

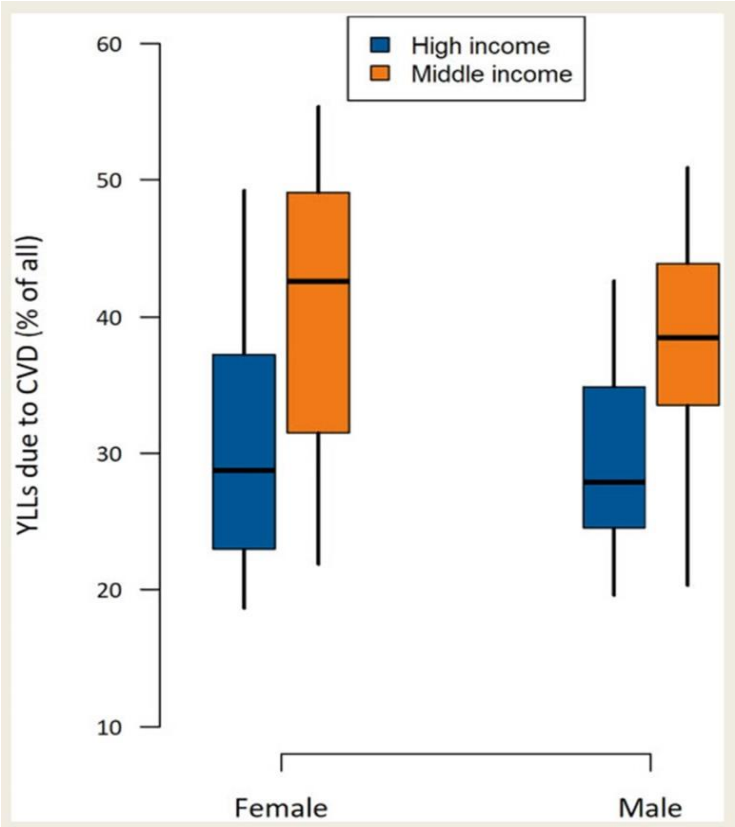

Figure 1.9 PYLLs attributed to cardiovascular disease in ESC member countries by sex and national income status (2017).

DALYs combine information regarding premature death (years of life lost) and disability caused by the CVD (years lived with CVD) to provide a summary measure of health lost due to that condition. Median age-standardized DALYs per 100,000 people due to CVD were 3,219 (IQR 1,597-5,324) for women. For men, the median number of DALYs per 100,000 people due to CVD was almost twice as high [5,925 (IQR 2,810-8124)]. IHD was the major contributor to the difference between women and men with a nearly three-fold difference in median values for DALYs: 1,384 (IQR 615-2,423) vs. 3,145 (IQR 1513-5261) per 100,000 people, respectively. For stroke, however, DALYs per 100,000 people were more comparable between the sexes at 951 (IQR 481-1730) for women vs. 1255 (IQR 612 – 2,426) for men.

1.4 The role of "sex" or "gender" in cardiometabolic mechanisms

1.4.1 Definition of "sex" and "gender"

In 2001, the IOM published the report "*Exploring the Biological Contributions to Human Health; Does Sex matter?*", one of the very first official recognitions of sex as variable with critical health impact (IOM, 2001). In 2015, the NIH with the notice "NOT-OD-15-102" required from investigators sex-specific reporting in any kind of study (NIH, 2015). It has not been a long time since CVD research has been oriented towards this approach. Emerging data convincingly demonstrate heterogeneities in CVD prevention, diagnosis and treatment between men and

women, attributed to biological and socially-constructed variances. The subsequent biological status, with different patterns in chromosomes, gene expression and hormone profile refer to the term "sex" while the broader, psychosocial, environmental, lifestyle and community indicators refer to the "gender" identity (EUGenMed 2016). Sex/gender discrepancies in CVD prevention and rehabilitation process are dependent on the complex interactions among genetic, metabolic and environmental factors (**Figure 1.10**) (Kouvari et al 2019).

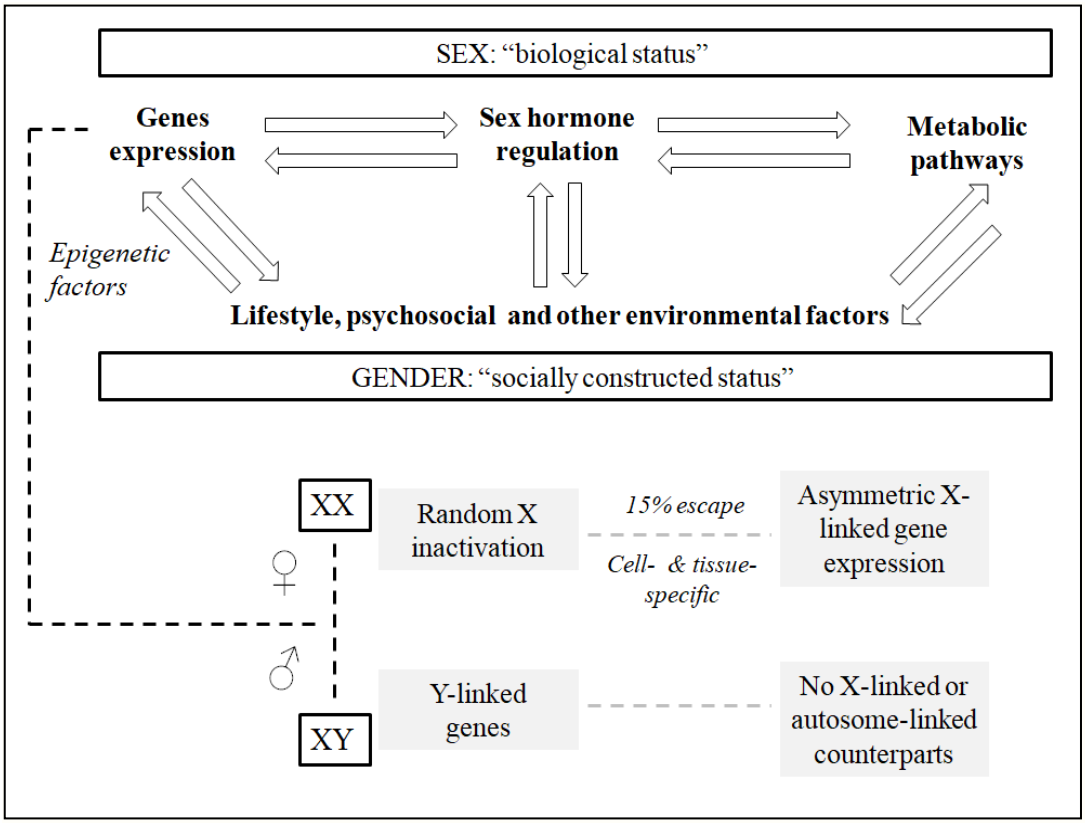

Figure 1.10 The complex interactions between sex- and gender-related characteristics that should be addressed in cardiovascular disease spectrum. **Source:** Angiology.2018;69:843-853.

1.4.2 Differences in CVD manifestation between men and women

IHD is the most frequent CVD manifestation in both men and women (Panagiotakos et al 2015). The first speculation about the "female" IHD pattern came from the Women's Ischemia Syndrome Evaluation study. The status of IHD women, namely the younger ones, is usually characterized by non-obstructive plaques, being independent of clinical features almost always presented in male patients such as coronary artery stenosis and plaque rupture (Gulati et al 2012); type II cardiovascular artery disease, outward remodelling, coronary microvascular dysfunction and non-obstructive coronary plaques are the predominant disease traits in middle-aged women (EUGenMed 2016; Sanghavi and Gulati 2015). Moreover, stress-mediated cardiomyopathy (takotsubo) is exclusively presented in postmenopausal women due to their autonomic regulation imbalance (EUGenMed 2016). Such sex-specific clinical features are commonly encountered challenges.

Other common-in-women CVD manifestations are the ischemic stroke and heart failure (EUGenMed 2016). Women are greater affected by stroke manifested with non-typical symptomatology. This usually leads to under-diagnosis and a later arrival in the emergency department. Additionally, their post-stroke period of life is accompanied by longer hospital accommodation, severe implications and permanent disabilities (Girijala et al 2017). Regarding heart failure, women have twice as high likelihood to be diagnosed with heart failure with preserved ejection fraction compared with men, for whom left ventricle dysfunction is more prevalent and with by far more efficient treatment (Azad et al 2011).

1.4.3 Cardiovascular system physiology and function in men and women: the non-genomic and genomic effects of sex hormones

Besides the overall common vasculature, notable anatomical differences have been observed between the two sexes (Garcia et al 2016, Blenck et al 2016). Women have smaller left anterior descending artery and right coronary artery diameters along with less atheromatous burden compared with men. Additionally, male sex is characterized by more sympathetic activity in contrast with the higher parasympathetic activity in women, highlighting sex-related differences in the autonomic nervous system (Huxley 2007).

Sex hormones (i.e. oestrogens, androgens, progesterone) are one of the most important biological factors determining the sex identity (Menazza et al 2016). The sex-specific hormone regulation and the attendant metabolic paths are to differentially affect CVD prevention and management determinants, including circulatory system function, cardiac remodelling and drugs metabolism. All these are highly dependent on sex-hormones and their receptors. More specifically, sex hormones have the potential of a rapid signalling cascade activation (non-genomic effect) or a long-term response (genomic effect) (Fazal et al 2014, Rallidis et al 2018).

The non-genomic response modulated by sex hormones is an important underlying mechanism through which the cardioprotective effects of oestrogens and the cardiotoxic effects of testosterone are exerted. Once oestrogens are linked with their receptor (i.e. G-protein coupled oestrogen receptor) a rapid activation of kinases is performed with a direct impact on vascular system; inducing vasodilation through the nitric oxide production, exerting anti-inflammatory and anti-apoptotic effects and inhibiting cardiac hypertrophy and fibrosis. Testosterone has exactly the opposite effect on vascular system (Salemi et al 2015).

Gene expression is subjected to hormonal regulation in different tissues and at different time of development (Tsiotra et al 2008). In this context, sex hormones exert their genomic effects acting as transcription factors for hormone-sensitive genes or as modulators of other transcription factors. In women, oestrogen receptors A and B lead to the expression of nitric

oxide synthase gene and other antimitogenic and antiapoptotic genes. Additionally, their long-term anti-inflammatory and antioxidant potential are partially attributed to the downregulation of genes encoding products which enhance the formulation of reactive oxygen species or cytotoxic compounds. On the other side, the genomic effects of testosterone have aggravating effects on cardiac health. For instance, the upregulation of genes encoding extracellular matrix and collagen enhances myocardial remodelling (Fazal et al 2014; Salemi et al 2015).

Hormonal status of the two sexes varies throughout lifespan. Additionally, genetic variants in enzymes involved in the synthesis and degradation process of steroid hormones and their nuclear receptors can differentially affect the endpoint. Oestrogens are not only produced by the women's ovary; since their receptors are also expressed in men, the local oestrogen synthesis through the conversion of androgens by aromatase has been suggested. However, oestrogens bioavailability in case of men is age-dependent while their synthesis is usually induced by adipose tissue of obese subjects; advanced age and obesity along with cardiomyocyte-specific effect of oestrogens in men's heart attenuate their cardioprotective effects (Fazal et al 2014). Women's reproductive life stage is postulated to be the main reason for the 7- to 10-year delay in CVD diagnosis. Nonetheless, this cannot be the finishing point; the disappointing outcomes of hormone replacement therapy as a preventive or management tool in women participating in Women Health Initiative Trial, imply the existence of more complex underlying mechanisms (Pal and Manson 2012) (**Figure 1.11**).

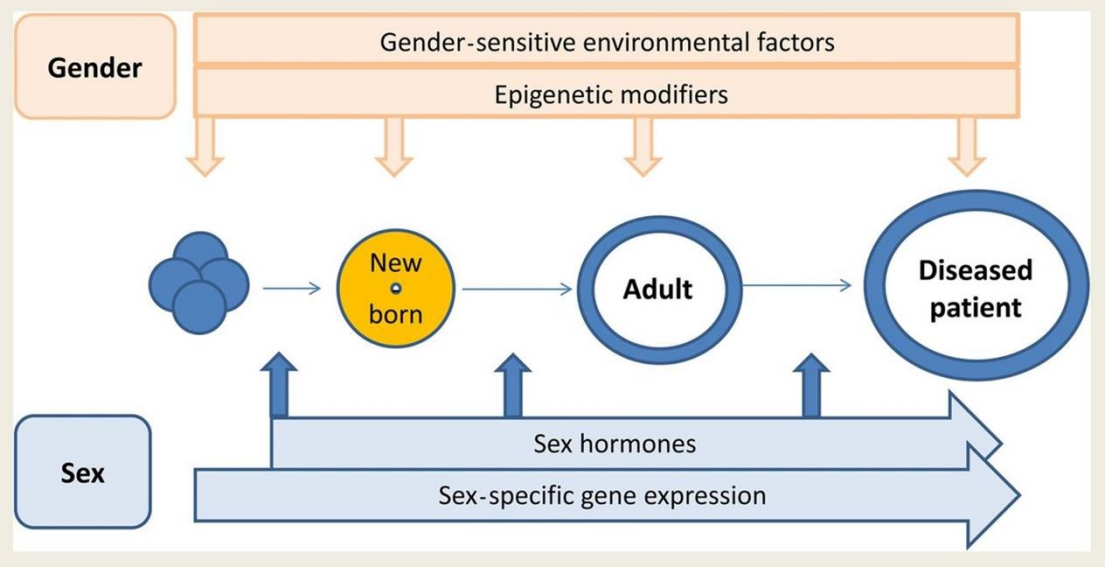

Figure 1.11 Interaction between sex and gender during lifetime: societal conditions (upper) as well as biological facts. **Source:** Eur Heart J.2016;37:24-34

1.4.4 Genetics in CVD spectrum: differences between the two sexes

GWASs with sex-specific orientation are the key to identify the genetic components of disease to better clarify prevention targets, to foresee progression mechanisms and to optimize

treatment. Biological sex is determined by the X and Y chromosomes particular combination; hence, this is justifiably the most challenging field to draw conclusions in disease spectrum. Sex chromosomes present indicative differences in genetic content. The Y-linked genes are exclusively presented in men and most of them have no X- or autosome- linked counterparts. This chromosome is expressed on its largest genetic part exhibiting regulatory functions in various biological processes. On the other hand, female karyotype has two X-chromosome copies. To manage the balance in allele dosage between the two sexes, a random tissue- and cell-specific inactivation process occurs in female tissues with the maternal or paternal copy being silenced in each somatic cell. This procedure results in a single dose of X-linked gene products. Nonetheless, 15% of X-linked genes escape from this inactivation process. Skewed inactivation usually occurs, leading to asymmetrical expression of X-linked genes; hence some X-linked genes are expressed at higher levels in women. Phenomena such as the different gene dosage between men and women, the mosaicism in women and the hemizygosity in men can explain the different effect of any variant in X-linked genes between the two sexes. These unequal patterns of expressions seem quite promising; lack of allele dosage compensation for cardiometabolic traits are speculated to account for sex-oriented pathophysiological phenotypes (Tukiainen et al 2014). Autosomes expression patterns also present variances in disease-related genes from the standpoint of sexual dimorphism (Mittelstrass et al 2011; Warren et al 2017).

1.4.4.1 Examples of sex-specific GWAS in the CVD spectrum

Genes linked with inflammatory pathways are to trigger atherosclerosis onset. In this case, some sexual dimorphisms have been revealed (Stamova et al 2012). Y- linked genes related with innate immunity are involved in macrophages activation and accumulation process. Additionally, in the X chromosome there are genes with apoptotic, lipid oxidation and free-radicals generation properties with greater upregulation in women (Stamova et al 2012). The macrophage migration inhibitory factor gene related with a proinflammatory state was associated with higher apolipoprotein B100, triglycerides levels and diabetes, only in men (Coban et al 2015). In Women's Genome Health study, seven genome loci associated with C-reactive protein plasma were identified; their protein products were involved in metabolic syndrome, insulin resistance, weight homeostasis and premature atherothrombosis (Ridker et al 2008).

In a Metabochip meta-analysis, 97 BMI associated loci were revealed with evidence of heterogeneity between men and women and in some cases with significantly stronger effect size for women (Locke et al 2015). Such genetic variants were differentially related with insulin sensitivity, blood pressure, inflammation and redox stress. Additionally, visceral adiposity is

strongly suggested as an independent CVD risk factor with higher effect size in women. A genetic basis towards this claim aroused when a QWAS descent to investigate the sex-specific loci of anthropometric traits; the entire loci associated with waist circumference were identified only in women (Randall et al 2013). In the context of Genetic Investigation of Anthropometric Traits, in nineteen waist-to-hip ratio loci, the effect was stronger for women while most of them were associated with high-density lipoprotein (HDL-C), triglycerides, fasting insulin and adiponectin (Shungin et al 2015, Winkler et al 2015). Another study revealed that 11β-hydroxysteroid dehydrogenase type 1 gene expression in adipocytes was higher in men. This gene was positively associated with HOMA-IR and haemoglobin A1c and negatively associated with HDL-C only in men (DeSchoolmeester et al 2013).

Sex-distinctive loci related with lipid metabolism have been identified in a large-scale European QWAS. Lipoprotein lipase gene was associated with HDL-C only in men (Aulchenko et al 2009). In two meta-analyses, stronger associations of genes related with triglycerides were revealed for women. Additionally, the effect of gene encoding 3-hyroxy-3-methylglutaryl-Coenzyme A reductase, the rate-limiting step in cholesterol synthesis, was stronger in women (Aulchenko et al., 2009). Hence, major doubts are revealed regarding the efficiency of statin hypolipidemic therapy in women. Additionally, in Women's Health Initiative, statins have been associated with higher diabetes mellitus rates. These hypolipidemic agents interact with the proliferator-activation receptor γ. This nuclear hormone receptor triggers insulin resistance. Considering the sex-specific PPARγ gene variants, statins as a treatment agent should be prescript with consciousness in women (Goodarzi et al 2013).

The Sry loci of the Y chromosome associated with greater activity in tyrosine hydroxylase, resulted in higher norepinephrine synthesis rate, predisposing men to hypertension in younger age than women. At the same time, genetic variants in alpha and beta adrenergic receptors have been accused for an excess risk in women for AMI, stroke and heart failure (Mittelstrass et al 2011, Warren et al 2017).

1.4.5 Sex-specific effect of clinical risk factors in primary CVD prevention

1.4.5.1 The INTERHEART study

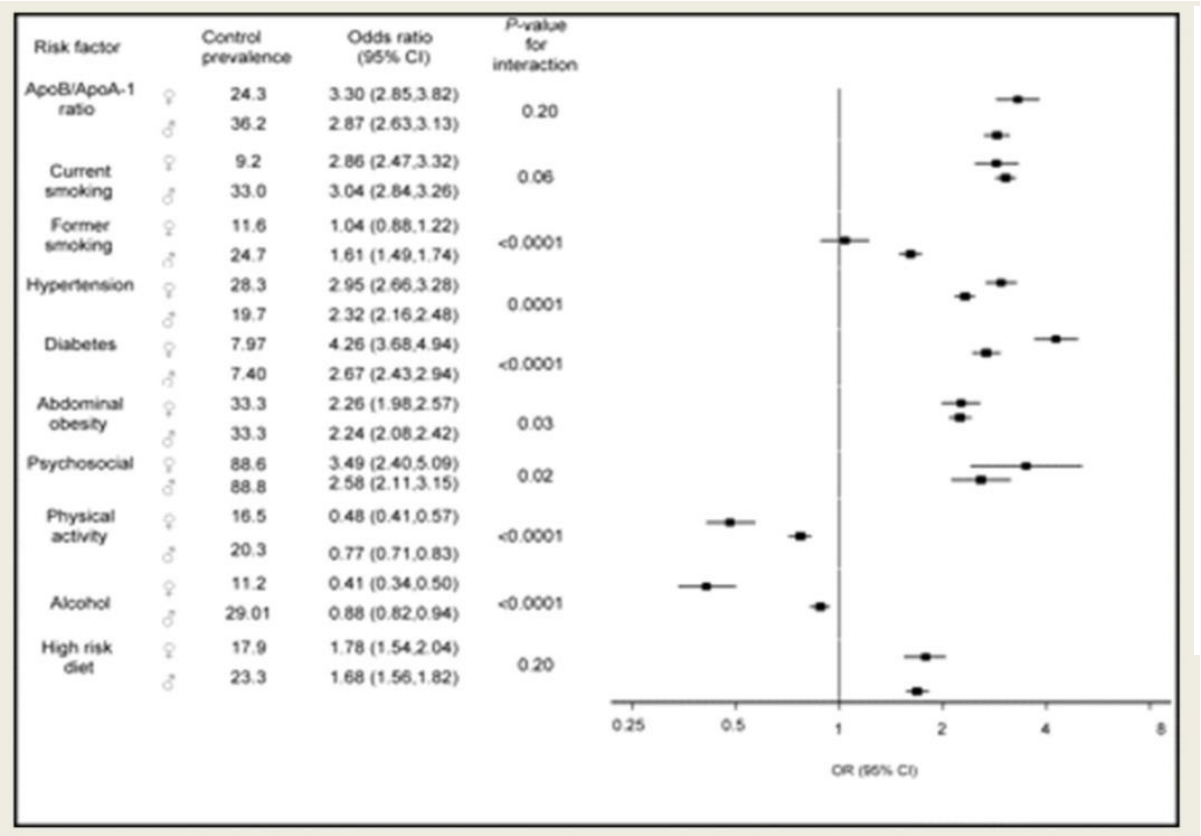

Figure 1.12 Comparison of population attributable risks between women and men. **Source:** Eur Heart J.2008;29(7):932-40

The INTERHEART global case-control study was one of the first large-scale studies in CVD epidemiology where the recognition of male sex as an independent risk factor in premature AMI was questioned. The results of that work suggested that clinical and lifestyle parameters alleviate this unanimously propagated association. Some of these factors were more potent in women, such as apolipoproteins, diabetes mellitus, abdminal obesity, psychological factors and dietary habits (Yusuf et al 2004, Anand et al 2008) (**Figure 1.12**).

Considering all nine risk factors, the collective PAR on AMI was 96% (95% CI: 94–98) in women compared to 93% (95% CI: 92–95) among men (**Figure 1.13**) (Anand et al 2008). What is more, the contribution of risk factors to the overall PAR varied between sexes due to the differences in ORs and prevalences of risk factors. The PARs of hypertension (35.8 vs. 19.5%) and diabetes (19.1 vs. 10.1%), physical inactivity (37.3 vs. 22.9), and alcohol use (46.9 vs. 10.5%) were significantly greater among women compared to men. Whereas among men, former smoking was associated with a higher PAR than it was among women (18.1 vs. 2.5). Interestingly, MetS-related risk factors (i.e. diabetes, hypertension, abnormal lipids, and abdominal obesity) contributed substantially to the risk of AMI among women (73%; 95% CI: 69–78) and men (68%; 95% CI: 65–71). The combined PAR for lifestyle factors including smoking, low alcohol use, high risk diet and physical inactivity was significantly higher among women than men [74.3 (95% CI: 67.9–80.7 vs. 67.3 (95% CI: 63.9–70.8)]. However, after removing alcohol, the combined lifestyle PAR was slightly lower among women compared to

men [55.2 (47.9–62.6) vs. 63.4 (60.1–66.8)]. The lifestyle PARs were quantitatively but not significantly greater among younger women and men compared to older women and men.

Risk factor		Odds ratio (95% CI)	PAR (95% CI)	*P* interaction
ApoB/ApoA1 ratio	♀	3.30 (2.85,3.82)	43.1 (38.2,48.1)	0.20
	♂	2.87 (2.63,3.13)	46.6 (43.3,50.0)	
Current smoking	♀	2.86 (2.47,3.32)	15.5 (13.5,17.5)	0.06
	♂	3.04 (2.84,3.26)	46.3 (44.1,48.4)	
Former smoking	♀	1.04 (0.88,1.22)	2.5 (0.6,4.5)	<0.0001
	♂	1.61 (1.49,1.74)	18.1 (15.2,21.1)	
Hypertension	♀	2.95 (2.66,3.28)	35.8 (33.0,38.6)	0.0001
	♂	2.32 (2.16,2.48)	19.5 (18.1,21.0)	
Diabetes	♀	4.26 (3.68,4.94)	19.1 (17.2,21.0)	<0.0001
	♂	2.67 (2.43,2.94)	10.1 (9.2,11.0)	
Abdominal obesity	♀	2.26 (1.98,2.57)	35.9 (30.3,41.5)	0.03
	♂	2.24 (2.08,2.42)	32.1 (28.8,35.4)	
Psychosocial	♀	3.49 (2.40,5.09)	25.7 (18.4,33.1)	0.02
	♂	2.58 (2.11,3.15)	21.7 (17.0,26.4)	
Physical inactivity	♀	2.07 (1.77,2.43)	37.3 (28.0,46.6)	<0.0001
	♂	1.30 (1.20,1.41)	22.9 (17.8,27.9)	
Lack of Alcohol intake	♀	2.42 (2.00,2.93)	46.9 (36.9,56.9)	<0.0001
	♂	1.13 (1.06,121)	10.5 (6.3,14.8)	
High risk diet	♀	1.78 (1.54,2.04)	25.5 (18.4,32.6)	0.20
	♂	1.68 (1.56,1.82)	26.8 (22.6,31.1)	

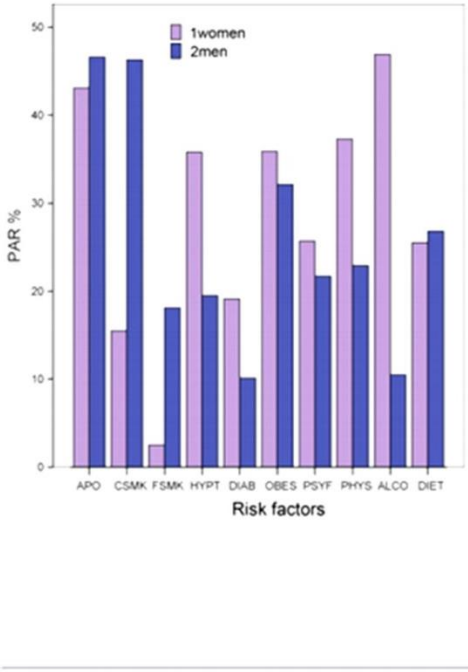

Figure 1.13 Comparison of risk factors related to acute MI between women and men. *Source: Eur Heart J.2008;29(7):932-40*

On the other side, the predicted probability of being an AMI case less than 60 years of age was substantially higher among men compared to women (60.6 vs. 33.0%, difference of 27.6%) (Anand et al 2008). This sex difference decreased to 23.2% after adjustment for regional differences, decreased to 18.2% after adjustment for region and smoking, and decreased to 4.7% after adjustment for all nine risk factors and region (**Figure 1.14**). Thus, more than 80% of the

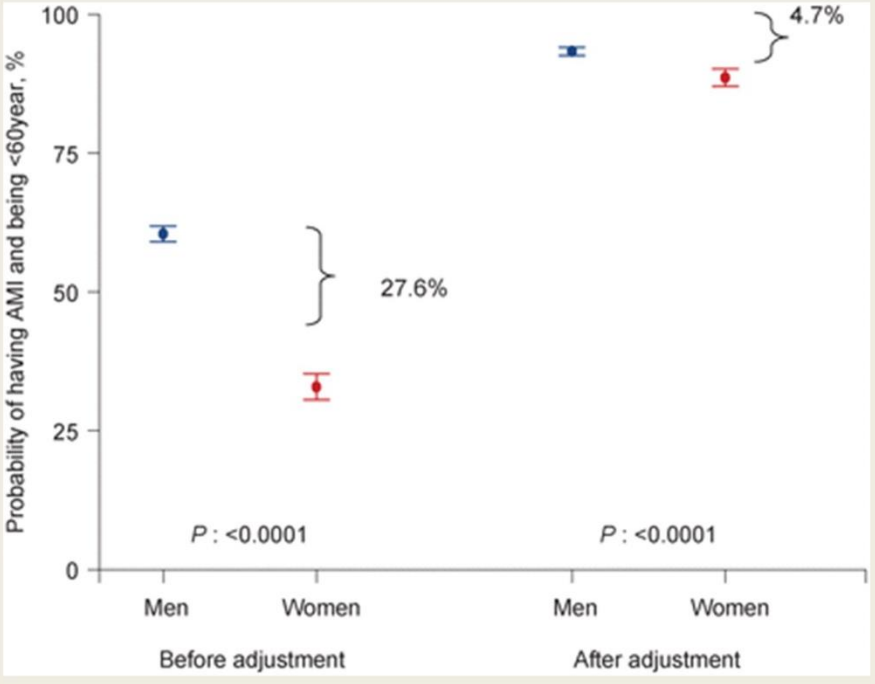

Figure 1.14 Differences in predicted probability of infarction cases <60 years comparing men and women. **Source:** Eur Heart J.2008;29(7):932-40

earlier age of first AMI in men compared to women was explained by the differences in the distribution of the nine risk factors. This suggests that the earlier age of acute AMI in men can largely be explained by the higher levels of some risk factors men possess at younger ages.

1.4.5.2 Abnormal glycemic profile

Diabetes mellitus

Diabetes mellitus is speculated to possess an increased burden on women's primary CVD risk (Huxley et al 2006, Lee et al 2000, Peters et al 2014a, Peters et al 2014b). In the most recent meta-analyses of longitudinal studies, women with diabetes had 44% and 27% higher risk of primary IHD (**Figure 1.15**) and stroke (**Figure 1.16**) respectively compared with their male counterparts (Peters et al 2014a, Peters et al 2014b).

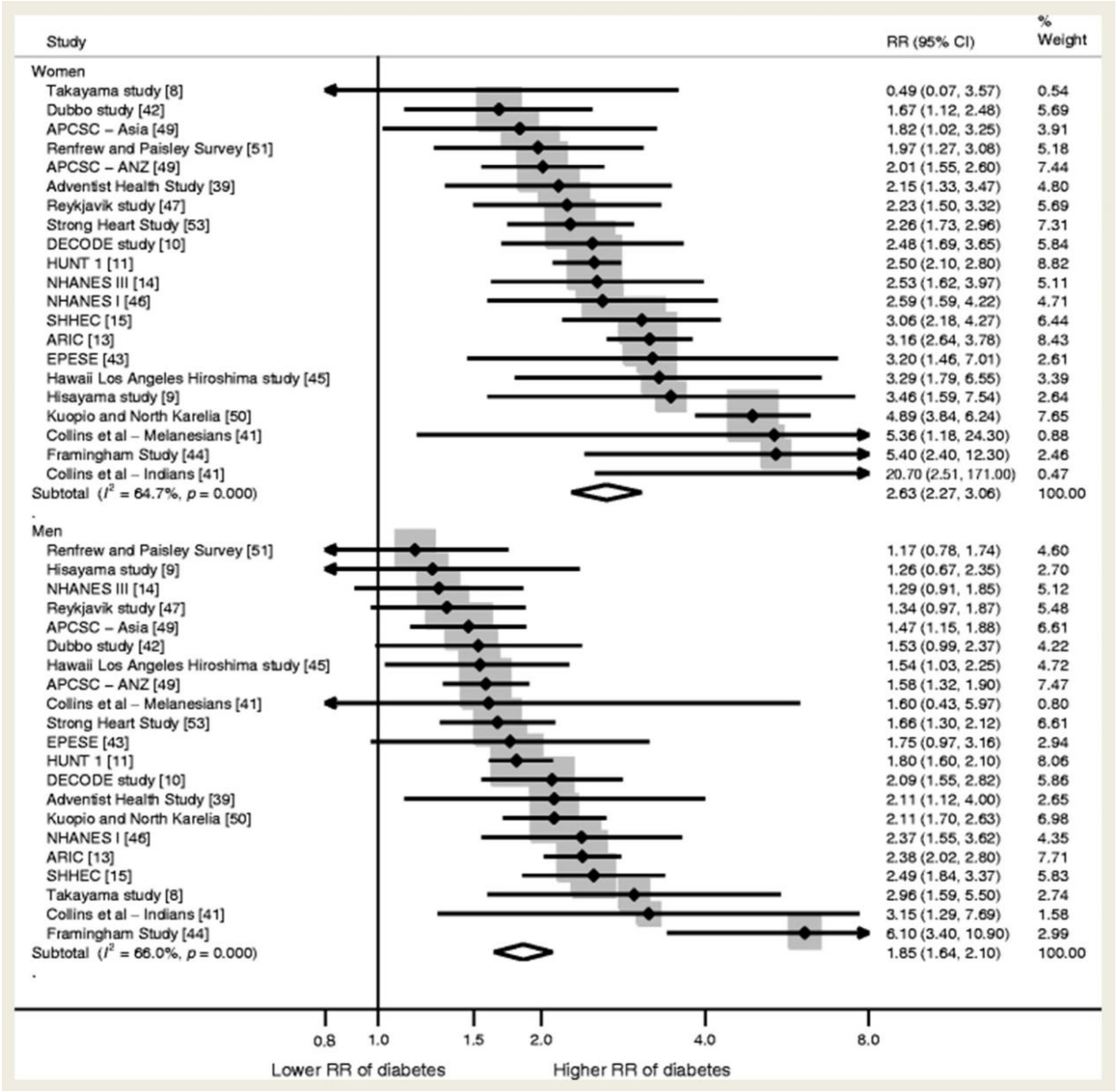

Figure 1.15 Multiple-adjusted pooled RR for incident CHD, comparing individuals with diabetes with those without diabetes. ANZ, Australia and New Zealand; EPESE, (National Institute on Aging) Established Populations for Epidemiologic Studies of the Elderly; HUNT, Nord-Trøndelag health study. **Source:** Diabetologia. 2014;57(8):1542-51

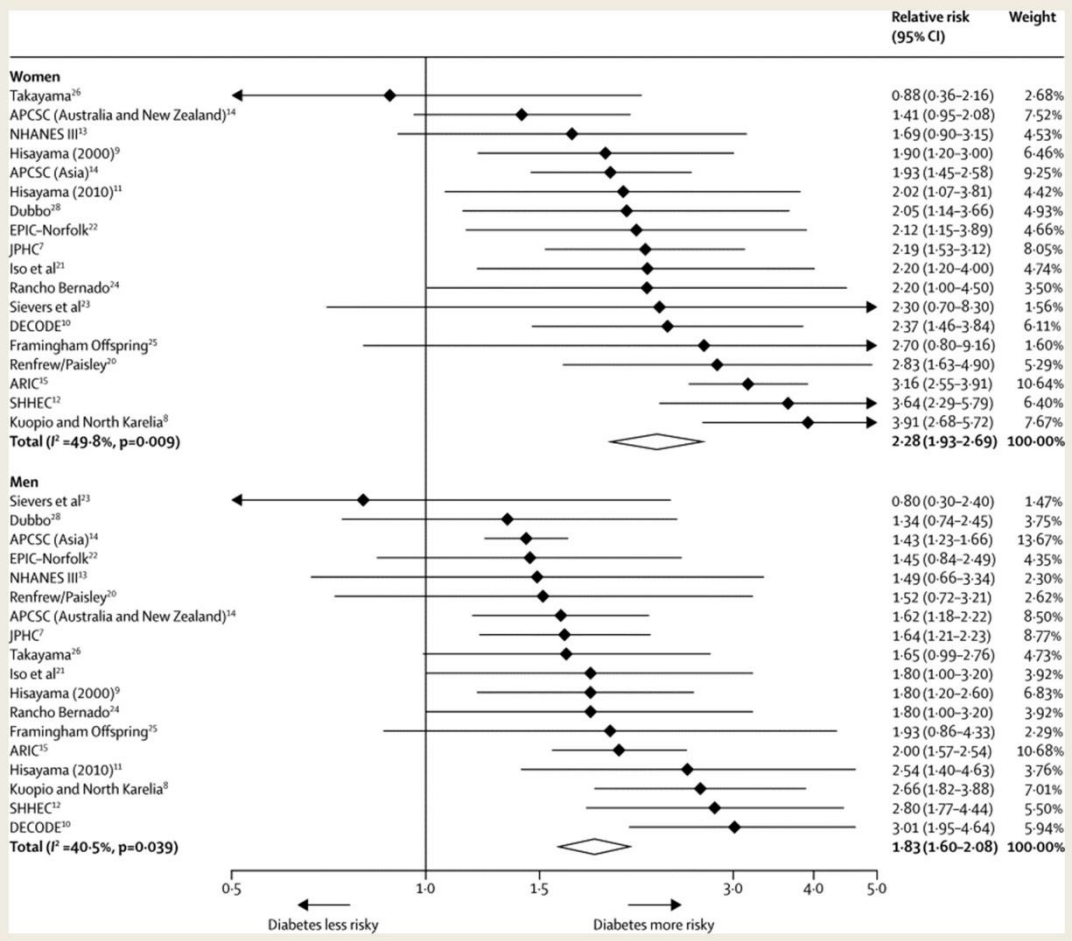

Figure 1.16 Maximum-adjusted pooled relative risk for any stroke, comparing individuals with diabetes to those without diabetes.
Source: Lancet.2014;383(9933):1973-80

Prediabetes

In a recent meta-analysis of cohorts the prediabetic state was suggested as an independent risk factor in primary CVD prevention (Huang et al 2016) (**Figure 1.17**). Based on the aforementioned female predominance in diabetes, a similar speculation could be performed regarding prediabetes. In a meta-analysis, the relative risk of hyperglycemia within a non-diabetic range was greater in cohorts including women than in cohorts with men (Levitan et al 2004). A sex gap in favour of women has been revealed, yet the hitherto sex-specific literature is scarce (Anagnostis et al 2014). The heterogeneity of prediabetes definitions (i.e. impaired fasting glucose, impaired glucose tolerance, increase HbA_{1c}) is a barrier to draw conclusions. A female predominance is observed in case of impaired glucose tolerance while in men impaired fasting glucose is the most prevalent prediabetic state (Manson and Bassuk 2015).

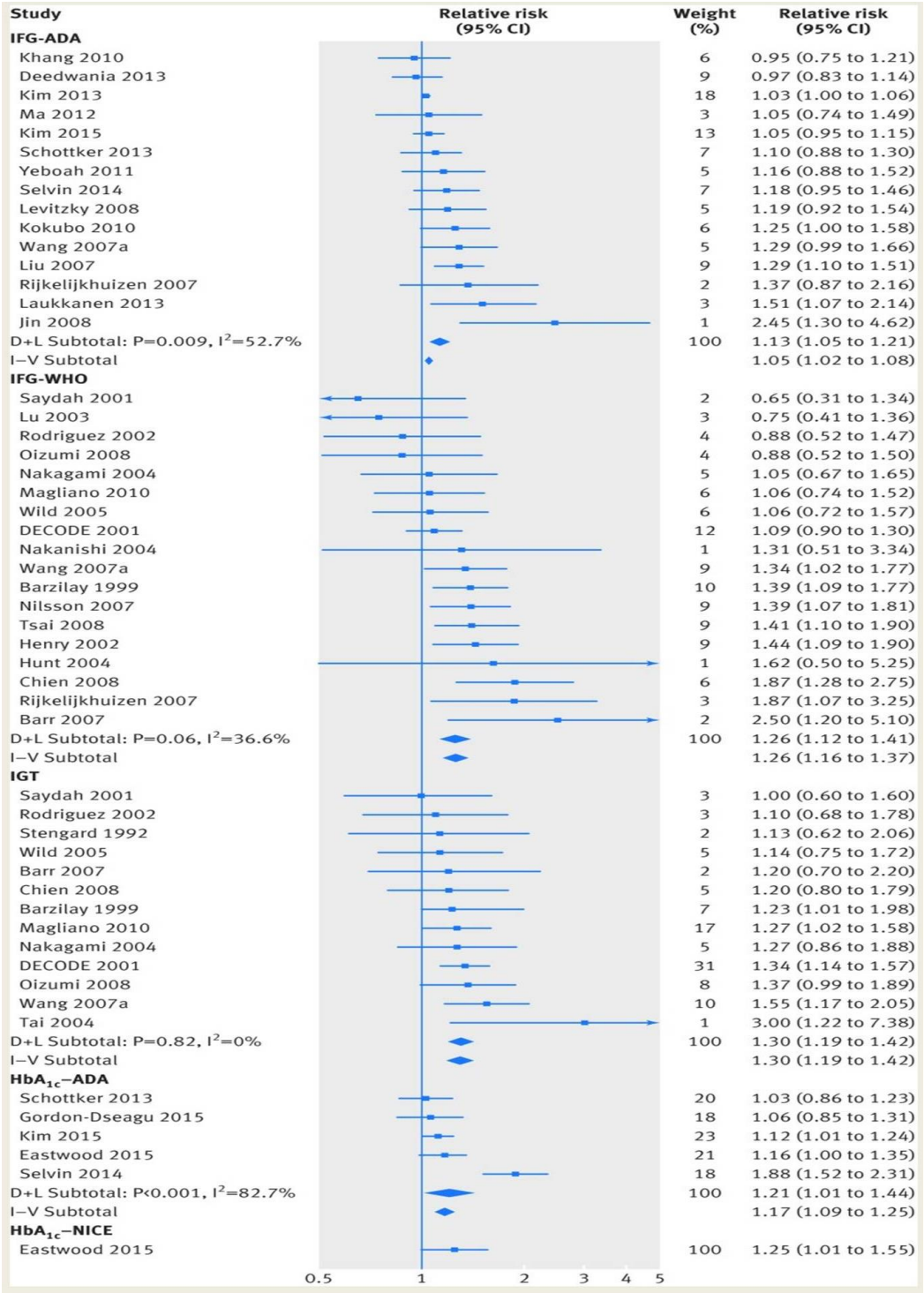

Figure 1.17 Association between prediabetes and composite cardiovascular events. **Source:** BMJ. 2016 Nov 23;355:i5953

Sex-specific metabolic explanation for abnormal glycaemic profile

In reproductive age, abnormal circulating glucose levels have been speculated to attenuate the cardioprotective properties of female sex hormones, reducing the vascular and platelet nitric oxide production and leading to increased vascular tone, platelet aggregation and vascular

proliferation. After menopause, a decrease in insulin elimination rate is observed (Takahashi et al 2013). Results regarding the direct association of this life stage with insulin resistance are less consistent. Thereby, the hypothesis of metabolic comorbidities has been revealed; visceral adiposity accompanied by low adiponectin levels may mediate menopause-insulin resistance association. Along with this, women are probably more prone to peripheral insulin resistance induced by free fatty acids (Stefanska et al 2015). Moreover, diabetes has been suggested as the strongest predictor of endothelial dysfunction in women, yet not in men for whom the obesity-induced endothelial dysfunction is more prevalent (Steinberg et al 2000). Other metabolic abnormalities in HDL-C, LDL-C, LDL particle size, apolipoprotein B100, A1, fibrinogen and lipid peroxidation process, usually recorded in women diagnosed with diabetes have been suggested to multiply the CVD risk (Stefanska et al 2015).

1.4.5.3 Abnormal blood pressure

In the INTERHEART study, women diagnosed with hypertension were at higher CVD risk compared with men having similar disease profile (Yusuf et al 2004). Nonetheless, in a recent meta-analysis of cohorts, the sex-specific systolic blood pressure attributable CVD risk was estimated without significant differences (Peters et al 2013b) (**Figure 1.18**). Raised blood pressure accounts for approximately 9.4 million CVD deaths worldwide every year. Men and women seem to be equally susceptible against this silent killer, yet the prevalence in the two genders strongly matters for decision policy makers. Hypertension increases with age. Men start with higher hypertension rates compared with women till menopausal life stage where these trends are reversed. This predominance becomes even more apparent due to women's increased lifespan (WHO 2013a).

Sex-specific metabolic explanation of raised blood pressure

Sex-based differences regarding renin-angiotensin-aldosterone system are hormones-mediated (Fazal et al 2014). Androgens stimulate this pathway leading to raised blood pressure while oestrogens down-regulate it through the reduction in renin/ angiotensin levels and receptors density and the inactivation of angiotensin-converting enzyme (Fazal et al 2014). Moreover, oestrogens have the potential to activate the nitric oxide synthase and the calcium-dependent potassium channels on endothelium inducing a rapid nitric oxide release. Along with the regulation of sympathetic nervous system these hormones enhance the vasodilation retention. These cardioprotective reactions are steeply erased in menopausal stage (Salemi et al 2015).

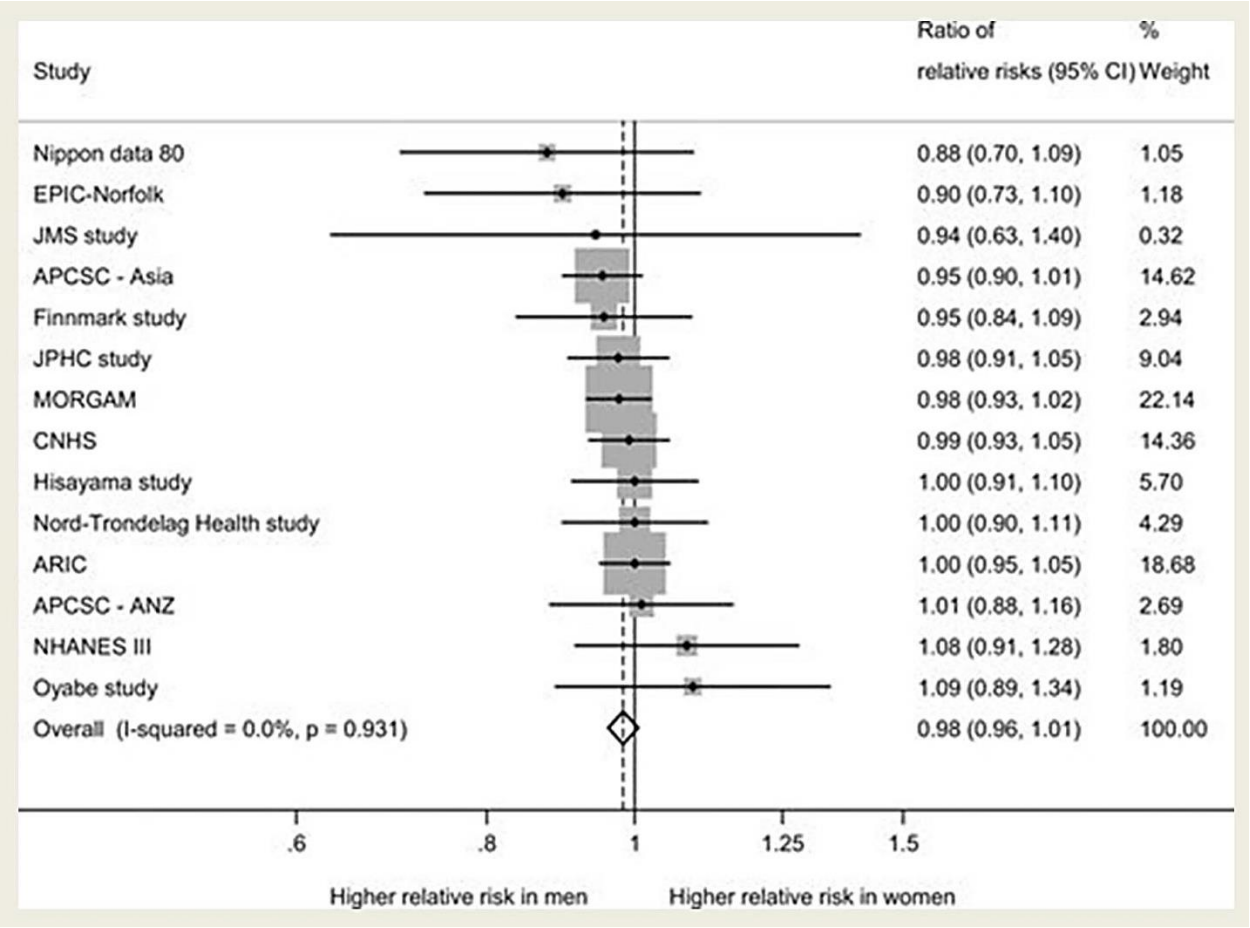

Figure 1.18 Multiple-adjusted women to men relative risk ratio for stroke for each 10 mm Hg increase in systolic blood pressure. **Source:** Stroke.2013;44(9):2394-401.

1.4.5.4 Abnormal lipid profile

Lipids/lipoproteins and their subclasses have been widely investigated regarding their contribution to CVD risk yet with inadequate sex-specific orientation. Raised LDL-C has been characterized as the key lipid parameter. Men have significantly higher LDL-C levels compared with the age-matched women, till the menopause stage; since then a steep raise in LDL-C levels occurs, predisposing women to an escalation in CVD risk (Mora et al 2011). Additionally, HDL-C and triglycerides have been suggested as stronger lipid indicators in case of women. More specifically, the addition of HDL-C in SCORE risk stratification led to a modest, yet significant improvement in its predictive ability for women (Cooney et al 2009). In Women's Health Study, the inverse association between HDL-C and primary CVD incidence was retained across all LDL-C and apolipoprotein B100 levels, with the exception of women with low total atherogenic particle burden (Mora et al 2011). As for triglycerides, in a meta-analysis of cohorts a stronger association of fasting blood triglycerides with CVD mortality in women was revealed (Liu et al 2013). In INTERHEART study, dyslipidaemia, defined as (apolipoprotein B100)/(apolipoprotein A1) ratio possessed the highest population-attributed risk in both genders (Yusuf et al 2004). In a meta-analysis of cohorts by the Emerging Risk Factors Collaboration, ApoB100 and ApoA1 had

the potential to improve the CVD risk discrimination only in men (ERFC, 2012). Other lipid-related markers such as Lp(a) and lipoprotein-associated phospholipase A2 have been suggested as emerging CVD risk factors, especially in women (Manson and Bassuk 2015).

Sex-specific metabolic explanation of abnormal lipid profile

The most evident changes in women's lipid profile starts in the menopause state (Gardner et al 2000). Menopause, namely through the accumulation of visceral fat, is associated with increased insulin resistance. Insulin resistance induces de novo lipogenesis and remnant lipoproteins uptake leading to triglycerides and triglycerides-rich very low-density lipoproteins overproduction. These are probably responsible for non-HDL-C raise and HDL-C decrease accompanied by the generation of small dense atherogenic HDL and LDL particles (Stefanska et al 2015). Moreover, ageing triggers the inactivation of CETP which naturally contributes to cholesterol reverse transport applied by HDL-C; this process has been postulated to be even more apparent in oestrogen-depleted postmenopausal women. Nonetheless, menopausal women still retain a modest survival advantage against age-matched men; the earlier peaking of the threatening-for-cardiac health lipoproteins in men along with the generally more buoyant – less atherogenic – lipoprotein particles in women are potential explanations (Salemi et al 2015).

1.4.5.5 Weight status and fat distribution

Women have slightly overlapped in overweight and obesity rates. In 2014, 39% of women and 38% of men were overweight while the respective estimations regarding obesity were 15% and 11%. According to estimates for 2025, the global obesity prevalence is to reach 18% in men while to surpass 23% in women (NCD-RisC, 2016). In women, the development of a weight status above the normal range is accelerated in postmenopausal life stage. Most importantly, different body composition patterns are presented between men and women; in men, adipose tissue is accrual in the trunk and abdomen while in women in the hips and thighs (Stefanska et al 2015). As for BMI sex-specific effect on IHD risk, in a very recent meta-analysis of cohorts, increments in BMI conferred a similar pooled risk in both men and women (**Figure 1.19**) (Mongraw-Chaffin et al 2015). Considering the different body composition between sexes along with the recognition that fat distribution –not adequately represented by BMI– is a better indicator of cardiac health this outcome raises doubts. However, research in the field of CVD and body fat distribution, separately in men and women is gravely lacking.

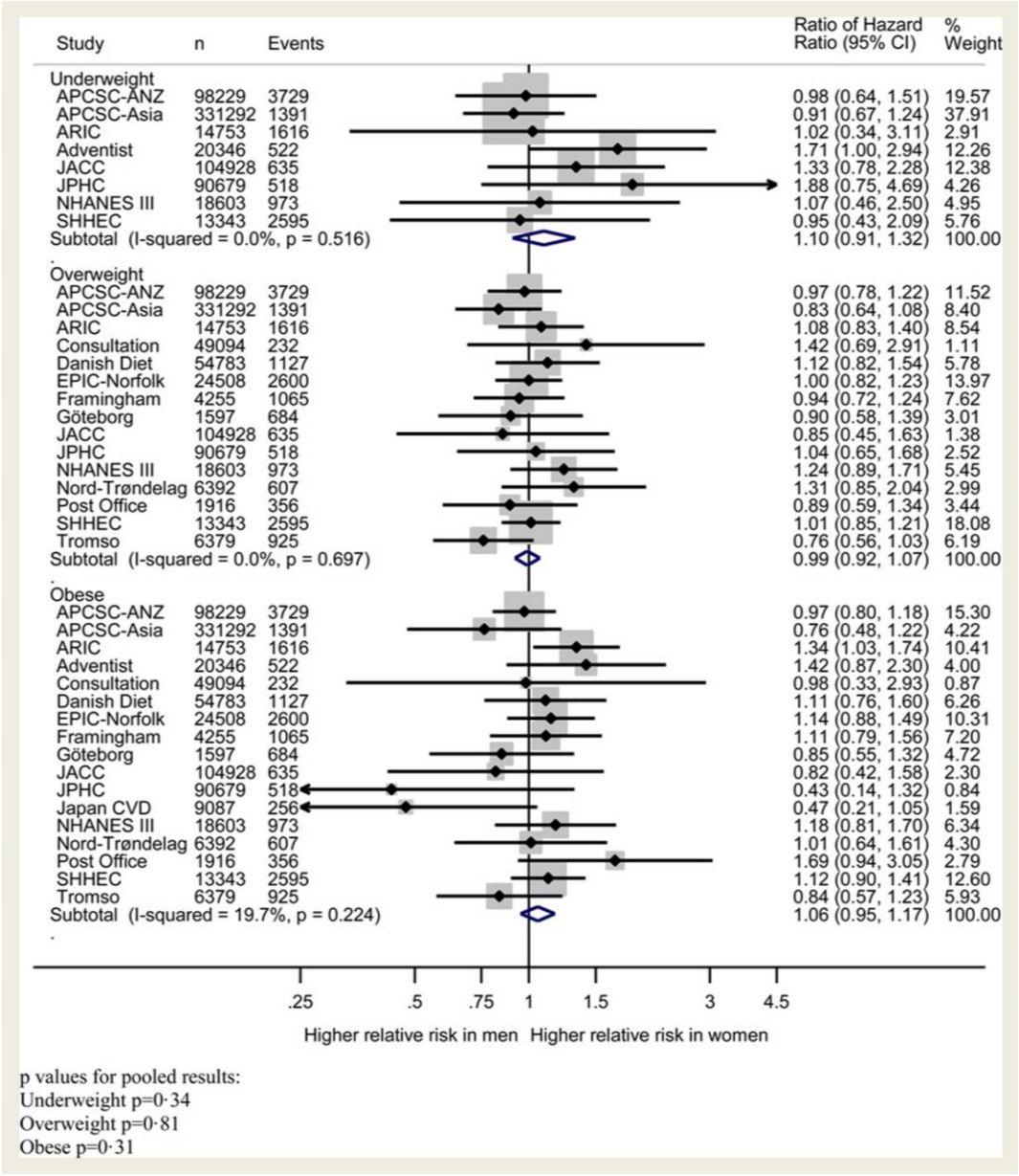

Figure 1.19 Women-to-men ratio of age-adjusted coronary heart disease hazard ratios and 95% confidence intervals for body mass index categories. **Source:** Lancet Diabetes Endocrinol. 2015;3(6):437-449

Sex-specific metabolic explanation for weight status and fat distribution

After puberty men have a higher basic metabolic rate compared with women because of their larger body size and higher lean mass. On the contrary, women are genetically predisposed to store more energy at a given level of intake (Kolovou et al 2014). Oestrogens and their receptors possess an indicative role in the regulation of body weight and fat distribution mainly in women prior to menopause, yet in men too. More specifically, oestrogens limit food intake through hunger and satiety signals either directly in central nervous system or indirectly through the interaction with hormonal regulators of appetite such as leptin, ghrelin and neuropeptide Y. Furthermore, oestrogens trigger fat deposition through a leptin-mediated mobilization from the atherogenic visceral to subcutaneous fat depots. On the contrary, testosterone in men inhibits this

procedure (Fazal et al 2014). Additionally, sex has been suggested to influence the adipocyte size in specific anatomic regions. Given the same weight gain, intraperitoneal visceral adipocytes size in men marginally increases compared with the indicative raise in case of women; large adipocytes present high lipolysis and proinflammatory adipokines secretion activity (Stefanska et al 2015). Finally, dietary patterns to which women usually adhere in their reproductive age such as frequent meals have been associated with body fatness in postmenopausal life stage (Yannakoulia et al 2007).

1.4.5.6 Female-specific risk factors

Pregnancy-related disorders

Pregnancy poses a substantial challenge to mother's vascular system. Pregnancy-related complications such as gestational diabetes and hypertension are usually the result of mother's inability to adapt to this cardiovascular and metabolic stress (Appelman et al 2015). Hypertensive disorders of pregnancy, in particular pregnancy-induced hypertension and preeclampsia, occur in 2–10% of women and are strongly associated with future maternal CVD risk. In a recent meta-analysis among 3.5 million women, preeclampsia was associated with two-fold higher risk for CHD at 14-year follow-up compared with women following normotensive pregnancy (Bellamy et al 2007). Gestational diabetes mellitus has a prevalence of 3–5% of all pregnancies and is similarly associated with an increased likelihood of CVD later in life (Kramer et al 2019). Most of this risk appears mediated by the development of type 2 diabetes, for which a meta-analysis estimated a lifetime relative risk of 7.4 for women who experience gestational diabetes, compared with women with normoglycemic pregnancies (Bellamy et al 2009).

Reproductive endocrine-related disorders

PCOS is the most common endocrine disorder in women, affecting around 5–10% of women of reproductive age. This syndrome is characterized by excessive production of androgens that leads to ovarian dysfunction and is often accompanied by insulin resistance (Bouchard et al 2014). Women with PCOS are predisposed to diabetes mellitus, hypertension, dyslipidaemia and other metabolic disorders related with increased cardiometabolic risk. To this issue, in a meta-analysis, it was revealed a two-fold increased relative risk for metabolic syndrome associated with PCOS (Moran et al 2010). Menopause signals the transition from reproductive to non-reproductive life and is associated with several biological changes to the endocrine system relevant to cardiac health. Age at menopause logically translates to the interval of oestrogen and androgen exposure and is associated with a modest risk difference in CVD (Appelman et al 2015). In a meta-

analysis, age at menopause of less than 50 years was independently associated with 38% higher CVD risk (Atsma et al 2006).

1.4.6 Gender identity in primary CVD prevention

Given that "sex", i.e., a biological dimension and "gender", i.e., a socially constructed dimension, are two different sides of the same coin, they may interact with each other. This time focus will be oriented on the gender-related characteristics. Men and women present different lifestyle, socioeconomic and psychological patterns which are to differentially affect cardiac health (Phillips, 2005).

Unhealthy lifestyle factors (i.e., rich in saturated and trans fatty acids dietary habits, sedentary lifestyle and smoking) remain huge driving forces of CVD onset and progression (Wilkins et al 2017). According to a very recent report from the European Health Network, the attributable disease burden to the aforementioned lifestyle risk factors exceeds 50% (Wilkins et al 2017). Lifestyle has a predominant role on cardiac health. In the INTERHEART global case-control study, unhealthy lifestyle was appeared to collectively account for almost the half of population attributable risk, from an AMI prevention standpoint in both genders (Anand et al 2008).

The healthy or unhealthy lifestyle patterns are highly dependent on gender attitudes and behaviours; "*we born men or women, we progressively become men and women who adapt different behaviours strongly influenced by the social context that ultimately result in the gender identity*" (Phillips et al 2005). Socially constructed attitudes and behaviours promote different patterns of healthy or unhealthy lifestyles between men and women (Phillips et al 2005). In this context, unhealthy behaviours may differentially affect men's and women's CVD risk on the grounds of their social as well as their biological basis (Kouvari et al 2018).

Other than the gender-specific perceptions and norms that interpret a large amount of daily behaviours in men and women, the actual CVD effect of these factors may be mediated by sex-driven biological mechanisms (Kouvari et al 2018, Agushi et al 2013). Under this context, lifestyle and other social features may exert their protective or aggravating CVD effect, through their acting as epigenetic factors; affecting the expression of oxidative stress-, inflammation- and insulin resistance- related genes (Whayne 2015). Numerous studies have tried to address potential sex differences on various aspects of CVDs as well as CVD risk factors (e.g. the excess risk of smoking in women is driven by women's greater absorption of toxic chemicals) (Kostapanos et al 2013); nevertheless, their interpretation is challenging and the hitherto evidence conflicting.

1.4.6.1 Lifestyle factors

Nutrition

Women are more likely to adhere to healthy dietary habits compared with men. In accordance with this generally accepted claim, in the Global Burden of Disease study dietary risks defined as diet high in sodium and low in fruits accounted for 12.2% in men and 9.0% in women of their total disability adjusted years of life, for 2015 (GBD 2015). Women usually relate a healthy diet with healthy weight maintenance and consequently with better body image, while appearance is always being recognized as a motivation to avoid unhealthy habits (Hattori et al 2017). On the contrary, it is documented that men are more likely to have poor dietary habits and alcohol overconsumption. In CARDIO2000 case-control study, the effect of Mediterranean diet on ACS onset likelihood was higher in women compared to men (Chrysohoou et al 2003). In the INTERHEART study, the effect of a "high risk" diet was as well slightly higher in women; yet in that case, "high risk" diet was defined, by the contributors of the study, only according to fruits and vegetables consumption instead of a holistic dietary pattern (**Figure 1.13**) (Anand et al 2008).

Physical activity

Women have lower rates of physical activity compared to men, with a possible explanation the social context under which they are motivated to participate in physical activities (Yahia et al 2016, Ashida et al 2012, van Uffelen et al 2017). In particular, women's role in both workplace and household tasks, within the recent decades, may lead to a lack of engagement in leisure time physical activities (Hattori et al 2017).

Smoking

It is a general belief that men are more prone to unhealthy habits and the example of active smoking is the most highlighted. Recent meta-analyses about the gender-specific effect of smoking on first CVD event suggested a higher effect size in women with CHD as main outcome (**Figure 1.20**) (Huxley et al 2011); women-smokers had 25% higher CHD risk compared with their men counterparts. No significant differences were observed for stroke (Peters et al 2013a).

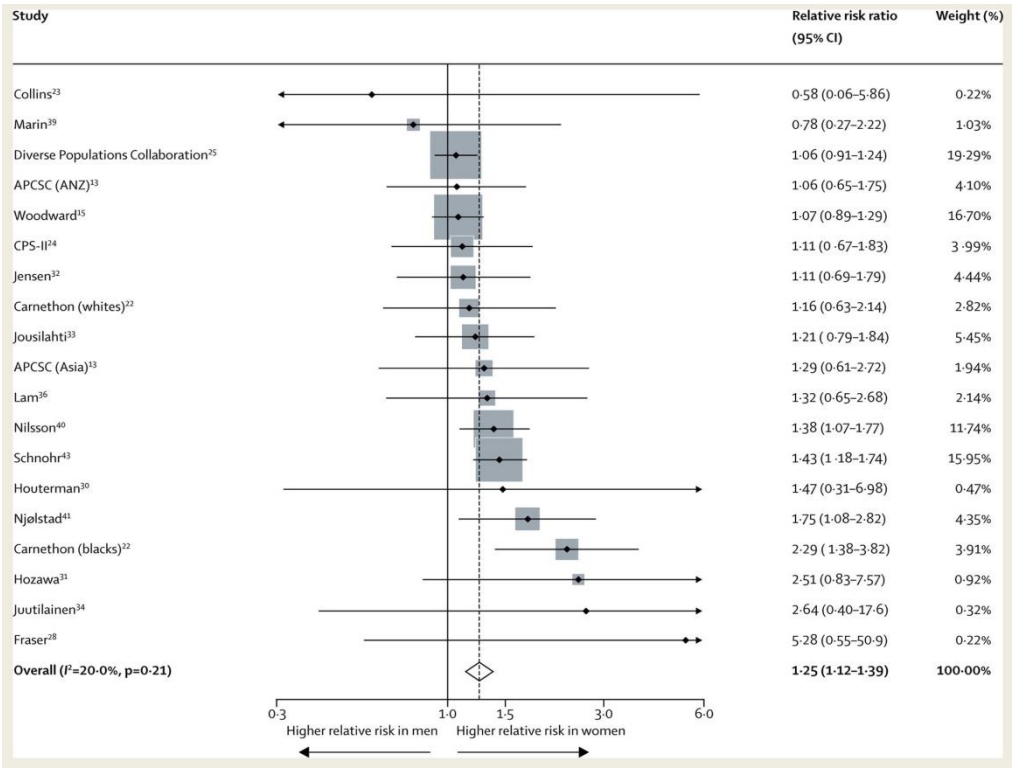

Figure 1.20 Multiple-adjusted female-to-male relative risk ratios for coronary heart disease, smoking compared with not smoking. **Source:** Lancet. 2011;378(9799):1297-305.

Subsequently, the positive effect of smoking cessation was similar in both genders with a small precedence of men (**Figure 1.21**) (Huxley et al 2011).

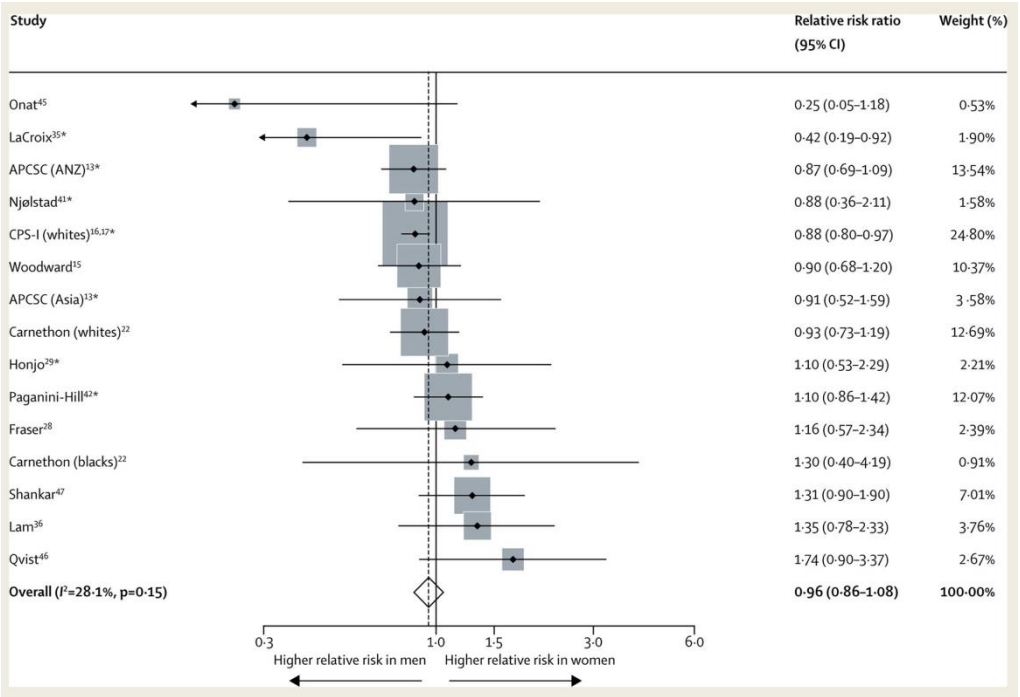

Figure 1.21 Female-to-male relative risk ratios for coronary heart disease, ex-smoking compared with never-smoking **Source:** Lancet. 2011;378(9799):1297-305.

The mechanism though which smoking may have a more aggravating effect on women probably is the higher nicotine metabolism as well as the greater difficulty in quitting it (Huxley et al 2011, Peters et al 2013, Smith et al 2016).

1.4.6.2 Psychological factors

Depression and CVD are the two main causes of disability in high income countries and is to become so for all income levels till 2030 (WHO 2008). It is well-established that depression is more prevalent in women. This has been highly attributed to psychosocial stressors, including low income and high unemployment rates (Lee et al 2017). INTERHEART is one of the few large-scale studies suggesting a higher magnitude of psychosocial factors-AMI association in favour of women (**Figure 1.13**) (Anand et al 2008). An updated meta-analysis revealed that subjects with depressive symptomatology had 1.3 times higher risk for coronary heart disease and AMI (Gan et al 2014). Beyond this pooled effect, that meta-analysis highlighted a remaining literature gap, related to sex-depressive symptoms interaction on CVD onset. Although men-to-women first-CVD relative risk ratio for depressive symptoms was 1.20 [men exceeded women], only eight out of twenty-four studies provided separate data for women while more than half of them presented men-specific outcomes; this is probably a bias from a sex-related standpoint which seems more evident in European populations (Gan et al 2013). Very few studies, after that meta-analysis, prospectively examined the sex-specific effect of depressive symptoms on CVD onset. Results are mixed ranging from a higher risk in women (Butnoriene et al 2015, Kozela et al 2016) to similar risk between sexes (Mejía-Lancheros et al 2014, Daskalopoulou et al 2016). Currently, Framingham study revealed an increase in predictive ability of Framingham Risk Equation in women after depressive symptomatology addition to the risk-assessment model (O'Neil et al 2016).

1.4.7 The role of sex/gender in secondary CVD prevention

Women suffering from a cardiac episode have significantly worse prognosis compared with men, with high short-term mortality rates (i.e. in hospital death, death within the first year after the episode) while they usually report low health related quality of life. Major challenges still exist in the secondary CVD prevention spectrum (Panagiotakos et al 2007b, Piepoli et al 2015).

As previously reported in the present manuscript, disease manifestation presents significant differences between sexes. An AMI is usually manifested with atypical symptoms and signs in case of women; younger women usually reports absence of chest pain/discomfort. This is an important barrier for the patient and its relatives to optimally recognize the episode leading to their delay in seeking for medical care and reducing the likelihood for timely management;

woman is to arrive 1 hour later at hospital than a man (Ferrari et al 2013). Another challenging issue has to do with the diagnostic criteria of a cardiac episode. In the AMI diagnosis the absence of sex-specific standards seems quite problematic. In this case, troponin I over a specific limit has been set as an important clinical criterion for definitive diagnosis. However, women are usually unlikely to reach this cut-off point being exposed to underdiagnosis. Even if the biological basis is not understood, several large-scale studies have recently suggested the high sensitivity cardiac troponin as a better diagnostic criterion for women. Hence, the available standards need to be better clarified for this group (Gulati et al 2012).

CVD rehabilitation program still remains challenging for health professionals (Crea et al 2015). The actual benefit of CVD interventions is debated especially for women. More specifically, the biological variances between sexes are justifiably associated with different pharmacokinetics (i.e. bioavailability, clearance, urinary excretion, plasma binding proteins, fat distribution). The appropriate type of active substance included in an agent along with the ideal dosage and duration of therapy may be different between men and women and possess a detrimental role in the effectiveness and mainly safety of the prescribed medicine (Garcia et al 2016). Moreover, there are disease manifestations (i.e. microvascular dysfunction, non-obstructive CHD) frequently presented in women, yet still inadequately understood. Currently, the case of heart failure with preserved ejection fraction management has become a very active area of research (Oren and Goldberg 2017).

Established clinical risk factors after a cardiac episode and their effect on the risk of rehospitalization, recurrent cardiac fatal/non-fatal episodes and unplanned revascularization have been inadequately investigated; even more when it comes to a sex-oriented approach. Clinical risk factors such as obesity, hypertension and diabetes seem more prevalent in women compared with men (Panagiotakos et al 2007b; Notara et al 2016a). Abnormal glycaemic status has a more aggravating effect on women's prognosis, as revealed in the recent literature (Liang et al 2014; Lin et al 2013). In women, a paradoxical association between BMI and CVD prognosis, yet with inconclusive evidence has been suggested (Kouvari et al 2017b). Depression, anxiety and stress are strongly underscored as factors with high prognostic ability in secondary prevention spectrum, especially in women; in the EUROASPIRE III these psychosocial parameters were highly associated with increased BMI, abnormal glycaemic status and poor quality of life probably due to their potential to discourage patients' adherence to recommended medical treatment and lifestyle modifications (Notara et al 2015, Notara et al 2016a, Notara et al 2016b, Pajak et al 2013). Considering the large economic and disability burden, a better clarification of such issues, separately for men and women, will contribute to the development of a post-

discharge screening system which will orient the specific targets and the intensity of the applied rehabilitation program.

1.5 Sex and cardiovascular disease prevention: policies and practices around the globe

1.5.1 Sex-specific reporting in global health agenda: policies and practices

The initiatives regarding the consideration of sex in global initiatives are summarized in **Table 1.1**. A reveal from the NIH was among the very first references regarding this issue; in 1994 a policy-guidance regarding the consideration of women in clinical trials was published (NIH 1994). Since then some updated versions were revealed in 2000 and 2001 (NIH 2000, NIH 2001). In 1995, the UN in a report from the fourth world conference on women, underscored the fact that a policy decision should be published only after a sex-specific evaluation regarding its effectiveness (UN 1995). Additionally, it has been about two decades since when the FDA established the Office of Women's Health (FDA OWA) targeting the need to manage the underrepresentation of women in general and women in special occasions (e.g. pregnancy) from medical clinical trials. In this context, in 1993 a guidance for researchers towards this issue was published, while in 2014 the FDA published an action plan to address the collection and availability of subgroup data, including the sex as strata (Federal Register: Department of Health and Human Services 1993, FDA 2014). The books "*Exploring the Biological Contributions to Human Health; Does Sex matter?"* and the *"Women's Health Research; Progress, pitfalls and Promise"* published by IOM in 2001 and 2010 respectively, were milestones for global health research and policy-making and one of the very first official recognitions of sex (i.e. the biological dimension) and gender (i.e., the socially constructed dimension) as variables with critical health impact, interacting with each other (IOM 2001, IOM 2010). Since then, in 2010, the Canadian Institutes of Health Research and its Institute of Gender and Health subdivision revealed a user-friendly tool for health researchers regarding the integration of sex and gender in their study design (CIHR Institute of Gender and Health 2010). In 2014, the WHO provided guidance on the integration of gender-responsive sustainable approaches and promoted disaggregated data analysis and health inequality monitoring (WHO 2015b). In 2015, the NIH with the notice *"NOT-OD-15-102"* required from investigators sex-specific reporting in any kind of study or an evidence-based justification in case of its omission (NIH 2015). At the same time, the UN underscored the necessity for gender-sensitive strategies in all sustainable development goals for 2030, while the 5th sustainable goal was incorporated to "achieve gender equality and empower all women and girls" (UN 2015a). In 2015 the League of European Research

Universities published a list of recommendations for universities, governments, funders and peer-reviewed journals to adopt strategies and policies towards a gendered research and innovation approach (LERU 2015). The Lancet Commission on Women and Health in 2015 recognized women's health as a key for sustainable development (Langer et al 2015). In 2016, the report "Women's Health: a new global agenda" provided a redefinition of the women's health agenda setting different priorities according to the reality depicted by the disease epidemiological data around the globe (Norton et al 2016). In this context, an updated version of the "Global Strategy for Women's, Children's and Adolescents' Health" (2016-2030) was launched by the WHO-UN Secretary General partnership; the roadmap report provided recommendations to diminish all preventable deaths in women, children and adolescents making a commitment to one-third reduction in premature NCDs mortality till 2030 (UN 2015b). At the same period of time, the European Association of Editors published a set of guidelines for reporting of "Sex and Gender Equity in Research (SAGER)" to provide to researchers and authors a tool to achieve sex- and gender- standardization in scientific publications while the European Commission in the context of the Horizon 2020 programme published a guidance to address the gaps in the participation of women in the Framework Programme's projects and to achieve a gender balance in the produced knowledge, innovation and technology (De Castro et al 2016, EC 2016).

Table 1.1 Global policies and practices to address gender disparities in health with scientific community as target audience

Organization	Year	Manuscript title	Manuscript main content
Food and Drug Administration (FDA)	1993	Guideline for the study and evaluation of gender differences in the clinical evaluation of drugs; Notice	Guidance on the consideration and evaluation of discrepancies of medicines effectiveness and appropriateness separately in men and women
National Institutes of Health, (NIH)	1994	NIH guidelines on the inclusion of women and minorities as subjects in clinical research – Federal Register Notice	Guidance on the inclusion of women and minorities in research
United Nations (UN)	1995	Report of the Fourth World Conference on Women.	The impact on women and men should be separately analysed, prior to the policy making
National Institutes of Health, (NIH)	2000	Notice "NOT-OD-00-048"	NIH guidelines on the inclusion of women and minorities as subjects in clinical research – Guide to Grants and Contracts – Update version

Institute of Medicine (IOM)	2001	Exploring the Biological Contributions to Human Health; Does Sex matter?	This book explores the health impact of sex and gender from behavioural characteristics to genetic and metabolic features
National Institutes of Health, (NIH)	2001	Notice "NOT-OD-02-001"	NIH Policy and Guidelines on The Inclusion of Women and Minorities as Subjects in Clinical Research: this update version provides additional recommendations on reporting, among others sex/gender discrepancies in the effects of interventions evaluated in the context of NIH-defined Phase III clinical trials
Institute of Medicine (IOM)	2010	Women's Health Research; Progress, pitfalls and Promise.	In this book policies and regulations launched to support women's health on community basis are discussed, highlighting their strengths and weaknesses
Canadian Institute of Health Research (CIHR)	2010	Sex, Gender and Health Research Guide: A Tool for CIHR Applicants	A guide to enhance all research applicants towards the integration of sex and gender in research designs
Food and Drug Administration (FDA)	2014	FDA Action plan to enhance the collection and availability of demographic subgroup data	Action plan to highlight the need of adequate representation and health data analysis of subgroups including among others both sexes
World Health Organization (WHO)	2015	Integrating equity, gender, human rights and social determinants into the work of WHO. Roadmap for Action (2014-2019).	a. guidance on the integration of gender responsive sustainable approaches in WHO programmes, institutional mechanisms and in national basis; b. enhance sex- and gender disaggregated data analysis and health inequality monitoring
National Institutes of Health (NIH)	2014	Notice "NOT-OD-14-085"	Transition Plans for Reporting Sex/Gender, Race, and Ethnicity Information in Non-Competing Type 5 Progress Reports
National Institutes of Health (NIH)	2015	Notice "NOT-OD-15-102"	NIH recommends that sex as a biological variable should be taken into serious consideration in research designs, analyses, and reporting in vertebrate animal and human studies. Strong evidence-based justification from the scientific literature, preliminary data, or other relevant considerations must be provided in case of applications where only the one sex is studied

United Nations (UN)	2015	Global Strategy for Women's, Children's and Adolescents' Health (2016-2030)	An update version of the "Global Strategy for Women's, Children's and Adolescents' Health (2010-2015): The strategy outlined what countries and health partners need to do to end "all preventable deaths of women, children and adolescents by 2030 and improve their health". A commitment was made to a one-third reduction in premature mortality from NCDs and to the promotion of mental health and wellbeing.
United Nations (UN)	2015	Sustainable Development Goals.	In all SDGs, terms such as "gender equality" and "gender-sensitive" strategies are underscored. Goal 5: "Achieve gender equality and empower all women and girls"
League of European Research Universities (LERU)	2015	Gendered research and innovation: integrating sex and gender analysis into the research process	This guidance provides a list of recommendations for universities, governments, funders and peer-reviewed journals to adopt strategies and policies towards a gendered research and innovation approach.
Lancet Commission on Women and Health	2015	Women and health; the key for sustainable development	This manuscript highlights health challenges that women share with men, yet with manifestations that affect women disproportionally owing to biological and environmental determinants
European Commission	2016	H2020 Programme. Guidance on Gender Equality in Horizon 2020.	This guidance provides recommendations to foster gender balance in Horizon 2020 research teams, to prevent underrepresentation of women and to integrate sex- and gender- specific analysis in research and innovation content aiming at ameliorating the scientific quality and the relevance of the produced knowledge
Oxford Martin School	2016	Women's Health; a new global agenda	A redefinition of women's health agenda, setting additional priorities, other than the women's sexual and reproductive health.
European Association of Editors	2016	Sex and Gender	A tool for researchers and

	Equity in Research (SAGER) guidelines	authors to standardize sex and gender in scientific publications in 4 levels: topic of the study, sex- and gender-disaggregated data report, study design, discussion/limitations

1.5.2 Women-cantered policies and practices in global CVD spectrum

The initiatives regarding the consideration of sex in global CVD spectrum are summarized in **Table 1.1**. In 1986, a workshop was converted by the NIH NHLBI to lay the groundwork for researchers and clinicians to perform endeavours towards the "cardiovascular disease in women" field, while in 1987 the key highlights of the workshop were summarized in a report being available in the scientific world (*Coronary Heart Disease in Women: Reviewing the Evidence, Identifying the Needs*). This was the very first initiative that emerged the "female heart" from the shadows (Eaker et al 1987, Hayes et al 2015).

1.5.2.1 Cardiovascular disease prevention and management guidelines in United States of America

The "Guide to Preventive Cardiology for Women" was the first official report with some recommendations regarding the CVDs prevention and management in women focusing on female-specific factors and medical treatments (e.g. hormone replacement therapy) conducted by AHA (Mosca et al 1999). However, the first set of evidence-based women-cantered guidelines regarding the primary and secondary prevention of chronic vascular atherosclerotic diseases came in 2004 (Mosca et al 2004a). Since then two updates of this guidance have been published. Initially, the AHA underscored the common misconception that women and men are equal against the disease and challenged the belief that the two sexes should be commonly treated (Mosca et al 2007, Mosca et al 2011b). The highlight of those guidelines was the underrepresentation of women in clinical trials. Since then, health professionals were triggered

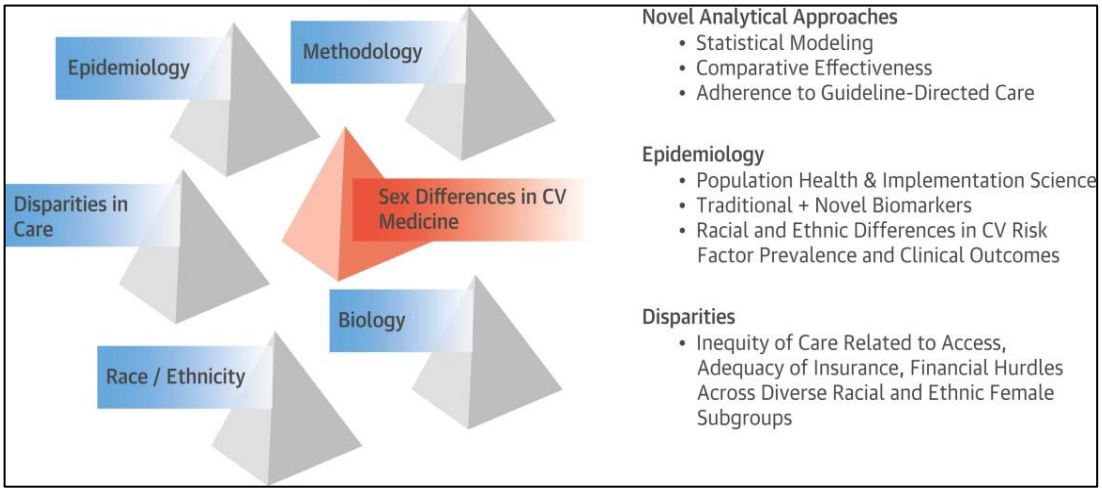

Figure 1.22 Understanding Sex and Gender Differences Requires Novel Methodologic Approaches That Consider Disparities in Care, as Well as Epidemiological and Statistical Limitations Within the Comparative Subgroup of Women Versus Men. **Source:** J Am Coll Cardiol. 2017;70(3):373-388

towards a more sex-specific research approach giving the potential for more definitive recommendations and passing from the evidence-based strategies to the effectiveness-based preventive action plans in 2011; notably, in the last AHA guidelines update, some primary prevention strategies were proved to be inappropriate for women (i.e. aspirin prescription) while it was underscored that women are susceptible to other comorbidities and conditions which multiply their CVD risk and challenge the effectiveness and appropriateness of the hitherto typical prevention and management strategies (Mosca et al 2011b). In addition to this, in 2014 the first set of guidelines related with stroke prevention in women was published by the AHA in collaboration with the American Stroke Association (Bushnell et al 2014).

1.5.2.2 Cardiovascular disease prevention and management guidelines in Europe

In 2008, a short guide called "Assessment and Management of Cardiovascular Risks in Women" was published by a joint workshop under the auspices of the ESC, European Society of Hypertension and International Menopause Society aiming at assisting menopause physicians in contributing to the overall management of women's cardiac health (ESC 2008). In 2011, the ESC published guidelines on the management of CVDs during pregnancy while an updated version was launched in 2018 (ESC 2011, Regitz-Zagrosek et al 2018). In the very recent prevention in clinical practice ESC guidelines some female-specific conditions have been reported (e.g., polycystic ovarian syndrome, pregnancy complications) yet a large scientific gap has been clearly stated; "*The young, women . . . continue to be underrepresented in clinical trials*", "*Information on whether female-specific conditions improve risk classification in women is unknown*") (Piepoli et al 2016).

1.5.2.3 Raising awareness campaigns, workshops, projects and other initiaves

In 2007, the unique aspects of nonclinical factors that affect the health of women, termed the "gendered structural determinants of health" in a 2007 report by the Women and Gender Equity Knowledge Network of WHO, affect the health and outcome of women at risk of or with CVD (WHO 2007). In the "Circulation" Journal, the journal of the AHA, a themed issue focusing on women's cardiac health (i.e. Cardiovascular Disease of women) highlighted major challenges and gaps in the sex- and gender- centered CVD prevention, diagnosis and treatment calling health professionals for additional research (Garcia et al 2015, Blenck et al 2016, Menazza et al 2016). In 2017, a state-of-the art review was published in the Journal of American College of Cardiology which synthesized evidence and discussed issues related to health care quality and equity for women, including minority population subgroups (**Figure 1.22**) as well as methodological issues in clinical research and practice (**Figure 1.23**) (Shaw et al 2017). The same year, the results from a survey conducted by the Women's Heart Alliance were revealed summarizing the knowledge,

perceptions and gaps of women as well as physicians regarding CVD in women (Bairey Merz et al 2017).

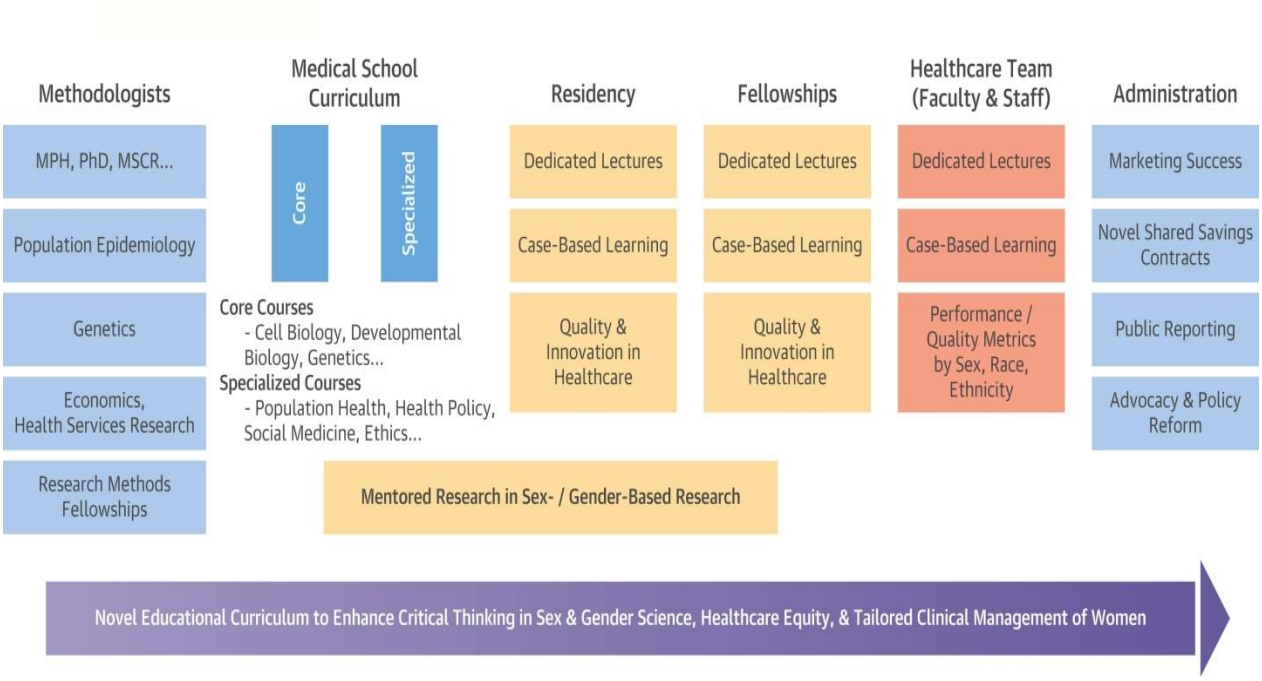

Figure 1.23 Medical Education to Develop Critical Thinking in Sex Biology and Effective Clinical Care of Women**. Source:** J Am Coll Cardiol. 2017;70(3):373-388

The FDA OWA funded projects have contributed to highlight major gaps in the field of CVDs in women (Elahi et al 2016). Nonetheless, apart from the limited number of high-quality sex-specific studies, the AHA recognized another challenge in the field of CVD in women; CVD was the first cause of death in women yet those were unaware of this threat (Mosca et al 2004b). In 2002, NHLBI along with, among others, the AHA teamed up to sponsor the "*Heart Truth*" awareness campaign with the "*Red Dress*" as centerpiece-symbol and the message "*Heart Disease Doesn't Care What You Wear – It's the #1 Killer of Women*" aiming at raising women's awareness regarding their cardiac health and enhancing the knowledge of health professionals and researchers regarding this issue (NHLBI 2016). In 2004 a national campaign in the United States called "Go Red for Women" was launched (AHA 2016). This campaign, which continues till today, includes passionate, emotional and social initiatives designed with a dual avail; **a.** to empower women to take charge of their own cardiac health and **b.** to support health professionals' daily clinical practice. More than one decade later this campaign has moved beyond the borders of the United States in more than 50 countries around the globe (AHA 2016). In 2006, the Society for Women's Health in collaboration with a non-profit organization, called "WomenHeart: The National Coalition for Women with Heart Disease" (http://www.womenheart.org/) revealed the report "10Q Report: Advancing Women's Heart Health through Improved Research, Diagnosis and Treatment" to enhance researchers and health

practitioners towards a female-specific cardiac care (Wenger et al 2013). In 2011, the "Make the Call, Don't Miss A Beat" campaign aimed at educating, engaging and empowering women and their families to recognize the seven symptoms of a heart attack that most commonly present themselves in women. This initiative included a comprehensive public service advertising campaign (AHA 2011).

Another initiative in the CVD spectrum focusing on women's health was the WISEWOMAN (Well-Integrated Screening and Evaluation for WOMenAcross the Nation) program started in 1993 (CDC 2014). This program is administrated CDC and specifically its division for heart disease and stroke prevention. The target group of the WISEWOMAN program is women with low financial status aged 40-64 years. The aim of the program is to provide free of charge heart disease and stroke risk factor screenings, namely blood pressure control, along with evidence-based methods enhancing women's adherence to healthier behaviours, so as to promote lifelong heart-healthy lifestyle changes. The contributors of this program focus on strategies being applicable to both health-care practitioners and on the basis of community targeting from clinicians and pharmacists to farmers' markets which were recently revealed in a technical assistance and guidance document (CDC 2014).

A bill to ameliorate the prevention, diagnosis and treatment of heart disease, stroke and other CVDs in women was introduced in the American House of Representatives in November 2011, called the "Heart Disease, Education, Analysis, Research, Treatment for Women Act" or the "Heart for Women Act" (House of Representatives 2011). This legislation was set to ensure the availability of gender-specific information in medical treatments to health care professionals, researchers and public, to expand the CDC-funded WISEWOMAN project to 20 additional states of America and to require the Secretary of Health and Human Services to perform an annual report for Congress regarding the quality of and access to health care services for women. This bill was highly supported, among other relevant associations and non-governmental organizations (NGOs), by the AHA and its American Stroke Association division (AHA/ASA 2011).

Recently, the Heart Centers for Women were developed as a response to the need for improved outcomes for women with CVD (Lundberg et al 2018). The Heart Centers for Women serve as a point of focus for the development of education and research programs to better address the unique features of CVD in women. The multidisciplinary approach to the care of women with or at risk for CVD has emphasized the importance of developing key clinical benchmarks to allow for standardization of care pathways. Stakeholders in such centers include representatives from internal medicine, family medicine, obstetrics and gynaecology departments, and nursing programs.

The FDA Office of Women's Health offers resources to help women and healthcare providers get informed about heart health including the Heart Health Social Media Toolkit to encourage women to protect their hearts. The toolkit includes resources for 'everyday' women and health professionals, including sample social media messages and blog posts (FDA 2020). What is more, the FDA Office of Women's Health is partnering with the NIH Office of Research on Women's Health to raise awareness about diverse women of different ages, races, ethnic backgrounds, and health conditions participating in clinical trials. The Diverse Women in Clinical Trials Initiative includes a consumer awareness campaign, as well as, resources and workshops for health professionals and researchers (FDA 2019).

In Europe important attempts have been performed towards the reformulation of women's health agenda. In 2004, a workshop was held at the 7th European Policy Forum based on which a report was revealed, underscoring the sex discrepancies in the CVD diagnosis and treatment methods and the challenges on community basis (EHN 2004). Among the very first initiatives of the European Society of Cardiology (ESC) was the "Women at Heart" program launched in 2005 with the aim to coordinate research and educational initiatives regarding CVDs in women (ESC 2004). The program started with a policy conference in June 2005, during which Experts' opinions were selected, the scientific gaps were underscored, and strategic plans were delineated to address this issue (ESC 2004). The highlights of the conference and the state-of-the art in Europe were summarized in a policy statement being available in various languages; in this policy statement a flowchart with synergic actions implemented by the ESC, the EU, the National Scientific Societies and the National Health Authorities at a European level was proposed to enhance researchers and other relevant scientific sectors (e.g., research funders) to cover the gender gaps in CVD investigation field (Stramba-Badiale et al 2006). On the 2005 ESC Congress a thematic subunit was deviated in "CVDs in women", so as to enhance the dissemination of this action in the scientific community while in 2006 an educational course was performed. In National Cardiac Societies (i.e. Swedish Cardiac Society, Polish Cardiac Society) roll outs of the "Women at Heart" program were revealed (Stramba-Badiale et al 2006). Under this perspective, the joint of the European Health Network and the ESC applied for a grant regarding the EuroHeart project, a consortium among 30 partners in 21 European countries (ESC/EHN 2007) among the primary purposes of this project presented in its work package n^{o} 6 was "*to question gender differences in the management of CVDs and consequently provide recommendations for research & regulatory policy makers*" (European Heart Health Strategy 2009). This survey highlighted significant gender biases in the use of investigations and evidence-based medical treatments (Daly et al 2006, Dagres et al 2007). In the context of this work package a report

called “Red Alert on Women’s Hearts” was revealed in November 2009 being available for the scientific community (Maas et al 2011). Additionally, 60 and 15 awareness campaigns for women and their physicians in the participated in the project countries were launched (Maas et al 2011).

The BHF has launched pages (i.e., the “Women’s Room”) in its website aiming at increasing the awareness of women regarding heart disease and stroke, related risk factors and symptomatology of a cardiac episode (BHF 2016). In 2015, the BHF launched a campaign, called “Bag it. Beat it” which continues till today, with the purpose to increase the funding of researches focusing on the prevention, diagnosis and treatment of CVDs in women. In 2010, a 2-day summit (*“Women and Heart Disease: A Summit to Eliminate Untimely Deaths in Women”*) was held in Minneapolis to give straightforward directions towards the focusing areas of strategies and policies to ameliorate the health outcomes of women with heart disease in Minessota (Lindquist et al 2012).

In 2000, the Heart and Stroke Foundation of Canada along with the University of Ottawa and the University of Alberta, revealed a statement report with recommendations concerning the policy development for a healthier female heart, so as to support researchers, health practitioners and policy makers to create a community where women, irrespective of their socioeconomic status, are to receive an effective medical care (Plotnikoff et al 2000). The Heart Institute in the University of Ottawa has launched the “Canadian Women’s Heart Health Centre” (https://cwhhc.ottawaheart.ca/) with the aim to reduce CVDs in women through achieving motivating individuals, health professionals and healthcare workers towards this public health concern. This initiative is disseminated in national events, social media to generate publicity of women’s health. In this context several training workshops have been organized by women having survived from a heart attack so as to motivate other women towards a healthier lifestyle and an adequate preventive medical control to avoid suffering from a heart attack. More specifically, the “Women@Heart” program has a duration of 12 two-hour sessions and it is held biweekly in community settings across the region while a similar program, called “IMPROVE Postpartum Program” has been launched for women having suffered from preeclampsia, eclampsia and gestational hypertension and as a result they are to have a high CVD risk (Canadian Women's Heart Health Centre). National cardiac/heart associations such as the Hellenic Cardiac Society, the Danish and the Italian Heart Foundation had taken small initiatives on this issue (HCS 2008).

In 2011 and 2012 two position statements were revealed by the International Council on Women’s Health Issues (ICWHI), an international non-profit organization aiming at empowering women’s health; in these two statements, the necessity for recognizing and addressing the needs

of women against chronic diseases with the focus oriented towards CVDs was underscored to researchers, health practitioners and policy makers (Davidson et al 2011).

Table 1.2 Global policies and practices to address cardiovascular diseases in women, on scientific and community basis.

Organization	Year	Title of policy/practice	Type of policy/practice	Target audiences
National Institutes of Health's National Heart, Lung, and Blood Institute (NHLBI)	1987	Coronary Heart Disease in Women: Reviewing the Evidence, Identifying the Needs	State-of-the art report	scientific community, policy makers
Centre of Control and Prevention of Disease – division for heart disease and stroke prevention	1993	WISEWOMAN (Well-Integrated Screening and Evaluation for WOMen Across the Nation)	awareness campaign	female citizens
American Heart Association	1999	Guide to Preventive Cardiology for Women	guidelines	scientific community
Heart and Stroke Foundation of Canada, University of Ottawa and University of Alberta	2000	Heart disease and stroke in Canadian women: policy development	position statement	scientific community, policy makers
National Heart, Lung and Blood Institute	2002	Heart Truth	awareness campaign	female citizens
Danish Heart Foundation	2003	Mind Yourself Woman!	awareness campaign	female citizens
European Health Network, European Health Management Association and Bristol-Myers Squibb	2004	Workshop from the 7th European Policy Forum: A healthy heart for European women	state-of-the art report	scientific community, policy makers
American Heart Association	2004	Evidence-Based Guidelines for Cardiovascular Disease Prevention in Women	guidelines	scientific community
American Heart Association	2004	Go Red for Women	awareness campaign	female citizens, scientific community, policy makers
European Society of	2005	Women at Heart	scientific community motivational program	scientific community

Cardiology				
European Society of Cardiology	2005	Cardiovascular diseases in women: a statement from the policy conference of the European Society of Cardiology	position statement	scientific community, policy makers
European Society of Cardiology & European Health Network	2006	EuroHeart project Work Package no 6: “to question gender differences in the management of CVDs and consequently provide recommendations for research & regulatory policy makers”	transnational project for prevention and management of cardiovascular diseases	scientific community, policy makers
WomenHeart: The National Coalition for Women with Heart Disease	2006	10Q Report: Advancing Women’s Heart Health through Improved Research, Diagnosis and Treatment	position statement	scientific community, policy makers
American Heart Association	2007	Evidence-Based Guidelines for Cardiovascular Disease Prevention in Women: 2007 Update	guidelines	scientific community
Hellenic Cardiac Society	2008	National Action Plan for Cardiovascular Diseases (2008-2012)	national action plan	scientific community, policy makers
European Society of Cardiology, European Society of Hypertension and International Menopause Society	2008	Assessment and Management of Cardiovascular Risks in Women	guidance	health professionals
European Society of Cardiology & European Health Network	2009	Red Alert for Women’s Hearts	report in the context of EuroHeart project	scientific community, policy makers

American Heart Association	2011	Evidence-Based Guidelines for the Cardiovascular Disease Prevention in Women: 2011 Update	guidelines	scientific community
American House of Representatives	2011	Heart Disease, Education, Analysis, Research, Treatment for Women Act" or the "Heart for Women Act"	legislation	scientific community, policy makers
International Council on Women's Health Issues	2011	The health of women and girls determines the health and well-being of our modern world: A white paper from the International Council on Women's Health Issues	white paper	scientific community, policy makers
Minneapolis Heart Institute, the Minneapolis Heart Institute Foundation and the University of Minnesota	2012	Women and Heart Disease: A Summit to Eliminate Untimely Deaths in Women	report from a 2-day summit	scientific community, policy makers
International Council on Women's Health Issues	2012	Improving women's cardiovascular health: a position statement from the International Council on Women's Health Issues.	position statement	scientific community, policy makers
European Society of Cardiology	2011	ESC Guidelines on the management of cardiovascular diseases during pregnancy: the Task Force on the Management of Cardiovascular Diseases during Pregnancy of the European Society of Cardiology (ESC)	guidelines	scientific community
Centre of Control and Prevention of Disease – division for heart disease and stroke prevention	2014	WISEWOMAN (Well-Integrated Screening and Evaluation for WOMen Across the Nation): Technical Assistance and	guidance	scientific community, policy makers

		Guidance Document		
British Heart Foundation	2015	Bag it. Beat it	awareness campaign	female citizens
Heart Institute in the University of Ottawa / Canadian Women's Heart Health Centre	2016	Women@Heart	peer support program	female citizens
Heart Institute in the University of Ottawa / Canadian Women's Heart Health Centre	2016	IMPROVE Postpartum Program	peer support program	female citizens

2 Scope and Objectives

2.1 General scope

The general scope of the present Ph.D. thesis was to evaluate the existence of sex-specific associations of lifestyle, psychological, clinical and biochemical factors with long-term CVD onset (1st fatal/non-fatal CVD event) (*primary prevention*) and recurrence (2nd or multiple fatal/non-fatal CVD event) (*secondary prevention*) using the samples of two large-scale epidemiological studies from Greece.

2.2 Specific objectives

- Estimation of the magnitude of association between conventional CVD risk factors and first or recurrent CVD events, separately for men and women suggesting potential underlying mechanisms.
- Development of research hypotheses on non-conventional or novel CVD risk factors in relation to first or recurrent CVD events based on recent literature reveals, highlighting sex-specific conclusions.
- Implementation of sex-oriented microsimulation scenarios for more effective primary and secondary CVD prevention and generation of sex-specific highlights for exploitation in policy making.

3 Materials and Methods

3.1 Sampling procedure

3.1.1 ATTICA study

ATTICA study is a prospective, observational cohort, established in 2001. The principal goal of the study was to investigate the prevalence of CVD risk factors, their associations with various socioeconomic, lifestyle and psychological characteristics and their prognostic value on CVD incidence (Pitsavos et al 2003). During 2001-2002 in the greater metropolitan Athens area (including 78% urban and 22% rural regions) *n*=3,042 apparently healthy volunteers agreed to participate (75% participation rate of the *n*=4056 participants initially approached); no significant differences regarding basic sociodemographic characteristics were observed between participants and non-participants (*all p's>0.05*). Of the enrolled patients, *n*=1,514 (50%) were men (46±13 years) and n=1,528 (50%) were women (45±14 years). During the baseline examination, a detailed clinical evaluation was performed by the study's physicians; all participants were free of CVD and other chronic diseases as per the protocol of the study.

3.1.2 GREECS study

The GREECS study is a prospective, observational cohort study, established in 2003. The main goal of the study was to evaluate the annual incidence of ACS and the role of various CVD risk factors on the development and prognosis of patients with established CVD (Pitsavos et al 2005). From October 2003 to September 2004, *n*=2,172 consecutive patients with discharge diagnosis of ACS (i.e., AMI or UA) that were hospitalized in the cardiology clinics or the emergency units of 6 major General Hospitals in Greece were enrolled in the study (participation rate varied 80-95% of the initially approached consecutive ACS patients). The particular hospitals were selected in order to represent populations in terms of different socio-economic, cultural and regional characteristics. Of the enrolled patients, *n*=1,649 (76%) were men (65 (13) years) and *n*=523 (24%) were women (62 (11) years). ACS was defined based on discharge diagnosis and following the guidelines given by the European Society of Cardiology (ESC/ACC 2000).

3.2 Bioethics

The ATTICA study was approved by the Bioethics Committee of Athens Medical School and the GREECS study was approved by the Medical Research Ethics Committee of the participated Institutions. Both studies were carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All participants were informed about the aims and procedures of the study and signed an informed consent.

3.3 Measurements at baseline examinations

In both cohorts, baseline examination included a range of participants' clinical, anthropometric, biochemical, socio-demographic and behavioural/lifestyle characteristics. It has to be noted here that similar methods, tools and criteria were used for the vast majority of measurements in both studies; all the exceptions are clarified in the following text.

3.3.1 Socio-demographic characteristics

Sociodemographic characteristics studied at baseline included among others; age, sex, educational and financial status. Educational level was measured in years of school. Financial status was defined according to the mean annual income during the last three years recorded through self-reports. Regarding people who were unemployed, the basic monthly allowance they received from the Social Service Office was considered to calculate annual income. Financial status was then classified as low/moderate (annual income <12.000€) and good/very good (>12.000€), following the tax policy of the Ministry of Economics.

3.3.2 Lifestyle characteristics

3.3.2.1 Dietary habits and level of adherence to Mediterranean diet

Dietary habits of the ATTICA study participants were evaluated through a semi-quantitative food-frequency questionnaire (FFQ), originally developed for the European Prospective Investigation into Cancer and Nutrition study and provided by the Unit of Nutrition of Athens Medical School in its Greek version (Katsouyanni et al 1997). Similarly, a validated FFQ was used to assess dietary habits of the GREECS study patients during the baseline examination. In both studies, participants' level of adherence to Mediterranean diet was evaluated through the MedDietScore (range 0-55) (Panagiotakos et al 2006, Panagiotakos et al 2007a); higher values of this score indicate greater adherence to the Mediterranean diet.

3.3.2.2 Physical activity status

In both studies, participants' physical activity status was evaluated using the translated and validated for the Greek population, short version (9 items) of the "International Physical Activity Questionnaire" (IPAQ) that follows the instructions given by the American College of Sports Medicine (Papathanasiou et al 2009). According to the reported physical activities, patients were classified into four categories: inactive, low (i.e., <150 metabolic equivalent – MET-minutes/week), moderate (150-300 MET-minutes/week), Healthy Engaged Physically Active – HEPA (>300 MET-minutes/week) (Papathanasiou et al 2009). In this work participants were classified into two main categories, inactive (sedentary) and physically active.

3.3.2.3 Smoking status

Participants' smoking habits were evaluated during the interview at enrolment through pack-years of smoking (a pack year was defined as twenty cigarettes smoked every day for one year). In both studies, current smokers were defined as those who reported smoking at least one cigarette or any type of tobacco per day at the time of the interview. Former smokers were defined as those who previously smoked but had quitted within the previous year before enrolment. Current and former smokers were combined as ever smokers. The rest of the participants were defined as non-smokers.

3.3.3 Psychological metrics

In ATTICA study, *n*=853 free-of-CVD participants underwent psychological evaluations through validated, self-reporting questionnaires. Depressive symptomatology was assessed using ZDRS (range 20–80). The validated-for-Greek population ZDRS cut-off score of 45 was used to dichotomize the sample to participants with (ZDRS≥45) and without (ZDRS<45) depressive symptomatology (Fountoulakis et al 2001). Anxiety symptomatology was assessed through the STAI (range 20-80) (Fountoulakis et al 2006). Subsequently, *n*=2,172 ACS patients from GREECS study were assessed for depressive symptomatology through CES-D scale (range 0–60), validated for Greek population. Because of non-available national thresholds for the reference population, patients were divided in three equal size categories (tertiles): (a) 1st tertile (CES-D<7), normal (b) 2nd tertile (7<CES-D<20), mild to moderate symptoms and (c) 3rd tertile (CES-D>20) severe symptoms (Notara et al 2016). The 2nd and 3rd tertile were merged and examined against the 1st.

3.3.4 Anthropometric characteristics

In both studies, weight status was defined using the BMI cut off points recommended by WHO (WHO 1998). BMI was calculated as weight (in kilograms) divided by height (in meters squared). Height was measured to the nearest 0.5 cm, with participants not wearing shoes, their backs square against the measuring wall tape, eyes looking straight ahead, with a right-angled triangle resting on the scalp and against the wall. Weight was measured with a lever balance, to the nearest 100 grams, without shoes and in light undergarments. Normal weight was defined as BMI between 18.5 and 25 kg/m^2, overweight as BMI between 25 and 29.9 kg/m^2 and obesity as BMI≥30 kg/m^2. Underweight was defined as BMI <18.5 kg/m^2.

3.3.5 Family and individual CVD history

Family history of CVD was recorded based on the medical history of first-degree relatives (biological parent, or brother, or sister). For the GREECS participants (i.e. ACS patients), the

baseline individual CVD history was also recorded; patients who had at least one cardiac episode – prior to the baseline episode – in their medical history were assigned to the group of positive individual CVD history, while the rest were defined as first diagnosed ACS patients.

3.3.6 Clinical characteristics

Baseline assessment of clinical characteristics, i.e., history of hypertension, dyslipidaemia and diabetes mellitus, was based on the information retrieved through established physical examination procedures, as well as participant's medical records regarding pharmaceutical treatment. Hypertension was defined as systolic blood pressure≥130mmHg and/or diastolic blood pressure≥85mmHg. Dyslipidaemia was defined as triglyceride levels≥150mg/dL and/or high-density lipoprotein levels <40 mg/dL in men and <50 mg/dL in women. Glycaemic abnormalities were defined as fasting glucose ≥100 mg/dL. Drug treatments for the aforementioned conditions were set as alternative indicators of metabolic abnormalities. Metabolic status was defined using the criteria suggested by *Lavie and colleagues* (Lavie et al 2018). In particular, healthy metabolic status was defined as absence of hypertension, dyslipidaemia and glycaemic abnormalities.

3.3.7 Biochemical measurements

Biochemical evaluation was carried out in the biochemistry laboratory of the First Cardiology Clinic of University of Athens School of Medicine, following the criteria of the World Health Organization Reference Laboratories. CRP was assayed by particle-enhanced immunonephelometry by a BNII Dade Behring automatic nephelometry. The intra- and interassay coefficients of variation for CRP were <5%. Blood glucose levels (mg/dl) were measured immediately with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA).

Only in case of ATTICA study, several additional metrics were performed. Beyond CRP other inflammation/coagulation markers (serum amyloid A, plasma fibrinogen, homocysteine, TNF-a, WBC, Il-6, Il-10) were evaluated. Serum insulin concentrations (μU/ml) were assayed by a radioimmunoassay (RIA100, Pharmacia Co., Erlangen, Germany). Precision was 12% for low (3 μU/ml) and 5% for high (90 μU/ml) serum levels. The intra-assay coefficient of variation was 9% and the limit of detection was 3 μU/ml. Insulin resistance was assessed by the calculation of the HOMA-IR: glucose (mmol/L) × insulin (μU/mL)/22.5. ALT and AST were measured using a chromatographic enzymic method in an automatic analyzer (RA-1000, Dade Behring, Marburg, Germany). Total adiponectin and leptin were measured by ELISA in duplicate using serum samples (R & D Systems Inc., Minneapolis, Minnesota). Serum TC, HDL-C and TAG were measured using a chromatographic enzymic method in a Technicon automatic analyzer RA-1000 (Dade Behring, Marburg, Germany). ApoA1 and ApoB100, as well as, Lp(a) were measured by a

latex enhanced turbidimetric immuno-assay. HDL-C was determined after precipitation of the ApoB100 containing lipoproteins with dextran-magnesium-chloride. Non-HDL-C was calculated by the formula: TC minus HDL-C. LDL-C was calculated with the Friedewald formula: {TC} - {HDL-C} - 1/5 (TAG). Lp(a) was measured by a latex enhanced turbidimetric immuno-assay. SUA was measured only at baseline in mg/dL (1 mg/dL = 59.48 μmol/L) using an enzymatic colorimetric test with the uricase-peroxidase method (UA plus, Roche Diagnostics, Mannheim, Germany). The measuring range was 0.2-25 mg/dL (0.01-1.49 mmol/L) and the inter- and intra-assay variability was 0.5 and 1.7%, respectively. Creatinine and urea were measured in serum, using a colorimetric method (BioAssay Systems, Hayward, CA). Renal function was assessed by the Cockcroft and Gault formula: CrCl = [(140 - age) × weight]/(72 × serum creatinine)] for men, whereas for women, the result of the above equation was multiplied by 0.85.

3.4 Endpoints and Follow-up evaluation

3.4.1 Follow-up in ATTICA study

The 10-year (2002-2012) follow-up of the ATTICA study participants was performed in 2012; in particular, *n*=2,020 participants of the *n*=3,042 enrolled, were found during the follow-up (66% participation rate). The rest *n*=1,022 (34%) of the participants that were lost in the follow-up were considered as censored (no differences were observed as regards social and clinical characteristics between those who lost to follow up and the rest).

3.4.2 Follow-up in GREECS study

The 10-year follow-up (2004-2014) of the GREECS study patients was performed in 2014; of the *n*=2,172 enrolled ACS patients, *n*=1,918 were found at the follow-up (88% participation rate). The rest *n*=254 (12%) of the patients that were lost in the 10-year follow-up were considered as censored (no differences were observed as regards social and clinical characteristics between those who lost to follow-up and the rest).

3.4.3 Endpoints

The combined endpoint studied in this work was the development of a fatal or non-fatal CVD event during the 10-year follow-up; specifically, a first event, for the ATTICA study apparently healthy participants and recurrent event, for the GREECS study ACS patients. A CVD event was defined as the development of: AMI, or angina pectoris, or other identified forms of ischemia (WHO-ICD coding 410-414.9, 427.2, 427.6), or heart failure of different types and chronic arrhythmias (WHO-ICD coding 400.0-404.9, 427.0 -427.5, 427.9-) or the development of stroke (WHO-ICD coding 430-438). For the participants who died during the follow-up, the information achieved from their relatives, as well as death certificates. As regards individuals who might first

suffered from stroke and then had coronary heart disease, it was a-priori decided the first outcome to be considered as the end-point, but also to record the consequent event for further testing of competing risks (however, there were no such cases in the sample).

3.5 Statistical Analysis

3.5.1 Descriptive statistics

Categorical variables are presented as absolute (n) and relative frequencies (%). Continuous variables are presented as mean values ± standard deviation. Associations between normally distributed variables and categorical variables were evaluated through one-way analysis of variance or Student's t-test for independent samples. Normal distribution of continuous variables was tested through P-P plot and equality of variances through Levene's test. For non-normally distributed variables, Kruskal-Wallis and Mann-Whitney tests were used. Associations among categorical variables were tested with the chi-squared test.

3.5.2 Multivariable analysis

To evaluate the magnitude of the association between categorical or continuous variables and the outcome of interest multivariable logistic or Cox regression analysis was performed. HRs and their corresponding 95%CIs for categorical or continuous variables in relation to the outcome of interest were evaluated through multivariable Cox-regression analysis. Proportional hazards' assumption was graphically tested. ORs and their corresponding 95%CIs for categorical or continuous variables in relation to the outcome of interest (e.g., 10-year CVD event) were evaluated through multivariable logistic regression analysis. Interactions between groups of participants were tested, and when significant (*p for interaction<0.05*) or borderline significant (*p for interaction<0.10*) the analyses were further stratified. STATA software, version 14 (MP & Associates, Sparta, Greece) was used for all statistical analyses.

3.5.3 Discriminant analysis

Discriminant analysis was performed to reveal the risk factors that had the best discriminative ability against the examined endpoint according to the Wilks' Lambda (Λ) (the lower the better discriminating ability).

3.5.4 C-statistics

The concordance statistics i.e. C-statistics was used to evaluate the discriminative accuracy of multivariable models against the examined endpoint. C-indices and the corresponding 95%CIs were equal to the areas under the curve obtained from the ROC analysis. Curves were constructed by plotting sensitivity against 1-specifity.

3.5.5 Multivariate analysis

FA using the extraction method of Principal Components was applied. To ensure suitability for conducting factor analysis, the Kaiser–Mayer–Olkin test and Bartlett's test of sphericity were used. In order to determine the number of patterns to be kept the criteria of eigenvalues >1.0 and the 'elbow' of the scree plot were used. The identified variables were orthogonally rotated to simplify the patterns' structure and to enhance their interpretability. The K-means algorithm of cluster analysis with the K-nearest-means classifier was applied to define the clusters of participants with common characteristics.

3.5.6 Microsimulation analysis

Microsimulation models are served as sophisticated tools well-suited to address such questions and guide evidence-based public health policies.

Further details on the methods and statistical analysis used in the present work are provided in the *Results section* specified for each research hypothesis.

4 Results

4.1 Summary of baseline and follow-up characteristics of the ATTICA and GREECS study participants

4.1.1 ATTICA study

4.1.1.1 Baseline characteristics of men and women participants according to their ten-year first CVD incidence

Baseline sociodemographic, lifestyle, clinical and psychological characteristics of ATTICA study participants according to their 10-year CVD status are summarized in **Table 4.1** separately for men and women.

Table 4.1 Baseline sociodemographic, anthropometric and clinical characteristics of men and women from the ATTICA study according to 10-year cardiovascular disease incidence (*n*=2,020).

Men	With 10-year CVD event	Without 10-year CVD event	*p-value*
N	198	808	
Age, years	56 (13)	43 (12)	*<0.001*
Years of school	9 (4)	12 (3)	*<0.001*
Body mass index, kg/m^2	28.3 (4.0)	27.1 (3.9)	*0.001*
Waist circumference, cm	101.5 (11.3)	97.0 (12.9)	*<0.001*
MedDietScore, range 0-55	22.4 (6.4)	24.5 (5.2)	*<0.001*
Physical activity, %	39	44	*0.24*
Current smoking, %	28	38	*<0.001*
History of hypertension, %	51	36	*<0.001*
History of diabetes mellitus, %	22	5	*<0.001*
History of hypercholesterolemia, %	58	44	*<0.001*
Family CVD history, %	29	26	*<0.001*
Baseline CVD history, %	0	0	-
Women	With 10-year CVD event	Without 10-year CVD event	*p-value*
N	119	895	
Age, years	59 (12)	42 (13)	*<0.001*
Years of school	9 (3)	12 (3)	*<0.001*
Body mass index, kg/m^2	27.3 (5.1)	24.9 (4.7)	*<0.001*
Waist circumference, cm	89.7 (14.1)	82.4 (13.3)	*<0.001*
MedDietScore, range 0-55	23.4 (6.4)	28.1 (6.6)	*<0.001*
Physical activity, %	38	43	*0.31*
Current smoking, %	39	45	*<0.001*

History of hypertension, %	49	20	*<0.001*
History of diabetes mellitus, %	19	3	*<0.001*
History of hypercholesterolemia, %	55	36	*<0.001*
Family CVD history, %	37	29	*<0.001*
Baseline CVD history, %	0	0	-

Data are presented as mean ± standard deviation (SD) or median (Interquartile Range) if normality was not met. P-values were obtained using Students' t-test for independent sample for the normally distributed variables (age, body mass index), Mann-Whitney test for the rest quantitative variables and chi-squared test for categorical variables. Abbreviations: Cardiovascular disease (CVD)

4.1.1.2 Kaplan-Meier curves and age distribution in relation to ten-year first CVD incidence in men and women participants of ATTICA study

The CVD event rate in the ATTICA study participants was 15.7% (n=317) [19.7% (n=198) in men and 11.7% (n=119) in women, *p<0.001*]. The man-to-woman CVD incidence ratio was 1.68. Median survival time was 9.7 years in men and 9.8 years in women (*p=0.*55) (**Figure 4.1**).

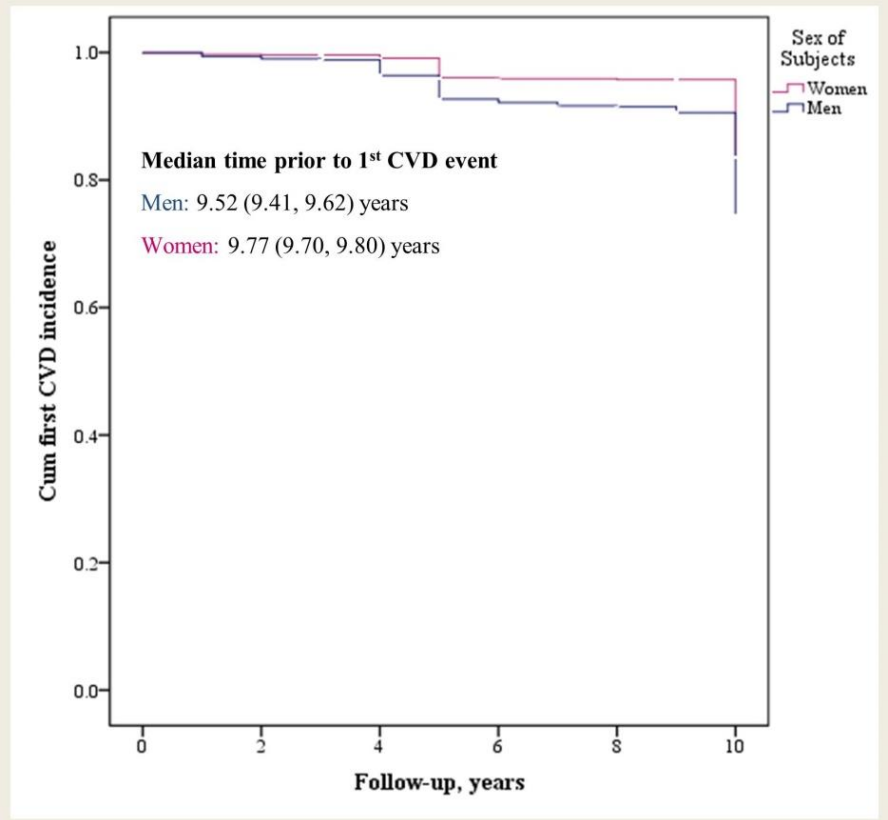

Figure 4.1 Kaplan-Meier curves in relation to ten-year first CVD incidence in men and women of ATTICA study. *Abbreviation:* Cardiovascular disease (CVD)

The mean age of 1st CVD event in men was 64±13 years while in women was about five years later i.e. 69±11 years (*p=0.01*) (**Figure 4.2**).

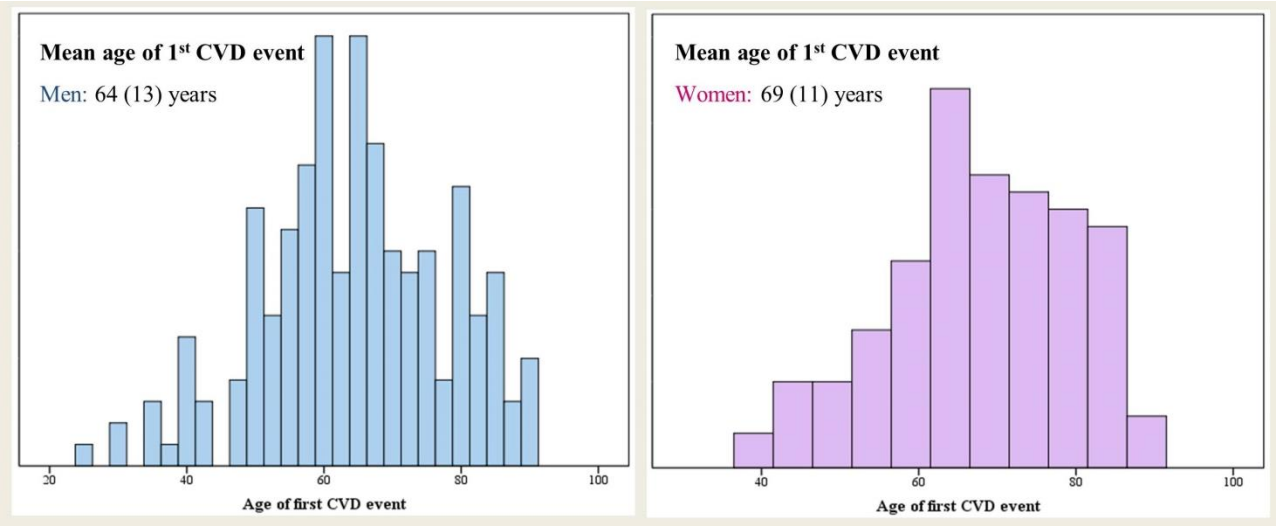

Figure 4.2 Distribution of men and women participants' age of ten-year first CVD event in ATTICA study. *Abbreviation:* Cardiovascular disease (CVD)

4.1.2 GREECS study

4.1.2.1 Baseline characteristics of men and women participants according to their ten-year first CVD incidence

Baseline sociodemographic, lifestyle, clinical and psychological characteristics of ATTICA study participants according to their 10-year CVD status are summarized in **Table 4.2** separately for men and women.

Table 4.2 Baseline sociodemographic, anthropometric and clinical characteristics of men and women from the GREECS study according to 10-year cardiovascular disease incidence (n=2,172).

Men	With 10-year CVD event	Without 10-year CVD event	p-*value*
N	639	1,010	
Age, years	65 (12)	63 (13)	*0.02*
Years of school	8 (4)	8 (4)	*0.27*
Body mass index, kg/m^2	27.3 (3.5)	27.5 (3.7)	*0.33*
Waist circumference, cm	-	-	-
MedDietScore, range 0-55	28.0 (5.7)	28.5 (5.7)	*0.06*
Physical activity, %	40	44	*0.11*
Current smoking, %	39	37	*0.78*
History of hypertension, %	50	46	*0.09*
History of diabetes mellitus, %	33	27	*0.01*
History of hypercholesterolemia, %	46	44	*0.60*
Family CVD history, %	39	35	*0.11*
Baseline CVD history, %	48	38	*<0.001*
Women	With 10-year CVD event	Without 10-year CVD event	*p-value*

N	172	351	
Age, years	73 (11)	69 (11)	*0.001*
Years of school	5 (4)	6 (3)	*0.25*
Body mass index, kg/m^2	28.0 (4.5)	27.6 (4.4)	*0.29*
Waist circumference, cm	-	-	-
MedDietScore, range 0-55	28.3 (5.5)	28.2 (5.5)	*0.95*
Physical activity, %	22	28	*0.20*
Current smoking, %	13	9	*0.10*
History of hypertension, %	71	69	*0.56*
History of diabetes mellitus, %	39	37	*0.69*
History of hypercholesterolemia, %	48	49	*0.89*
Family CVD history, %	35	34	*0.85*
Baseline CVD history, %	38	38	*0.88*

Data are presented as mean ± standard deviation (SD) or median (Interquartile Range) if normality was not met. P-values were obtained using Students' t-test for independent sample for the normally distributed variables (age, body mass index), Mann-Whitney test for the rest quantitative variables and chi-squared test for categorical variables. ***Abbreviations***: Cardiovascular disease (CVD)

4.1.2.2 Kaplan-Meier curves and age distribution in relation to ten-year recurrent CVD incidence in men and women participants of GREECS study

The ten-year CVD event rate in ACS patients of the GREECS study, was 37.3% (n=811) (38.8% in men and 32.9% in women, *p=0.016*). Ten-year CVD mortality rate was 17.7% (17.3% in men and 18.7% in women, *p=0.71*). Median survival time was 8.1 years in men and 7.8 years in women (*p*=0.002) (**Figure 4.3**). The man-to-woman CVD incidence ratio was 1.18.

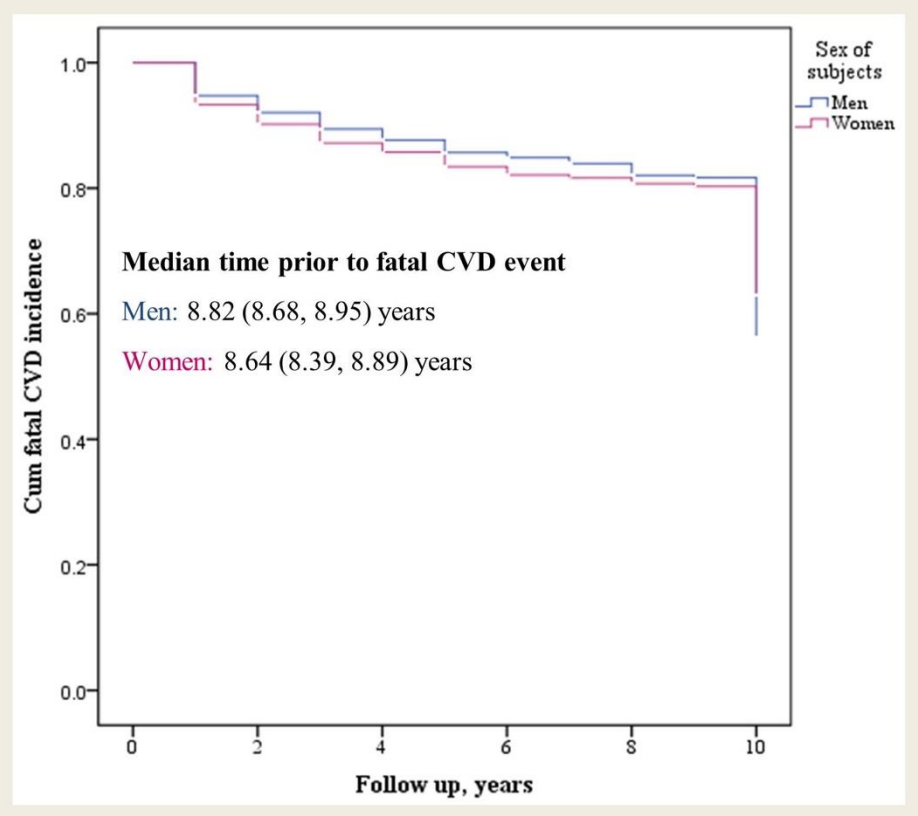

Figure 4.3 Kaplan-Meier curves in relation to ten-year recurrent CVD incidence in men and women of GREECS study. *Abbreviation:* Cardiovascular disease (CVD)

The mean age of recurrent CVD event in men was 72±11 years while in women 78±10 years (*p=0.01*) (**Figure 4.4**).

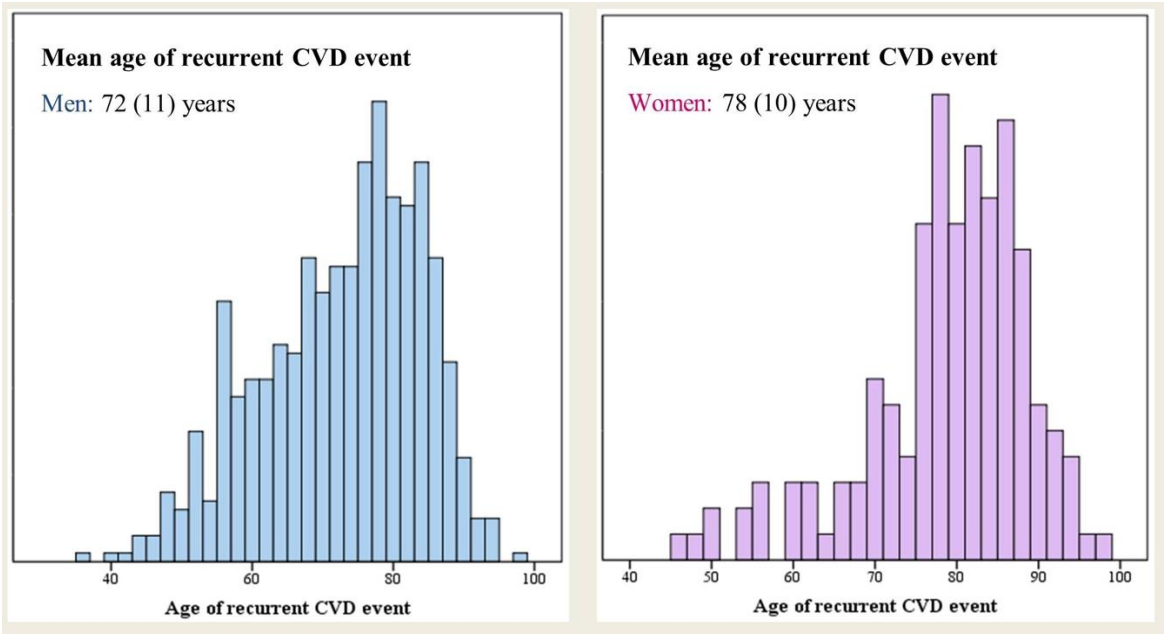

Figure 4.4 Distribution of men and women participants' age of ten-year recurrent CVD event in GREECS study. *Abbreviation:* Cardiovascular disease (CVD)

4.2 The sex-specific role of lifestyle and psychological factors in relation to ten-year first and recurrent CVD incidence

4.2.1 Level of adherence to Mediterranean diet, physical activity and smoking status: results from ATTICA and GREECS study

4.2.1.1 Scope and research hypothesis

The scope here was to perform a sex-specific analysis of lifestyle CVD risk factors (diet, physical activity and smoking status) on 10-year risk for developing a first or a recurrent CVD event, in a sample of apparently healthy individuals (i.e. from the ATTICA Study) and a sample of patients with established ACS (from the GREECS study). The research hypothesis tested was whether sex interacts with lifestyle parameters on the long-term prognosis of CVD in primary or secondary CVD prevention setting.

4.2.1.2 Methods and Statistican analysis

Multi-adjusted logistic regression analysis was performed to evaluate the association between lifestyle factors, principally nutrition, physical activity and smoking, and 10-year first and recurrent CVD event. Interactions between lifestyle factors and sex on 10-year CVD event were also examined, and in the case of significant interactions (*p for interaction<0.10*), stratified analysis was performed with sex as strata. Sex-specific discriminant analysis was performed and Wilks' Lambda (Λ) (the lower the better discriminating ability) was calculated to reveal which of the investigated lifestyle factors had the better discriminating ability over the 10-year CVD event, separately in men and women. PARs for each risk factor, as well as for their combinations, were calculated using a standard methodology already described elsewhere (Yusuf et al 2004), whereas their 95%CI were based using a *logit* transformation approach, by the exception of the case where PAR estimates were negative, in which the Wald type CIs were used.

4.2.1.3 Results

In

Table 4.3 the age-adjusted 10-year CVD event rates are presented separately, in apparently healthy individuals from the ATTICA study and in the ACS patients from the GREECS study, overall, as well as according to various unhealthy lifestyle behaviours. In particular, in the overall sample the man-to-woman age-adjusted ratio was consistently higher to 1 (i.e. men exhibited higher incidence compared with women); this was much higher when a first CVD was studied compared with a recurrent event (i.e. 1.66 *vs.* 1.18, $p<0.001$). The same sex-related pattern was observed when the analysis focused on sedentary participants or smokers. However, as regards dietary habits, among those who followed an unhealthy diet (i.e. low adherence to the Mediterranean diet), women showed higher incidence compared with men as regards a first CVD event, whereas the "superiority" of men was evident only when recurrent CVD events were considered.

Sex-specific, discriminant analysis (using Wilk's Λ; i.e. the lower the value the better the discriminating ability of the factor) over the 10-year follow-up in both cohorts revealed different lifestyle patterns as regards first and recurrent CVD events. In particular, in men, physical activity (Λ=0.977, *p<0.001*) and smoking (Λ=0.997, *p=0.07*) had the best discriminating ability in correctly classifying a potential candidate for a first CVD event, followed by adherence to Mediterranean diet as evaluated through the MedDietScore (Λ=0.999, *p=0.23*), whereas in women, the dominant discriminating factor was the level of adherence to Mediterranean diet (Λ=0.952, *p<0.001*), followed by smoking (Λ=0.996, *p=0.03*) and physical activity (Λ=0.999, *p=0.31*). As regards the recurrent CVD events among ACS patients, it was observed that for men patients, physical activity (Λ=0.997, *p=0.08*) and adherence to Mediterranean diet (Λ=0.998, *p=0.09*) were the dominant discriminating factors, followed by smoking habits (Λ=0.999, *p=0.85*), while for women, MedDietScore (Λ=0.997, *p=0.07*) and current smoking (Λ=0.997, *p=0.09*) were the best discriminating factors, followed by physical activity (Λ=0.999, *p=0.26*).

Table 4.3 Distribution of the 10-year incidence of cardiovascular disease (CVD) in men and women participated in the ATTICA study (*n*=2,020) and in the GREECS study (*n*=2,172), overall and according to their baseline lifestyle characteristics.

ATTICA study			
First CVD event, % (n)	**Men** *(n=1,015)*	**Women** *(n=1,005)*	**Men-to-women C VD incidence rate ratio**
Overall, % (n)	19.7% (198)	11.7% (119)	1.66
Lifestyle behaviours			
Low adherence to Mediterranean diet*, % (n)	22.0% (188)	25.0% (92)	0.88
Sedentary lifestyle, % (n)	20.9% (120)	10.9% (68)	1.92
Current smoking, % (n)	17.5% (78)	8.9% (34)	1.97
GREECS study			
Recurrent CVD event, % (n)	**Men** *(n=1,649)*	**Women** *(n=523)*	**Men-to-women C VD incidence rate ratio**
Overall, %(n)	38.8 (640)	32.9 (172)	1.18

Lifestyle behaviours			
Low adherence to Mediterranean diet*, % (n)	40.9 (252)	33.5 (58)	1.22
Sedentary lifestyle, % (n)	40.6 (355)	33.8 (122)	1.20
Current smoking, % (n)	39.3 (240)	25.8 (16)	1.52

In **Table 4.4**, two epidemiologic models are presented, regarding first and recurrent 10-year CVD events. As it can be seen, the effect-size measures of male sex as well as smoking habits was higher for first CVD events compared with recurrent CVD events ($p<0.01$), whereas, physical activity status seemed to play a more significant role for recurrent CVD events compared with first CVD events. Moreover, significant interactions between sex and level of adherence to Mediterranean diet, physical activity status and smoking on the 10-year first and recurrent CVD event were observed (*p-values for interaction ≤0.10*).

Table 4.4 Results from logistic regression analysis presenting the association of socio-demographic, lifestyle, and clinical characteristics on 10-year cardiovascular disease event, in apparently healthy individuals (ATTICA study) and patients with diagnosed acute coronary syndrome (GREECS study).

	ATTICA study (n=2,020)	**GREECS study (n=2,172)**
	OR (95% CI)	**OR (95% CI)**
Age (per 1 year)	1.05 (1.01, 1.09)	1.01 (1.00, 1.02)
Male sex	2.09 (1.10, 2.75)	1.58 (1.20, 2.08)
MedDietScore (≥27 vs. <27, range 0-55)	0.85 (0.79, 0.99)	0.96 (0.92, 1.09)
Low adherence to Mediterranean diet (MedDietScore <27 vs. ≥27, range 0-55)	1.18 (1.01, 1.27)	1.04 (0.92, 1.09)
Physical activity (sedentary vs. active)	1.06 (0.99, 1.49)	1.27 (1.01, 1.61)
Current smoking (yes vs. no)	1.40 (1.01, 2.05)	0.96 (0.73, 1.25)
Body mass index (per 1 kg/m^2)	1.11 (1.02, 1.21)	1.01 (0.98, 1.04)
Hypertension (yes vs. no)	2.46 (1.03, 5.86)	0.89 (0.70, 1.12)
Hypercholesterolemia (yes vs. no)	1.16 (0.54, 2.51)	1.06 (0.85, 1.33)
Diabetes mellitus (yes vs. no)	1.56 (0.84, 2.90)	1.17 (0.92, 1.48)
Years of school (per 1 year)	0.91 (0.82, 1.01)	0.99 (0.96, 1.02)
Family CVD history (yes vs. no)	1.47 (0.67, 3.23)	1.25 (1.00, 1.57)

Abbreviations: Odds Ratio (OR); 95% Confidence Interval (95%CI)

Based on these sex-lifestyle interactions, stratified analyses were performed, and the results are illustrated in **Figure 4.5**. As it can be seen, low adherence to Mediterranean diet was associated with 22% higher first CVD event risk (OR=1.22, 95%CI 1.03, 1.51) in women, while in men, sedentary lifestyle was associated with 35% higher first CVD event risk (OR=1.35, 95%CI 1.01, 1.85), whereas smoking with 28% higher risk (OR=1.28, 95%CI 1.04, 1.82). As for the recurrent CVD events, low adherence to Mediterranean diet (OR=1.25, 95%CI 1.01, 2.50) and sedentary lifestyle (OR=1.25, 95%CI 1.01, 1.30) were associated with 25% higher 10-year recurrent CVD events risk in women, as well as smoking (OR=1.15, 95%CI 1.01, 1.30), which was associated with 15% increased risk. In men, low adherence to Mediterranean diet (OR=1.27,

95%CI 1.01, 1.35) and physical activity (OR=1.27, 95%CI 1.02, 1.59) were associated with 27% higher 10-year recurrent CVD events risk. The calculation of the PARs per lifestyle risk factor revealed that the relative importance of each factor varied while it was highly related to its prevalence. In all prevention stages (i.e. first or recurrent CVD events) and irrespective to the sex, dietary habits in terms of adherence to Mediterranean diet was the most important risk factor even from smoking while physical activity status was as well important in case of apparently healthy men. Finally, it is noteworthy that in case of smoking in the prognosis of ACS patients, even if the frequency of men smokers was almost 3-fold higher than their women's counterparts the respective PARs were quite similar; potentially, confirming the initially observed higher odds of smoking in women patients.

Then, the cumulative effect of the studied lifestyle risk factors was assessed on first and recurrent CVD event outcomes. Specifically, in men, together, current smoking, low adherence to Mediterranean diet, and sedentary lifestyle increased the risk for a first CVD event to 1.83 (0.94, 4.21) compared with those without these risk factors, and they accounted for 41% of the PAR of CVD, whereas in women, the aforementioned accumulated risk was 1.65 (1.34, 3.47) compared with those without these risk factors and accounted for 23% of the PAR of CVD. Additionally, the cumulative risk was recalculated after taking into account the sleep duration; in men the cumulative risk presented 17% raise, reaching 2.21 (0.96, 5.05), while the respective increase in women was 33% reaching 2.49 (0.73, 6.76).

Regarding recurrent cardiac events, together, current smoking, low adherence to Mediterranean diet, and sedentary lifestyle increased the risk for a first CVD event in men to 1.76 (0.76, 2.17) compared with those without these risk factors, and they accounted for 25% of the PAR of CVD, whereas in women, the aforementioned accumulated risk was 1.84 (1.03, 5.17) compared with those without these risk factors and accounted for 25% of the PAR of CVD. Thereby, the aforementioned lifestyle risk factors seem to have a more pivotal role in case of apparently healthy men compared with ACS men patients. On the other side, women that had suffered from a cardiac episode were almost twice as high affected by an unhealthy lifestyle pattern compared with their free-of-CVD period of life.

Lifestyle risk factor	Gender	CVD event	Cases, % (n)	OR (95%CI)	PAR (95% CI)
Low adherence to Mediterranean diet	Men	First	22.0 (188)	1,.06 (0.90, 1.25)	41% (0, 73)
		Recurrent	40.9 (252)	1.27 (1.01, 1.35)	25% (0, 33)
	Women	First	25.0 (92)	1.22 (1.03, 1.51)	23 % (13, 53)
		Recurrent	33.5 (58)	1.28 (1.01, 1.59)	25% (1, 62)
Sedentary lifestyle	Men	First	20.9 (120)	1.35 (1.01, 1.85)	23% (8, 42)
		Recurrent	39.6 (355)	1.27 (1.02, 1.59)	13% (1, 25)
	Women	First	10.9 (68)	1.11 (0.90, 1.21)	6% (0, 12)
		Recurrent	33.8 (122)	1.25 (1.01, 2.50)	15% (7, 52)
Current Smoking	Men	First	17.5 (78)	1.28 (1.04, 1.82)	19% (3, 41)
		Recurrent	19.3 (240)	1.09 (0.74, 1.61)	3% (0, 18)
	Women	First	8.9 (34)	1.22 (0.78, 1.90)	8% (0, 25)
		Recurrent	25.8 (16)	1.15 (1.01, 1.59)	2% (1, 3)

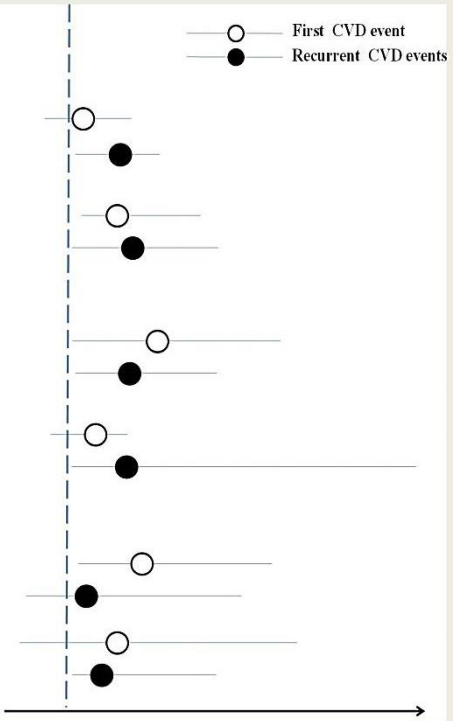

Figure 4.5 Sex-specific analysis of lifestyle risk factors as regards first and recurrent CVD risk, in the ATTICA study participants and GREECS study patients.

Odds ratios (dots) and their 95% confidence intervals (horizontal lines) were obtained for a. low adherence to Mediterranean diet (MedDietScore <27 vs. ≥27, range 0-55), b. physical activity status (sedentary vs. physically active lifestyle) & c. smoking status (current vs. never) through multivariate logistic regression analysis adjusted for age, body mass index, educational status, history of hypertension, hypercholesterolemia, diabetes mellitus and family CVD history. Vertical and horizontal axes are intersected in the value 1; values in the right side indicate a harmful effect while values in the left side indicate a protective effect. Odds Ratio (OR); 95% Confidence Interval (95%CI); Population Attributable Risk (PAR).

4.2.2 Clustering of components of Mediterranean diet in relation to ten-year first and recurrent CVD event: identifying sex-specific features: results from ATTICA and GREECS study

4.2.2.1 Scope and research hypothesis

The scope here was to define the patterns of Mediterranean diet that result in better CVD outcomes, as well as, the sex distribution in each pattern. The research hypothesis was that the aforementioned differences in the association between Mediterranean diet and long-term CVD onset or recurrence between men and women were attributed to differences within the level of consumption in the separate components of this dietary pattern between sexes.

4.2.2.2 Methods and statistical analysis

To evaluate the different factors of Mediterranean diet in terms of prioritizing the food components that contribute more or less to the MedDietScore, FA using the extraction method of Principal Components was applied. For each factor, food items with loadings greater or equal than |0.4| were considered to contribute significantly and were used for calculation of the patterns' scores, based on the regression method. HRs and the corresponding 95%CIs for the factors' effect on CVD onset, recurrence or mortality were evaluated through Cox proportional hazards models. In addition, the K-means algorithm of cluster analysis with the K-nearest-means classifier was

applied to define the clusters of participants with common dietary habits. The frequency of the 11 food items were converted to Z- scores (standardized) and entered into the cluster algorithm, which was run 100 times in order to reduce the effect of random splitting. The analyses were performed for two to five clusters, and the best cluster solution was chosen in terms of the amount of explained variation, the size (≥10% of the sample) and interpretation of each cluster, as well as, its stability. A two-cluster solution was decided to be the optimal, as they were best interpretable and to check whether FA and cluster analysis extract comparable patterns. Finally, the sex distribution in each cluster was checked and it is presented in the form of women-to-men ratio.

4.2.2.3 Results

FA identified two dietary factors, both in the ATTICA and in the GREECS epidemiological study (**Figure 4.6**). The two factors had eigenvalues> 1.0 and each accounted for at least 10% of the common variance. The first factor with high loading coefficients for fruits, vegetables, legumes, non- refined cereals and potatoes was labelled as "Factor 1 - factor with higher score in plant-based food items", while the second factor with high loading coefficients for red mead & meat products, poultry, full fat dairy products, fish and alcoholic beverages, was labelled as "Factor 2 - factor with higher score in animal-based food items". Similar clusters were generated from the K-means cluster analysis. Regarding the sex distribution, the vast majority of women were found to be assigned in the 1st cluster (woman-to-man ratio= 3:1) while most men were assigned to the 2nd cluster (woman-to-man ratio= 1:4).

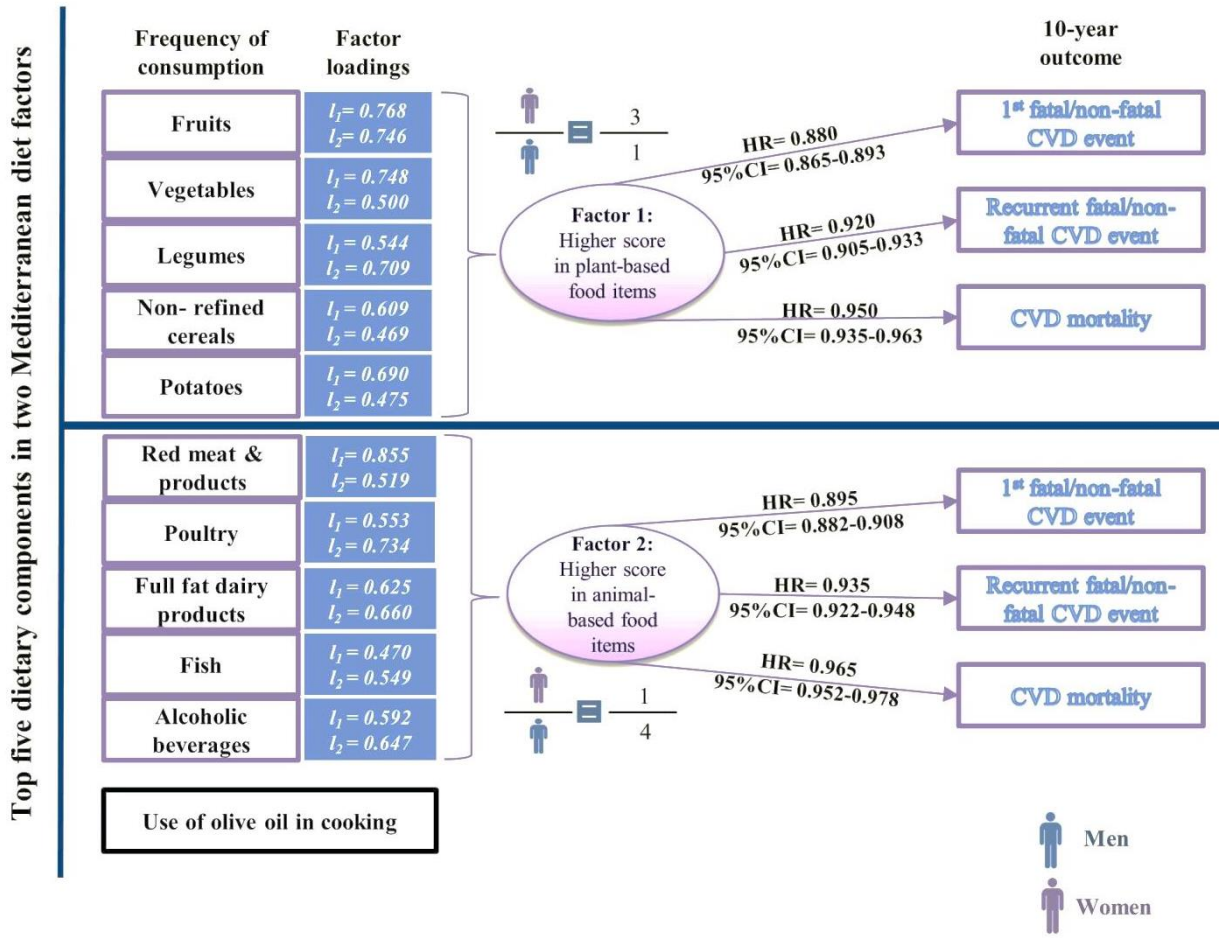

Figure 4.6 Different clusters of Mediterranean diet according to the score in plant- or animal- based food items and their effect on the 10-year risk of first fatal/non-fatal CVD event in apparently healthy men and women as well as on the 10-year risk of recurrent fatal/non-fatal CVD event and CVD mortality in men and women with established acute coronary syndrome.

Level of adherence to the Mediterranean diet was estimated by the MedDietScore scale; The identification of two different patterns of Mediterranean diet was based on Factor analysis with Varimax rotation; l1= factor loading of each food item in the context of the ATTICA epidemiological study and l2= factor loading of each food item in the context of the GREECS epidemiological study; N= 2,172 patients with diagnosed acute coronary syndrome (1,649 men and 523 women) participated in the 10-year follow up of the GREECS epidemiological study and N= 2,020 apparently healthy individuals (1,006 men and 1,014 women) participated in the 10- year follow- up of the ATTICA epidemiological study; The hazard ratios and the respective confidence intervals are presented for a 2- unit increment in the plant and the animal- based components; Abbreviations: CVD= Cardiovascular disease, HR= Hazard Ratio, CI= Confidence Interval.

4.2.3 The sex-specific role of dairy products consumption on ten-year first CVD event: results from ATTICA study

4.2.3.1 Scope and research hypothesis

The scope here was to examine the total as well as sex-specific association between dairy products (total and subtypes) and 10-year first fatal/non-fatal CVD incidence in apparently healthy men and women from a Mediterranean region, Greece. Three a priori research hypotheses were explored separately for men and women; *firstly*, dairy consumption protects against CVD onset irrespective to its fat content, *secondly*, participants with a regular consumption of fermented milk-derived products exhibit lower risk to experience from a major cardiac episode

and *thirdly*, cardiometabolic risk factors and surrogate markers related with insulin resistance may interact with dairy products intake on the primary endpoint (i.e. CVD).

4.2.3.2 Methods and statistical analysis

ATTICA study participants were specifically asked for the consumption of low fat or full fat milk and yogurt as well as the different types of cheese (i.e. feta cheese, semi-hard cheese, hard cheese and soft cheese); focusing on cheese, the vast majority of the kinds consumed in Greek traditional diet have a mean fat content of 20-30% with some cheeses reaching even the 40-50% [21]. Hence, it was decided not to separate them in low fat and full fat. The consumption of butter and cream were also recorded. Fat-free products consumption was very low in ATTICA study sample (<5%); hence these were excluded from the analyses. Dairy-product consumption categories were defined as <1 serving/day, 1-2 servings/day and >2 servings/day. Frequency of consumption was quantified in terms of the number of times a month a food was consumed in small, medium or large portion sizes. Then this information was transformed to the consumption of standard servings per day. Standard serving/portion sizes (such as a glass of milk (240mL), or a cup of yoghurt (200g), or a slice of cheese (30g), or a teaspoon of butter (5g)) were assigned to each food item. HRs and their corresponding 95%CIs for the dairy product intake in relation to the examined endpoint were evaluated through multivariable Cox-regression analysis in the total sample, as well as separately in men and women. Furthermore, an official interaction analysis was performed to identify the potential synergistic effect of various clinical, biochemical and lifestyle factors on the association between dairy consumption and CVD onset; subgroup analysis was performed using as strata factors that presented a significant interacting effect on the examined association i.e. *p for interaction*<0.05.

4.2.3.3 Findings

The baseline sociodemographic, clinical, anthropometric, biochemical and lifestyle characteristics of men and women from the ATTICA study across the dairy product consumption level are summarized in *Table 1*. Participants assigned to the group with the highest level of consumption (i.e. >2servings/day) presented significantly lower insulin resistance depicted by HOMA-IR (*p=0.03*), lower values in inflammatory markers i.e. CRP (*p=0.03*) and IL-6 (*p=0.02*) as well as lower levels of AST (*p=0.01*) and ALT (*p=0.05*). Further focus on fatty liver assessed through HSI revealed that both men and women at the highest level of dairy consumption had better liver health compared with their counterparts at lower intake level (*all p-values<0.05*). Ten-year CVD incidence rate according to dairy consumption level is also summarized in **Table 4.5**. Regular dairy product consumption i.e. >2 servings/day in women presented about two- to three- fold lower risk for CVD onset within the decade compared with rare consumption (*p=0.02*) while in men no significant trends were observed.

Table 4.6 summarizes the consumption level of specific food groups, total daily energy and macronutrients intake according to total dairy intake level separately in men and women. Ranking from the low to the high level of consumption both men and women seemed to adapt a plant-based dietary pattern with higher consumption of fruits, vegetables and legumes as well as the use of olive oil exclusively on daily food preparation (all *p-values<0.05*). Within the highest level of dairy intake, the men-to-women ratio regarding low-fat dairy intake was about 1:2 (3.3 servings/day in women *vs* 1.9 servings/day in men) while in case of full-fat dairy products no sex-related differences were observed. In the same subgroup, men consumed twice as high cheese compared with women. As for the yogurt intake the level of consumption was about 1 serving higher in women compared with men. Overall, ranking from the lowest to the highest dairy consumption higher total daily energy and saturated fatty acid intake was observed (all *p-values<0.001*).

Findings from nested models that evaluated the association between total, full-fat and low-fat dairy consumption level and CVD event in the total sample as well as separately for men and women are presented in **Table 4.7**. In the unadjusted models, it was revealed that >2servings/day dairy products of any kind resulted in about 56% lower risk to develop CVD, compared with participants reported rare consumption; this was more evident in women starting even from a moderate level of consumption (i.e. 1-2 servings/day). Similar associations were revealed for low-fat dairy products while in case of full-fat products no significant associations were observed. Further adjustment for sociodemographic, clinical and lifestyle factors revealed that the aforementioned associations remained significant only in case of women and only on a regular-consumption basis of >2 servings/day irrespective to the fat content. Interestingly, this independently protective effect was lost when inflammation-, insulin resistance- and liver function- related biomarkers were taken into account.

Table 4.5 Baseline sociodemographic, lifestyle, clinical, biochemical characteristics and 10-year first cardiovascular disease incidence according to total dairy product consumption in apparently healthy men and women of ATTICA study (*n*=1,885)

	Men (*n*=1,012)				**Women (*n*=873)**			
	Total dairy product consumption (servings[b]/day)			*p-value*	**Total dairy product consumption (servings[b]/day)**			*p-value*
	<1	**1-2**	**>2**		**<1**	**1-2**	**>2**	
N	*194*	*556*	*262*		*121*	*480*	*272*	
Baseline metrics								
Sociodemographic factors								
Age, years; mean (SD)[c]	45 (9)	41 (9)	38 (11)	*<0.001*	39 (9)	39 (11)	36 (11)	*0.13*
Years of school; median (IR)[d]	12 (4)	11 (3)	11 (3)	*0.85*	10 (4)	11 (3)	10 (3)	*0.91*
Anthropometric factors								
Body mass index, kg/m^2; mean (SD)	27.0 (3.4)	27.1 (4.1)	27.1 (3.8)	*0.99*	25.1 (4.4)	24.4 (4.7)	23.9 (4.8)	*0.26*
Waist circumference, cm; mean (SD)	97 (11)	97 (12)	96 (14)	*0.96*	81 (13)	81 (15)	78 (12)	*0.17*
Lifestyle factors								
Physical activity, n (%)	68 (35)	211 (38)	149 (57)	*<0.001*	38 (32)	216 (45)	146 (54)	*0.01*
MedDietScore[e], range 0-55; median (IR)	22.6 (4.6)	24.2 (4.6)	24.4 (5.1)	*0.005*	29.5 (7.6)	29.3 (8.3)	33.3 (11.9)	*<0.001*
Current smoking, n %	83 (43)	278 (50)	120 (46)	*0.44*	59 (49)	201 (42)	108 (40)	*0.45*
Clinical factors								
History of hypertension, n (%)	77 (40)	194 (35)	94 (36)	*0.69*	16 (13)	81 (17)	35 (13)	*0.54*
History of diabetes mellitus, n (%)	25 (13)	22 (4)	13 (5)	*0.003*	0 (0)	10 (2)	8 (3)	*0.42*
History of hypercholesterolemia, n (%)	98 (51)	216 (39)	57 (22)	*<0.001*	31 (26)	124 (26)	46 (17)	*0.15*

Family CVD history, n (%)	36 (19)	166 (30)	60 (23)	*0.07*	32 (27)	139 (29)	62 (23)	*0.51*
Inflammation/coagulation markers								
C-Reactive Protein, mg/dL[f]; median (IR)	0.24 (0.26)	0.20 (0.25)	0.18 (0.24)	*0.03*	0.19 (0.29)	0.19 (0.23)	0.15 (0.25)	*0.03*
Interleukin 6, pg/mL; median (IR)	1.5 (0.3)	1.5 (0.3)	1.4 (0.3)	*0.008*	1.3 (0.3)	1.3 (0.3)	1.3 (0.3)	*0.15*
TNF-a[g], pg/mL; median (IR)	7.9 (2.1)	7.6 (2.4)	7.0 (2.2)	*0.007*	5.7 (3.6)	5.2 (3.1)	4.2 (2.6)	*0.002*
Liver function markers								
Alanine transaminase, U/L[h]; median (IR)	24.0 (13.6)	23.3 (14.3)	20.1 (11.2)	*0.67*	20.0 (13.4)	23.0 (14.2)	19.2 (11.2)	*0.08*
Aspartate transaminase, U/L[i]; median (IR)	24.9 (11.4)	23.7 (11.1)	24.7 (10.9)	*0.95*	23.9 (11.0)	23.1 (11.0)	18.1 (10.5)	*0.05*
Hepatic steatosis index[j]; median (IR)	39 (5.7)	36 (5.2)	31 (7.8)	*0.03*	38 (5.6)	34 (5.4)	29 (8.9)	*<0.001*
Glucose/insulin homeostasis markers								
HOMA-IR[k]; median (IR)	4.0 (3.2)	3.3 (3.7)	3.3 (2.3)	*0.02*	2.4 (0.5)	2.7 (2.1)	2.8 (2.1)	*0.45*
Follow-up metrics								
10-year fatal/non-fatal CVD[l] event, n (%)	34 (17.8)	83 (15.0)	28 (10.9)	*0.41*	17 (14.0)	31 (6.4)	16 (5.7)	*0.02*

[a] Data are presented as mean ± SD or median (IR) if normality was not met. P-values were obtained using one way ANOVA for the normally distributed variables (age, body mass index), Kruskal Wallis test for the rest quantitative variables and chi-squared test for categorical variables; [b] Standard serving/portion sizes were a glass of milk (240mL), or a cup of yoghurt (200g), or a slice of cheese (30g)), or a teaspoon of butter (5g); [c] SD=Standard Deviation; [d] IR=Interquartile range; [e] MedDietScore is a Mediterranean diet score with a theoretical range of 0 to 55. The index is calculated based on participants' responses to a set of 11 questions regarding the monthly consumption of various food groups; [f] To convert mg/dL CRP to mg/L, multiply by 10; [g] Tumor necrosis factor-alpha (TNF-a); [h] To convert U/L alanine transaminase to μkat/L, multiply by 0.0167; i To convert U/L aspartate transaminase to μkat/L, multiply by 0.0167; [j] Hepatic Steatosis Index (HSI) is an index for non-alcoholic fatty liver disease, calculated using the following formula: HSI = 8 × ((Alanine transaminase)/(Aspartate transaminase) ratio) + (body mass index) (+2, if female; +2, if participant has diabetes mellitus). Participants with HSI measurements of >36 were categorized in the hepatic steatosis group; [k] HOMA-IR=Homeostatic Model Assessment of Insulin Resistance; [l] CVD-Cardiovascular disease.

Table 4.6[a] Food group, total energy and nutrients intake according to total dairy product consumption in apparently healthy men and women of ATTICA study (*n*=1,885)

	Men (*n*=1,012)				**Women (*n*=873)**			
	Total dairy product consumption (servings[b]/day)			*p-value*	**Total dairy product consumption (servings[b]/day)**			*p-value*
	<1	**1-2**	**>2**		**<1**	**1-2**	**>2**	
N	*194*	*556*	*262*		*121*	*480*	*272*	
Food group, consumption level								
Dairy subcategories, servings/day;								
Full-fat dairy	1.0 (0.4)	2.0 (0.8)	2.4 (0.7)	*<0.001*	0.7 (0.4)	2.0 (0.8)	2.4 (0.7)	*<0.001*
Low-fat dairy	0.9 (0.5)	1.4 (1.1)	1.9 (1.2)	*<0.001*	0.4 (1.8)	1.8 (2.0)	3.3 (3.9)	*<0.001*
Milk	0.2 (1.4)	1.3 (1.0)	1.7 (2.4)	*<0.001*	0.5 (1.6)	1.5 (1.1)	1.9 (2.5)	*<0.001*
Yogurt	0.5 (1.4)	1.4 (1.6)	1.9 (2.7)	*<0.001*	0.7 (1.9)	1.7 (1.5)	2.8 (2.6)	*<0.001*
Cheese	0.8 (1.3)	1.0 (1.2)	2.0 (1.4)	*<0.001*	0.3 (1.4)	0.5 (1.1)	1.0 (1.3)	*<0.001*
Butter	0.4 (1.1)	0.8 (1.0)	1.5 (1.6)	*<0.001*	0.5 (1.2)	0.6 (0.9)	1.1 (1.5)	*<0.001*
Fruits, servings/week; median (IR)	19.5 (12.0)	24.0 (12.5)	30.6 (14.9)	*<0.001*	22.2 (12.6)	27.3 (13.3)	31.2 (13.4)	*<0.001*
Vegetables, servings/week; median	30.1 (14.6)	32.2 (13.1)	38.9 (17.9)	*<0.001*	29.2 (11.7)	33.9 (13.2)	38.4 (13.8)	*<0.001*
Total grains, servings/week; median	46.9 (15.6)	50.7 (15.5)	59.0 (22.6)	*<0.001*	44.2 (17.9)	51.4 (16.2)	58.7 (19.1)	*<0.001*
Non-refined grains, servings/week,	15.9 (8.7)	20.4 (10.1)	19.6 (12.2)	*0.24*	18.8 (9.5)	25.7 (12.3)	18.9 (13.1)	*0.14*
Potatoes, servings/week; median (IR)	9.3 (4.7)	12.6 (6.6)	14.1 (8.6)	*<0.001*	10.8 (7.4)	10.5 (5.7)	11.9 (7.6)	*0.11*
Legumes, servings/week; median (IR)	5.3 (4.3)	5.1 (2.3)	5.9 (3.3)	*0.07*	3.7 (1.7)	4.6 (2.3)	5.1 (2.4)	*<0.001*

Red meat, servings/week; median (IR)	4.2 (2.1)	4.8 (2.0)	4.4 (3.1)	*0.08*	3.3 (1.5)	4.1 (2.0)	4.6 (2.7)	*0.07*
Poultry, servings/week; median (IR)	1.2 (0.7)	1.3 (0.6)	1.3 (0.9)	*0.48*	1.2 (0.7)	1.2 (0.8)	1.4 (1.0)	*0.22*
Eggs, servings/week; median (IR)	0.7 (0.8)	1.1 (1.0)	1.7 (1.1)	*<0.001*	0.8 (0.5)	0.9 (0.8)	1.3 (0.1)	*<0.001*
Fish and shellfish, servings/week;	1.9 (1.1)	2.0 (0.9)	2.3 (1.4)	*0.10*	1.8 (0.8)	1.9 (0.9)	2.1 (1.3)	*0.09*
Nuts, servings/week; median (IR)	1.1 (0.9)	1.6 (1.4)	1.2 (2.2)	*<0.001*	1.1 (1.1)	1.3 (1.3)	1.6 (1.7)	*0.07*
Sweets, servings/week; median (IR)	3.8 (1.7)	3.9 (2.2)	3.7 (2.9)	*0.54*	4.2 (2.2)	4.5 (2.2)	4.5 (2.5)	*0.32*
Alcohol, servings/week; median (IR)	12.2 (14.8)	13.0 (16.5)	11.4 (14.3)	*0.59*	4.8 (8.9)	5.5 (10.9)	5.6 (13.5)	*0.88*
Exclusive use of olive oil, %	45	53	61	*0.07*	61	65	70	*0.001*
Fast food, rare consumption, %	53	51	52	*0.20*	61	57	59	*0.55*
Total energy intake, kcal/day; median (IR)	1,759 (611)	2,330 (753)	2,885 (884)	*<0.001*	1,608 (719)	1,980 (693)	2,572 (865)	*<0.001*
total carbohydrates, % total energy intake; median (IR)	37 (7)	36 (5)	35 (6)	*0.21*	39 (6)	37 (6)	36 (5)	*0.001*
total protein, % total energy intake; median (IR)	14 (2)	14 (1)	14 (2)	*0.18*	13 (2)	14 (1)	15 (1)	*<0.001*
total fat, % total energy intake; median (IR)	33 (4)	34 (3)	35 (4)	*<0.001*	34 (4)	35 (4)	36 (4)	*0.016*
saturated fatty acids, % total energy intake; median (IR)	11 (3)	13 (2)	15 (3)	*<0.001*	12 (2)	14 (2)	15 (2)	*<0.001*

[a] Data are presented as median (Interquartile Range) since normality was not met. P-values were obtained using Kruskal Wallis test for the quantitative variables and chi-squared test for categorical variables. [b] Standard serving/portion sizes were a glass of milk (240mL), or a cup of yoghurt (200g), or a slice of cheese (30g)), or a teaspoon of butter (5g).[c] IR=Interquartile Range

Table 4.7 Total and sex-based nested Cox-regression analyses to evaluate the HRs and their corresponding 95%CIs of total, full-fat and low-fat dairy products in relation to 10-year cardiovascular disease event in the ATTICA study (*n*=1,885).

			Total (n/cases)	**Men (n/cases)**	**Women (n/cases)**	
			1,885/277	1,012/175	873/102	
	Model with	**Consumption categories**	HR[a] (95%CI)[b]	HR (95%CI)	HR (95%CI)	Model adjusted for
Model 1	**a.** Total dairy products	<1 serving[c]/day	*Ref*	*Ref*	*Ref*	Crude model
		1-2 servings/day	0.63 (0.35, 1.14)	0.81 (0.40, 1.66)	**0.35 (0.10, 0.51)**	
		>2 servings/day	**0.45 (0.22, 0.92)**	0.56 (0.23, 1.34)	**0.30 (0.09, 0.55)**	
	b. Full-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.74 (0.46, 1.21)	0.91 (0.50, 1.76)	0.56 (0.20, 1.41)	
		>2 servings/day	0.65 (0.31, 1.32)	0.79 (0.43, 1.49)	0.67 (0.39, 1.45)	
	c. Low-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.59 (0.38, 1.10)	0.79 (0.35, 1.60)	**0.28 (0.08, 0.45)**	
		>2 servings/day	**0.40 (0.19, 0.85)**	0.51 (0.20, 1.30)	**0.25 (0.08, 0.49)**	
Model 2	**a.** Total dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	Age, (sex), menopause status (*only in women*)
		1-2 servings/day	0.83 (0.44, 1.55)	1.05 (0.50, 2.21)	**0.42 (0.14, 0.65)**	
		>2 servings/day	0.73 (0.34, 1.53)	0.85 (0.34, 2.10)	**0.33 (0.10, 0.59)**	
	b. Full-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.84 (0.55, 1.30)	1.01 (0.61, 1.80)	0.66 (0.30, 1.51)	
		>2 servings/day	0.75 (0.40, 1.33)	0.98 (0.54, 1.69)	0.77 (0.41, 1.55)	
	c. Low-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.59 (0.38, 1.10)	0.79 (0.35, 1.60)	**0.35 (0.18, 0.50)**	
		>2 servings/day	**0.50 (0.23, 0.99)**	0.55 (0.25, 1.34)	**0.30 (0.10, 0.54)**	

Model 3	**a.** Total dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	Model 2 plus body mass index, physical activity, current smoking, years of school, MedDietScore[d], total energy intake, saturated fatty acid intake
		1-2 servings/day	0.91 (0.47, 1.95)	1.08 (0.49, 2.39)	**0.46 (0.11, 0.77)**	
		>2 servings/day	0.84 (0.38, 1.85)	0.92 (0.34, 2.43)	**0.37 (0.11, 0.60)**	
	b. Full-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.83 (0.55, 1.30)	1.01 (0.63, 1.80)	0.67 (0.30, 1.51)	
		>2 servings/day	0.74 (0.40, 1.33)	0.99 (0.54, 1.69)	0.77 (0.41, 1.55)	
	c. Low-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.60 (0.38, 1.10)	0.79 (0.35, 1.60)	**0.40 (0.18, 0.70)**	
		>2 servings/day	**0.533 (0.26, 1.02)**	0.55 (0.25, 1.34)	**0.39 (0.12, 0.66)**	
Model 4	**a.** Total dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	Model 3 plus history of hypertension, hypercholesterolemia and diabetes mellitus, family history of CVD[e]
		1-2 servings/day	0.83 (0.41, 1.67)	1.11 (0.47, 2.62)	0.59 (0.21, 1.03)	
		>2 servings/day	0.73 (0.31, 1.71)	0.88 (0.31, 2.52)	**0.48 (0.23, 0.90)**	
	b. Full-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.83 (0.55, 1.30)	1.01 (0.63, 1.80)	0.67 (0.30, 1.51)	
		>2 servings/day	0.74 (0.40, 1.33)	0.99 (0.54, 1.69)	0.77 (0.41, 1.55)	
	c. Low-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.59 (0.38, 1.10)	0.79 (0.35, 1.60)	0.55 (0.20, 1.10)	
		>2 servings/day	**0.50 (0.23, 0.99)**	0.55 (0.25, 1.34)	0.50 (0.12, 1.01)	
Model 5	**a.** Total dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	Model 4 plus CRP[f], IL-6[g], TNF-a[h], HSI[i], HOMA-IR[j], waist circumference
		1-2 servings/day	0.93 (0.44, 1.97)	1.18 (0.47, 2.95)	0.62 (0.24, 1.05)	
		>2 servings/day	0.72 (0.29, 1.80)	0.89 (0.28, 2.87)	0.54 (0.27, 1.01)	
	b. Full-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.83 (0.55, 1.30)	1.01 (0.63, 1.80)	0.67 (0.30, 1.51)	
		>2 servings/day	0.74 (0.40, 1.33)	0.99 (0.54, 1.69)	0.77 (0.41, 1.55)	
	c. Low-fat dairy products	<1 serving/day	*Ref*	*Ref*	*Ref*	
		1-2 servings/day	0.59 (0.38, 1.10)	0.79 (0.35, 1.60)	0.60 (0.25, 1.13)	

	>2 servings/day	0.53 (0.25, 1.03)	0.55 (0.25, 1.34)	0.55 (0.15, 1.06)

[a] HR=Hazard ratio (HRs and their corresponding 95%CIs were obtained from Cox regression analysis. **Bold** indicates statistically significant outcomes i.e. $p<0.05$); [b]95%CI=95% Confidence Interval; [c] Serving size was defined as follows: serving for milk=240mL; serving for yogurt=200g; serving for cheese=30g; serving for butter=5g; [d] MedDietScore is a Mediterranean diet score with a theoretical range of 0 to 55. The index is calculated based on participants' responses to a set of 11 questions regarding the monthly consumption of various food groups; [e] CVD=Cardiovascular disease; [f] CRP=C-Reactive Protein; [g] IL-6=Interleukin 6; [h] TNF-a=Tumor necrosis factor-alpha; [i] HSI=Hepatic Steatosis Index; [j] HOMA-IR=Homeostatic Model Assessment of Insulin Resistance.

In **Table 4.8** the dose-response relationship between specific types of dairy products and 10-year CVD event in the total sample as well as separately for men and women is presented. Even if no significant associations were revealed in case of milk, fermented dairy products (i.e. yogurt and cheese) seemed to present an independent protective effect against the examined endpoint; per 200g/day yogurt consumption, the risk to develop CVD was 20-30% lower with this claim being more evident in women. The analyses were repeated according to the fat content of milk and yogurt revealing similar outcomes in the multi-adjusted analysis (on the basis of Model 4, total sample); per 240mL/day full fat milk HR=1.01 95%CI (0.90, 1.20), per 240mL/day low fat milk HR=0.98 95%CI (0.84, 1.10), per 200g/day full fat yogurt HR=0.75 95%CI (0.30, 0.92), per 200gr low fat yogurt HR=0.73 95%CI (0.27, 0.89) (*data not shown on table*). As for cheese, per 30g/day intake, about 5% lower CVD risk was observed, especially in men. Interestingly, in case of butter non-significant associations were highlighted.

Furthermore, an interaction analysis was also performed to identify the potential synergistic effect of various clinical, biochemical and lifestyle factors on the association between dairy consumption and CVD onset and results are summarized in **Table 4.9**. Dairy consumption level was interacted with HSI (*p for interaction=0.02*), HOMA-IR (*p for interaction=0.001*) and CRP (*p for interaction=0.07*). In the total sample multi-adjusted analysis, a protective effect of total, low-fat and fermented dairy products was observed in case of NAFLD – based on HSI – and insulin resistance. In case of systemic inflammation only yogurt consumption seemed to independently protect against CVD. The dairy products with promising cardioprotective properties were yogurt and cheese (i.e. fermented dairy products) for men and total sample as well as low-fat dairy products and yogurt for women, yet only in case of established NAFLD, insulin resistance and systemic inflammation (all *p-values for interaction<0.10*).

Table 4.8 Total and sex-based dose-response nested Cox-regression analyses to evaluate the association between dairy products with 10-year cardiovascular disease event (n=1,885).

			Total (n/cases)	**Men (n/cases)**	**Women (n/cases)**	
			1,885/277	1,012/175	873/102	
	Model with	**Consumption**	HR[a] (95%CI)[b]	HR (95%CI)	HR (95%CI)	*Model adjusted for*
Model 1	**a.** Milk	per 240mL/day[c]	**0.85 (0.74, 0.97)**	0.87 (0.74, 1.01)	0.80 (0.60, 1.07)	Crude model
	b. Yogurt	per 200g/day	**0.76 (0.41, 0.92)**	0.77 (0.83, 1.03)	**0.67 (0.24, 0.86)**	
	c. Cheese	per 30g/day	**0.97 (0.95, 0.99)**	**0.97 (0.94, 0.99)**	0.98 (0.94, 1.01)	
	d. Butter	per 10g/day	0.88 (0.68, 1.14)	0.87 (0.64, 1.17)	0.90 (0.56, 1.46)	
Model 2	**a.** Milk	per 240mL/day	0.92 (0.80, 1.06)	0.93 (0.80, 1.09)	0.82 (0.58, 1.15)	Age, (sex), menopause status (*only in women*)
	b. Yogurt	per 200g/day	**0.76 (0.41, 0.92)**	0.81 (0.62, 1.06)	**0.67 (0.26, 0.86)**	
	c. Cheese	per 30g/day	**0.98 (0.96, 1.00)**	**0.97 (0.95, 1.00)**	0.99 (0.95, 1.03)	
	d. Butter	per 10g/day	1.13 (0.86, 1.47)	1.06 (0.78, 1.45)	1.26 (0.75, 2.12)	
Model 3	**a.** Milk	per 240mL/day	0.92 (0.80, 1.06)	0.93 (0.80, 1.09)	0.82 (0.58, 1.16)	Model 2 plus body mass index, physical activity, current smoking, years of school, MedDietScore[d], total energy intake, saturated fatty acid intake
	b. Yogurt	per 200g/day	**0.74 (0.36, 0.91)**	0.82 (0.62, 1.07)	0.72 (0.33, 0.91)	
	c. Cheese	per 30g/day	**0.98 (0.96, 1.00)**	**0.98 (0.95, 1.00)**	0.99 (0.95, 1.04)	
	d. Butter	per 10g/day	1.13 (0.87, 1.49)	1.06 (0.76, 2.47)	1.26 (0.74, 2.14)	
Model 4	**a.** Milk	per 240mL/day	0.92 (0.79, 1.07)	0.96 (0.82, 1.12)	0.71 (0.45, 1.12)	Model 3 plus history of hypertension, hypercholesterolemia and diabetes mellitus, family history of CVD[e]
	b. Yogurt	per 200g/day	**0.72 (0.29, 0.89)**	0.90 (0.68, 1.08)	**0.86 (0.49, 0.98)**	
	c. Cheese	per 30g/day	**0.98 (0.96, 1.00)**	**0.97 (0.94, 1.00)**	1.01 (0.96, 1.04)	
	d. Butter	per 10g/day	1.07 (0.79, 1.46)	1.09 (0.78, 1.53)	0.91 (0.42, 1.98)	
Model 5	**a.** Milk	per 240mL/day	0.97 (0.83, 1.12)	1.01 (0.85, 1.18)	0.73 (0.46,1.18)	Model 4 plus CRP[f], IL-6[g], TNF-a[h], HSI[i], HOMA-IR[j], waist circumference
	b. Yogurt	per 200g/day	**0.70 (0.28, 0.88)**	0.98 (0.75, 1.08)	0.92 (0.58, 1.03)	
	c. Cheese	per 30g/day	**0.97 (0.95, 1.00)**	**0.96 (0.92, 0.99)**	1.01 (0.96, 1.06)	
	d. Butter	per 10g/day	1.06 (0.77, 1.47)	1.13 (0.79, 1.61)	0.69 (0.27, 1.80)	

[a] HR=Hazard ratio (HRs and their corresponding 95%CIs were obtained from Cox regression analysis. **Bold** indicates statistically significant outcomes i.e. $p<0.05$); [b] 95%CI=95% Confidence Interval

[c] Daily consumption was evaluated according to the assessed weekly consumption divided by 7; [d] MedDietScore is a Mediterranean diet score with a theoretical range of 0 to 55. The index is calculated based on participants' responses to a set of 11 questions regarding the monthly consumption of various food groups; [e] CVD=Cardiovascular disease; [f] CRP=C-Reactive Protein; [g] IL-6=Interleukin 6; [h]TNF-a=Tumor necrosis factor-alpha; [i] HSI=Hepatic Steatosis Index; [j] HOMA-IR=Homeostatic Model Assessment of Insulin Resistance

Table 4.9 Total and sex-based sensitivity analyses to evaluate the association of dairy product consumption (in servings) with 10-year cardiovascular disease event (n=1,885).

HSI[b] (*p for interaction with total dairy products=0.02*)

		Total (n/cases)		**Men (n/cases)**		**Women (n/cases)**	
		1,885/277		1,012/175		873/102	
		HR[c] (95%CI)[d]		HR (95%CI)		HR (95%CI)	
Model with	**Consumption categories**	**HSI>36**	**HSI≤36**	**HSI>36**	**HSI≤36**	**HSI>36**	**HSI≤36**
a. total dairy products	per 1 serving/day	**0.91 (0.80, 0.98)**	1.01 (0.84, 1.13)	0.99 (0.88, 1.20)	1.04 (0.80, 1.11)	**0.86 (0.75, 0.93)**	0.98 (0.82, 1.09)
b. full-fat dairy products	per 1 serving/day	0.89 (0.78, 1.06)	1.06 (0.84, 1.19)	1.05 (0.78, 1.17)	1.05 (0.65, 1.27)	0.90 (0.81, 1.08)	1.07 (0.95, 1.22)
c. low-fat dairy	per 1 serving/day	**0.93 (0.82, 0.99)**	1.04 (0.80, 1.24)	1.03 (0.76, 1.15)	1.08 (0.68, 1.30)	**0.89 (0.78, 0.94)**	1.01 (0.89, 1.17)

products							
d. milk	per 240mL/day	0.96 (0.84, 1.09)	1.06 (0.94, 1.19)	1.03 (0.57,1.20)	1.12 (0.73,1.25)	0.83 (0.68, 1.01)	0.93 (0.77, 1.11)
e. yogurt	per 200g/day	**0.75 (0.23, 0.93)**	0.75 (0.32, 1.04)	**0.80 (0.59, 0.98)**	0.93 (0.72, 1.10)	**0.79 (0.51, 0.94)**	0.89 (0.61, 1.03)
f. cheese	per 30g/day	**0.95 (0.90, 0.99)**	1.05 (0.93, 1.10)	**0.89 (0.84, 0.97)**	1.02 (0.98, 1.07)	1.04 (0.97, 1.10)	1.00 (0.92, 1.04)
g. butter	per 10g/day	1.10 (0.80, 1.55)	1.02 (0.78, 1.40)	1.11 (0.77, 1.62)	1.17 (0.83, 1.70)	0.65 (0.30, 1.74)	0.59 (0.27, 1.81)
Insulin resistance (*p for interaction with total dairy products=0.001*)							
		Total		**Men**		**Women**	
		1,885/277		1,012/175		873/102	
		HR (95%CI)		HR (95%CI)		HR (95%CI)	
Model with	**Consumption categories**	**HOMA IR[e]>2.78**	**HOMA IR≤2.78**	**HOMA IR>2.78**	**HOMA IR≤2.78**	**HOMA IR>2.78**	**HOMA IR≤2.78**
a. total dairy products	per 1 serving/day	**0.87 (0.76, 0.95)**	0.98 (0.81, 1.10)	1.03 (0.92, 1.24)	1.08 (0.84, 1.15)	**0.90 (0.79, 0.97)**	1.02 (0.86, 1.13)
b. full-fat dairy products	per 1 serving/day	0.85 (0.74, 1.02)	1.02 (0.80, 1.15)	1.01 (0.74, 1.13)	1.01 (0.61, 1.23)	0.85 (0.77, 1.04)	1.03 (0.91, 1.18)
c. low-fat dairy products	per 1 serving/day	**0.89 (0.78, 0.95)**	1.00 (0.76, 1.20)	0.99 (0.72, 1.11)	1.04 (0.64, 1.26)	**0.85 (0.74, 0.90)**	0.97 (0.85, 1.13)

d. milk	per 240mL/day	0.92 (0.80, 1.05)	1.02 (0.90, 1.15)	0.99 (0.53,1.16)	1.08 (0.69,1.21)	0.79 (0.64, 1.01)	0.89 (0.73, 1.07)
e. yogurt	per 200g/day	**0.71 (0.19, 0.89)**	0.71 (0.28, 1.01)	**0.76 (0.55, 0.94)**	0.89 (0.68, 1.06)	**0.75 (0.49, 0.90)**	0.85 (0.59, 1.06)
f. cheese	per 30g/day	**0.91 (0.86, 0.95)**	1.01 (0.89, 1.06)	**0.85 (0.80, 0.93)**	0.98 (0.94, 1.03)	1.00 (0.93, 1.06)	**0.96 (0.88, 1.00)**
g. butter	per 10g/day	1.06 (0.87, 1.51)	0.98 (0.74, 1.36)	1.08 (0.75, 1.63)	1.13 (0.79, 1.66)	0.70 (0.35, 1.75)	0.64 (0.22, 1.87)

Systemic inflammation (*p for interaction with total dairy products=0.07*)

		Total (n/cases)		**Men (n/cases)**		**Women (n/cases)**	
		1,885/277		1,012/175		873/102	
		HR (95%CI)		HR (95%CI)		HR (95%CI)	
Model with	**Consumption categories**	**CRP[f]>0.11mg/dL**	**CRP≤0.11mg/dL**	**CRP>0.11mg/dL**	**CRP≤0.11mg/dL**	**CRP>0.11mg/dL**	**CRP≤0.11mg/dL**
a. total dairy products	per 1 serving/day	0.94 (0.83, 1.01)	1.04 (0.87, 1.16)	1.02 (0.91, 1.23)	1.07 (0.83, 1.14)	**0.87 (0.78, 0.96)**	1.01 (0.85, 1.12)
b. full-fat dairy products	per 1 serving/day	1.01 (0.81, 1.09)	1.09 (0.87, 1.22)	1.08 (0.81, 1.20)	1.08 (0.68, 1.29)	0.93 (0.83, 1.11)	1.10 (0.98, 1.18)
c. low-fat dairy products	per 1 serving/day	0.96 (0.85, 1.02)	1.07 (0.83, 1.27)	1.06 (0.79, 1.18)	1.11 (0.71, 1.33)	**0.91 (0.81, 0.97)**	1.04 (0.93, 1.19)
d. milk	per 240mL/day	0.99	1.09	1.06	1.15	0.86	0.96

		(0.87, 1.13)	(0.97, 1.23)	(0.60,1.23)	(0.76,1.20)	(0.65, 1.04)	(0.80, 1.13)
e. yogurt	per 200g/day	**0.78 (0.26, 0.97)**	0.78 (0.35, 1.07)	0.83 (0.62, 1.01)	0.96 (0.75, 1.13)	**0.81 (0.55, 0.97)**	0.91 (0.65, 1.07)
f. cheese	per 30g/day	0.98 (0.93, 1.02)	1.08 (0.96, 1.13)	**0.91 (0.87, 1.00)**	1.05 (0.97, 1.08)	1.03 (0.94, 1.11)	1.03 (0.95, 1.07)
g. butter	per 10g/day	1.12 (0.82, 1.57)	1.04 (0.81, 1.42)	1.15 (0.81, 1.68)	1.21 (0.84, 1.71)	0.67 (0.32, 1.76)	0.63 (0.30, 1.87)

[a] Standard serving/portion sizes were a glass of milk (240mL), or a cup of yoghurt (200g), or a slice of cheese (30g)), or a teaspoon of butter (5g); [b] HSI=Hepatic Steatosis Index; [c] HR=Hazard ratio (HRs and their corresponding 95%CIs were obtained from multivariate Cox regression analysis adjusted for age, (sex), menopause status (only in women), body mass index, physical activity, current smoking, years of school, MedDietScore, total energy intake, saturated fatty acid intake, history of hypertension, hypercholesterolemia and diabetes mellitus, family history of cardiovascular disease. **Bold** indicates statistically significant outcomes i.e. $p<0.05$.); [d] 95%CI=95% Confidence Interval; [e] HOMA-IR=Homeostatic Model Assessment of Insulin Resistance; [f] CRP=C-Reactive Protein.

4.2.4 The sex-specific role of dietary vitamin D intake on ten-year first CVD event: results from ATTICA study

4.2.4.1 Scope and research hypothesis

The scope here was to examine the association between dietary vitamin D intake on 10-year first fatal/non-fatal CVD incidence, conventional CVD risk factors and surrogate markers. Based on the hitherto generated outcomes on this issue three a priori research hypotheses were performed; *firstly*, dietary vitamin D intake does not independently affect the risk for hard cardiac endpoints, *secondly*, participants with low dietary vitamin D intake are at higher risk to develop cardiometabolic risk factors and subsequently exhibit high CVD risk and *thirdly*, the genetic, metabolic and lifestyle differences between men and women result in sex-mediated associations between this dietary factor and the examined endpoints.

4.2.4.2 Methods and analysis

Daily intake of vitamin D was calculated by means of the food database provided by the United States Department of Agriculture Agricultural Research Service accompanied by adjustments on recent data regarding the vitamin D content of animal-origin foods (USDA 2019, Liu et al 2015, Schmid et al 2013). The food sources that contributed mostly to dietary vitamin D intake in the present sample was primarily fish and eggs. Vitamin D supplements were not considered to quantify vitamin D intake; participants who reported supplementary intake of vitamin D were excluded from the analysis. HRs and their corresponding 95%CIs for the dietary vitamin D intake in relation to the examined endpoints within the decade were evaluated through multivariable Cox-regression analysis in the total sample, as well as separately in men and women. Multi-adjusted linear regression models were applied to test the association between the dietary vitamin D intake (per 1μg) and various biomarkers (per 1 unit).

4.2.4.3 Findings

Baseline characteristics of men and women participants across vitamin D intake tertiles are summarized in **Table 4.10**. Results from unadjusted analysis for the role of vitamin D on hard and intermediate CVD-related endpoints are presented in

Table 4.11 Ten-year incidence of first fatal/non-fatal cardiovascular disease incidence and intermediate cardiometabolic conditions according to dietary vitamin D tertiles, in apparently healthy men and women (*n*=1,885).

. Men in the lowest category of dietary vitamin D intake had about twice as high risk to develop CVD compared with their counterparts in the highest level of intake (*p=0.002*) while in women no significant trend was observed (*p>0.05*). In man sample, hypertension incidence in the 1st vitamin D tertile was about 20% higher compared with those assigned to the 3rd tertile (*p=0.03*). In women, ranking from 1st to 3rd vitamin D tertile, significant trends were observed for diabetes, hypercholesterolemia and transition to unhealthy metabolic status with participants at the highest vs. the lowest level of consumption having about 6%, 17% and 21% lower rates of the respective endpoints (*all p-values<0.05*).

Table 4.10 Baseline sociodemographic, clinical, anthropometric, biochemical and lifestyle characteristics of men and women from the ATTICA study according to dietary vitamin D intake tertiles (*n*=1,885).

Baseline characteristics	**Dietary vitamin D intake tertiles**			
	1st tertile	**2nd tertile**	**3rd tertile**	***p-value***
N	*618*	*635*	*632*	-
Dietary vitamin D intake, μg/d	1.6 (0.3)	2.6 (0.2)	3.3 (0.9)	-
Sociodemographic factors				
Age, years	41 (10)	39 (11)	38 (10)	*0.03*
Male sex, %	50	53	58	*0.12*
Anthropometric factors				
Body mass index, kg/m^2	25.8 (4.3)	25.7 (4.4)	25.9 (4.8)	*0.92*
Waist circumference, cm	89 (15)	88 (14)	90 (17)	*0.20*
Lifestyle factors				
Physical activity, %	38	45	48	*0.02*
MedDietScore, range 0-55	25 (7)	26 (6)	28 (9)	*<0.001*
Current smoking, %	48	42	45	*0.33*
Clinical factors				
History of hypertension, %	28	24	27	*0.53*
History of diabetes mellitus, %	5	4	3	*0.69*
History of hypercholesterolemia, %	37	30	25	*0.005*
Metabolically healthy status, %	46	52	57	*0.02*
Family CVD history, %	29	26	25	*0.51*
Inflammation/coagulation markers				
C-Reactive Protein, mg/L	2.0 (2.5)	1.8 (2.5)	1.6 (2.4)	*0.03*
Interleukin 6, pg/dL	1.9 (0.3)	1.6 (0.3)	1.4 (0.3)	*0.02*
Tumor necrosis factor-alpha, pg/mL	6.2 (2.9)	5.8 (1.3)	5.1 (2.4)	*0.09*
White blood cells, 10^{3}counts	8.0 (2.1)	6.7 (1.9)	6.7 (2.0)	*0.01*
Amyloid A, mg//L	4.2 (4.9)	4.0 (5.0)	3.8 (4.2)	*0.67*
Homocysteine, μmol/L	11.6 (6.9)	11.7 (6.0)	11.6 (5.5)	*0.94*
Fibrinogen, mg/dL	317 (65)	302 (63)	303 (65)	*0.04*
Liver function markers				
Alanine transaminase, U/L	24.00 (13.68)	23.35 (14.34)	20.08 (11.25)	*0.67*
Aspartate transaminase, U/L	24.91 (11.44)	23.77 (11.06)	24.74 (10.93)	*0.95*

Glucose/insulin homeostasis markers				
HOMA-IR	3.5 (2.3)	3.2 (2.6)	3.0 (1.2)	*0.04*
Renal function markers				
Creatinine clearance, mL/min/1.73m^2	98 (28)	98 (25)	104 (30)	*0.004*

Data are presented as mean ± standard deviation (SD) or median (Interquartile Range) if normality was not met. P-values were obtained using one way ANOVA for the normally distributed variables (age, body mass index), Kruskal Wallis test for the rest quantitative variables and chi-squared test for categorical variables. ***Abbreviations***: Cardiovascular disease (CVD); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Table 4.11 Ten-year incidence of first fatal/non-fatal cardiovascular disease incidence and intermediate cardiometabolic conditions according to dietary vitamin D tertiles, in apparently healthy men and women (*n*=1,885).

10-year endpoint, %	**Men, dietary vitamin D tertiles**			*p-value*	**Women, dietary vitamin D tertiles**			*p-value*
	1st	*2nd*	*3rd*		*1st*	*2nd*	*3rd*	
Fatal/non-fatal CVD event	24	17	12	*0.002*	14	10	11	*0.59*
Hypertension	33	23	14	*0.03*	28	21	25	*0.21*
Hypercholesterolemia	40	44	37	*0.68*	29	32	12	*0.008*
Diabetes mellitus	9	10	8	*0.59*	13	8	7	*0.001*
Metabolically unhealthy status	28	24	28	*0.81*	48	40	27	*<0.001*

P-values were obtained using chi-squared test. ***Abbreviations***: Cardiovascular disease (CVD).

Nested Cox regression models to evaluate the association between daily vitamin D intake and CVD event are presented in **Table 4.12**. In unadjusted models, 1μg raise in daily dietary vitamin D intake was linked with 31% lower risk to develop a cardiac episode; sex-based stratified analysis revealed that this association reached the level of significance in both sexes with the man-to-woman rate ratio being 4:1 (*p for interaction=0.001*). In age-, sex- and (for women) menopause- adjusted model the aforementioned association in total sample analysis was attenuated yet retained the level of significance; however, when the sample was stratified, this was not the case in women (*p>0.05*). After adjusting for anthropometric, lifestyle and clinical factors, 1μg raise in the estimated dietary vitamin D intake resulted in about 24% lower 10-year CVD risk while in men a stronger association was observed i.e. 1μg raise in vitamin D corresponded to 33% decrease in 10-year CVD risk. Adjustment for various surrogate CVD markers revealed that inflammatory markers strongly mediated the examined association either in the total or in the man sample analysis; the inverse association between vitamin D and CVD incidence was retained yet without being significant (*p>0.05*).

Table 4.12 Total and sex-based sensitivity analyses to evaluate the association of dietary vitamin D intake with 10-year cardiovascular disease event (n=1,885).

	Total	**Men**	**Women**	
N, cases	1,885/277	1,012/175	873/102	
	HR (95%CI)	HR (95%CI)	HR (95%CI)	**Models adjusted for**
Model with dietary vitamin D as continuous variable				
per 1µg (40IU)	0.69 (0.51, 0.92)*	0.56 (0.38, 0.82)*	0.89 (0.58, 1.00)*	
Model with dietary vitamin D tertiles				Crude model
1st	Ref	Ref	Ref	
2nd	0.51 (0.29, 0.89)*	0.42 (0.21, 0.84)*	0.64 (0.23, 1.75)	
3rd	0.43 (0.23, 0.77)*	0.29 (0.11, 0.75)*	0.63 (0.22, 1.01)	
Model with dietary vitamin D as continuous variable				
per 1µg (40IU)	0.74 (0.54, 0.96)*	0.61 (0.44, 0.86)*	1.09 (0.67, 1.78)	
Model with dietary vitamin D tertiles				Model 1: Age, (sex), menopause status (*only in women*)
1st	Ref	Ref	Ref	
2nd	0.50 (0.28, 0.91)*	0.43 (0.21, 0.87)*	0.72 (0.24, 2.12)	
3rd	0.48 (0.26, 0.90)*	0.31 (0.15, 0.65)*	1.06 (0.34, 3.30)	
Model with dietary vitamin D as continuous variable				
per 1µg (40IU)	0.75 (0.59, 0.95)*	0.64 (0.48, 0.80)*	0.94 (0.63, 1.41)	Model 2: Model 1 plus body mass index, physical activity, current smoking, MedDietScore
Model with dietary vitamin D tertiles				

	1st	Ref	Ref	Ref	
	2nd	0.58 (0.31, 1.07)	0.48 (0.23, 1.01)	0.83 (0.27, 2.54)	
	3rd	0.52 (0.27, 1.00)*	0.32 (0.14, 0.74)*	1.24 (0.37, 4.15)	
Model with dietary vitamin D as continuous variable					
	per 1μg (40IU)	0.76 (0.60, 0.97)*	0.66 (0.49, 0.89)*	1.03 (0.69, 1.55)	Model 3: Model 2 plus history of hypertension, hypercholesterolemia and diabetes mellitus, family history of CVD
Model with dietary vitamin D tertiles					
	1st	Ref	Ref	Ref	
	2nd	0.67 (0.35, 1.28)	0.61 (0.28, 1.33)	0.82 (0.23, 2.94)	
	3rd	0.47 (0.23, 0.96)*	0.35 (0.14, 0.74)*	1.44 (0.36, 5.68)	
Model with dietary vitamin D as continuous variable					
	per 1μg (40IU)	0.79 (0.62, 1.03)	0.70 (0.57, 1.01)	1.09 (0.77, 1.62)	Model 4: *Model 3* plus **inflammation/coagulation**-related surrogate CVD markers i.e. CRP, WBC, IL-6, TNF-a, amyloid A, fibrinogen, homocysteine
Model with dietary vitamin D tertiles					
	1st	Ref	Ref	Ref	
	2nd	0.78 (0.39, 1.54)	0.80 (0.35, 1.83)	0.92 (0.23, 3.57)	
	3rd	0.54 (0.31, 1.01)	0.42 (0.20, 0.98)*	1.51 (0.40, 5.81)	
Model with dietary vitamin D as continuous variable					
	per 1μg (40IU)	0.79 (0.62, 0.98)*	0.66 (0.49, 0.90)*	1.09 (0.71, 1.07)	Model 5: *Model 3* plus **liver function**-related surrogate CVD markers i.e. ALT, AST,
Model with dietary vitamin D tertiles					
	1st	Ref	Ref	Ref	

	2nd	0.78 (0.39, 1.54)	0.80 (0.35, 1.83)	0.92 (0.23, 3.57)	
	3rd	0.41 (0.19, 0.89)*	0.35 (0.16, 0.76)*	1.31 (0.29, 5.81)	
Model with dietary vitamin D as continuous variable					
	per 1µg (40IU)	0.80 (0.63, 0.99)*	0.66 (0.50, 0.91)*	1.10 (0.72, 1.07)	Model 6: *Model 3* plus **insulin resistance**-related surrogate CVD markers i.e. HOMA-IR
Model with dietary vitamin D tertiles					
	1st	Ref	Ref	Ref	
	2nd	0.77 (0.39, 1.55)	0.79 (0.34, 1.83)	0.92 (0.23, 3.57)	
	3rd	0.43 (0.21, 0.92)*	0.35 (0.16, 0.76)*	1.30 (0.27, 5.81)	
Model with dietary vitamin D as continuous variable					
	per 1µg (40IU)	0.79 (0.62, 0.98)*	0.66 (0.49, 0.90)*	1.09 (0.71, 1.07)	Model 7: *Model 3* plus **renal function**-related surrogate CVD markers i.e. creatinine clearance
Model with dietary vitamin D tertiles					
	1st	Ref	Ref	Ref	
	2nd	0.78 (0.39, 1.54)	0.80 (0.35, 1.83)	0.92 (0.23, 3.57)	
	3rd	0.41 (0.19, 0.89)*	0.35 (0.16, 0.76)*	1.31 (0.29, 5.81)	

*****p<0.05. HRs and their corresponding 95%CIs were obtained from Cox regression analysis. **Bold** indicates statistically significant outcomes i.e. p<0.05. **Abbreviations:** Alanine transaminase (ALT); Aspartate transaminase (AST); Cardiovascular disease (CVD); Confidence Interval (CI); C-Reactive Protein (CRP); estimated glomerular filtration rate (eGFR); Hazard Ratio (HR); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR); Interleukin 6 (IL-6); Tumor necrosis factor-alpha (TNF-a) White blood cells (WBC).

Figure 4.7 illustrates the hazard ratios of dietary vitamin D intake in relation to the 10-year onset of intermediate cardiometabolic conditions in the total sample as well as separately for men and women. Increased dietary vitamin D intake was inversely associated with 10-year onset of hypertension in men and transition to metabolically unhealthy status in women.

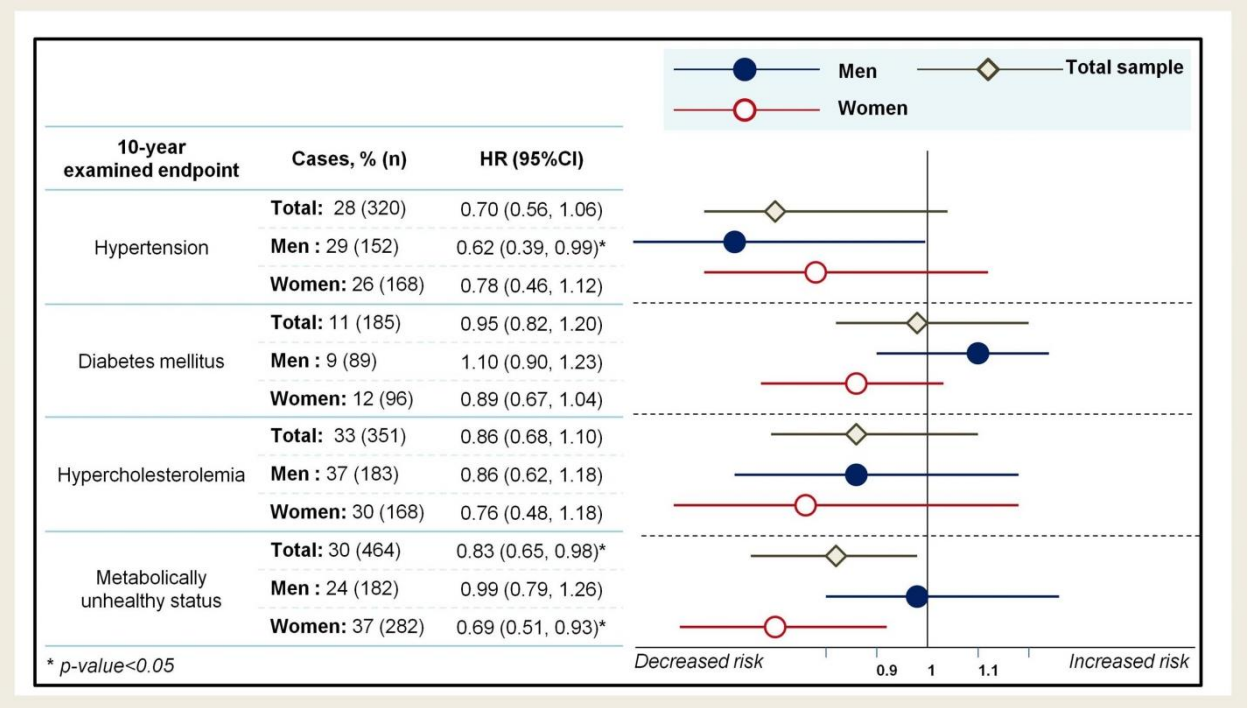

Figure 4.7 Hazard ratios and 95% Confidence Intervals of dietary vitamin D intake (per 1 μg/d) in relation to 10-year incidence of intermediate cardiometabolic conditions.

Hazard ratios (dots) and their corresponding 95% Confidence Intervals (vertical lines) for dietary vitamin D intake (per 1 μg/d) were obtained through Cox regression analysis. Models were adjusted for age, (sex), menopause status (for women), body mass index, physical activity, current smoking, MedDietScore and family history of CVD.

The multi-adjusted associations between various surrogate markers and the dietary vitamin D intake in apparently healthy men and women are summarized in **Table 4.13**. In the context of inflammation/coagulation-related biomarkers, significant inverse associations were observed for both men and women in terms of CRP, IL-6 and fibrinogen (*all p-values<0.05*). Additionally, HOMA-IR and vitamin D intake were inversely associated yet only in case of women (*p=0.01*).

Table 4.13 Results from multivariate linear regression analysis regarding the association between dietary vitamin D intake (per 1µg raise) and surrogate cardiovascular disease markers in the total sample and separately for men and women (*n*=1,885).

	Total sample	**Men**	**Women**
	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)
Inflammation/coagulation markers			
C-Reactive Protein, per 1 mg/L	−0.24 (0.13)*	−0.25 (0.13)*	−0.29 (0.13)*
Interleukin 6, per 1 pg/dL	−0.27 (0.31)*	−0.17 (0.34)*	−0.28 (0.26)*
Tumor necrosis factor-alpha, per 1 pg/mL	−0.19 (1.18)	−0.17 (1.68)	−0.18 (0.91)
White blood cells, per 10^3counts	−0.29 (0.10)*	−0.24 (0.13)*	−0.19 (0.11)*
Amyloid A, per 1 mg//L	−0.10 (0.32)	−0.13 (0.67)	−0.12 (0.71)
Homocysteine, per 1 µmol/L	−0.16 (0.86)	−0.15 (0.83)	−0.17 (0.84)
Fibrinogen, per 1 mg/dL	−11.2 (1.10)*	−12.9 (1.20)*	−10.4 (1.12)*
Liver function markers			
Alanine transaminase, per 1 U/L	−0.11 (0.29)	−0.13 (0.30)	−0.10 (0.27)
Aspartate transaminase, per 1 U/L	−0.10 (0.25)	−0.11 (0.28)	−0.10 (0.24)
Glucose/insulin homeostasis markers			
HOMA-IR, per 1 unit	−0.75 (0.24)	−0.77 (0.83)	−0.83 (0.25)*
Renal function markers			
Creatinine clearance, per 1 mL/min/1.73m^2	−0.15 (0.92)	−0.21 (0.96)	−0.16 (0.83)

p<0.05. Beta-Coefficients and their corresponding standard error were obtained from linear regression analysis after adjusting for age, (sex), body mass index, physical activity, current smoking, MedDietScore, history of hypertension, diabetes mellitus and hypercholesterolemia and family history of cardiovascular disease. ***Abbreviations:*** Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

4.2.5 The sex-specific role of dietary inflammatory index on ten-year first CVD event: results from ATTICA study

4.2.5.1 Scope and research hypothesis

The scope here was to evaluate the association between inflammatory load of diet expressed through an index of separate foods and nutrients and; **a.** the presence of MetS using different sets of criteria to define it, **b.** the 10-year incidence of diabetes mellitus, hypertension, dyslipidaemia, and transition from healthy to unhealthy metabolic status. The research hypothesis was that dietary patterns with increased anti-inflammatory potential are inversely associated with MetS as well as the 10-year transition to unhealthy metabolic status and separate intermediate cardiometabolic risk factors with specific highlights for men and women.

4.2.5.2 Methods and statistical analysis

Calculation of the dietary inflammatory load of participants' diet was according to the methodology and the rationale of the Dietary Inflammation Index that have been previously proposed by Shivappa and colleagues (Shivappa et al 2014). Thus, a D-AII was developed based on participants' dietary habits (Georgousopoulou et al 2016; Tyrovolas et al 2019). In particular, various foods of the study's database were scored with − 1, + 1 and 0 if they increased, decreased or had no effect, respectively, on specific inflammatory biomarkers (i.e., CRP, IL-6, Interleukine-10 (IL-10)TNF-α etc.) according to the previous literature (Georgousopoulou et al 2016; Tyrovolas et al 2019). Following to this procedure a specific Z-score for each food item according to participants' consumption was calculated and then was multiplied by the aforementioned scoring. The sum of these products was used to generate the total dietary anti-inflammatory index score for each participant. The D-AII score ranged from 10 to 77 (Georgousopoulou et al 2016; Tyrovolas et al 2019). ORs and their corresponding 95%CIs of D-AII tertiles and the examined endpoints were evaluated through multivariable logistic regression analysis. Multi-adjusted linear regression models were applied to test the association between the D-AII (per 1 unit) and MetS components (per 1 unit).

4.2.5.3 Results

In the sample used for this research hypothesis (n=2,992), the prevalence of MetS was 19.9% (man-to-woman MetS prevalence ratio=1.69, $p<0.001$) based on the revised NCEP ATP III criteria, 49.0% (man-to-woman MetS prevalence ratio=1.18, $p<0.001$) based on IDF criteria and 49.1% (man-to-woman MetS prevalence ratio=1.15, $p<0.001$) based on harmonized definition.

Ranking from the 1st (lowest dietary anti-inflammatory load) to the 3rd (highest dietary anti-inflammatory load) tertile, significantly lower levels of inflammatory markers i.e. CRP, IL-6, IL-10, TNF-a (All $ps\leq0.05$) were observed. Additionally, participants assigned to the highest D-AII tertile had significantly lower systolic ($p<0.001$) and diastolic ($p=0.004$) blood pressure,

better lipidemic profile, mainly in terms of HDL-C (*p=0.05*) and TG (*p=0.02*), lower hepatic steatosis index (*p<0.001*) and borderline significantly lower insulin resistance in terms of HOMA-IR (*p=0.06*). As for the MetS prevalence, only when the set of IDF criteria or harmonized definition was used significant trends were observed with participants assigned to the 3rd D-AII tertile having about 10 points lower MetS rate compared with their 1st tertile counterparts (*p<0.001*) (*data not shown on table*).

The multi-adjusted ORs and the corresponding 95% CIs of D-AII in relation to the MetS prevalence defined according to three different set of criteria are summarized in **Table 4.14**. In total sample analysis the D-AII (in terms of tertiles) presented from a borderline significant association in case of the revised NCEP ATP III criteria –defined MetS (i.e. *p<0.10*) to a neutral association (i.e. *p≥0.10*) when the rest definitions were used.

Multi-adjusted associations between MetS components and the D-AII in apparently healthy men and women are summarized in **Table 4.15**. Significant positive and inverse associations were observed in the total sample and principally in women regarding HDL-C and TAG levels, respectively (*all p-values<0.05*). Additionally, fasting glucose levels were inversely associated with D-AII in both men and women (*all p-values<0.05*).

Table 4.14 Total and sex-based sensitivity logistic regression analyses to evaluate the association of dietary anti-inflammatory index tertiles with metabolic syndrome (*n*=2,992).

		Total	**Men**	**Women**	
		OR (95%CI)	OR (95%CI)	OR (95%CI)	
Metabolic syndrome based on **revised NCEP ATP III criteria**		*N, cases*	*N, cases*	*N, cases*	**Model adjusted for**
		2,992/596	*1,479/372*	*1,513/224*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)		0.98 (0.96, 0.99)**	0.98 (0.95, 1.01)	0.98 (0.96, 1.00)	
Model with dietary anti-inflammatory index tertiles					Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, low density lipoprotein.
	1st	*Ref*	*Ref*	*Ref*	
	2nd	0.72 (0.51, 1.01)*	0.71 (0.46, 1.09)*	0.76 (0.41, 1.39)	
	3rd	0.81 (0.58, 1.11)	0.90 (0.61, 1.33)	0.71 (0.38, 1.34)	
Metabolic syndrome based on **IDF criteria**		N, cases	N, cases	N, cases	
		2,992/1,467	*1,479/787*	*1,513/680*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)		0.98 (0.97, 0.99)**	0.98 (0.96, 1.00)*	0.98 (0.96, 1.01)	
Model with dietary anti-inflammatory index tertiles					Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, low density lipoprotein.
	1st	*Ref*	*Ref*	*Ref*	

	2nd	0.97 (0.73, 1.29)	1.15 (0.78, 1.68)	0.80 (0.52, 1.23)	
	3rd	0.96 (0.73, 1.25)	1.06 (0.74, 1.52)	0.89 (0.58, 1.36)	
Metabolic syndrome based on **harmonized definition**		N, cases	N, cases	N, cases	
		2,992/1,470	*1,479/780*	*1,513/690*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)		0.97 (0.96, 0.98)**	0.98 (0.96, 1.00)*	0.98 (0.96, 1.01)	
Model with dietary anti-inflammatory index tertiles					Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, low density lipoprotein.
	1st	*Ref*	*Ref*	*Ref*	
	2nd	0.95 (0.72, 1.26)	1.14 (0.78, 1.63)	0.76 (0.51, 1.19)	
	3rd	0.98 (0.74, 1.25)	1.08 (0.740, 1.52)	0.90 (0.61, 1.34)	

p<0.05, *p<0.10.* ORs and their corresponding 95%CIs were obtained from logistic regression analysis. Higher values of dietary anti-inflammatory index indicated higher dietary anti-inflammatory load (1st tertile corresponded to lower dietary anti-inflammatory index and 3rd tertile to higher dietary anti-inflammatory index values). ***Abbreviations: Confidence Interval (CI); C-Reactive Protein (CRP); Odds Ratio (OR); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Table 4.15 Results from multivariate linear regression analysis regarding the association between dietary anti-inflammatory index (per 1 point raise, range 10-77) and metabolic syndrome components in the total sample and separately for men and women (*n*=2,992).

	Total sample	**Men**	**Women**
	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)
Metabolic syndrome components			
Waist circumference, per 1 cm	+0.04 (0.12)	−0.01(0.10)	+0.07 (0.14)
Fasting blood glucose levels, per 1	−0.41 (0.21)*	−0.38 (0.25)*	−0.45 (0.33)*

mg/dL			
High density lipoprotein, per 1 mg/dL	+0.29 (0.10)*	+0.15 (0.12)	+0.33 (0.13)*
Triglycerides, per 1 mg/dL	−0.28 (0.15)*	−0.22 (0.19)	−0.25 (0.26)*
Systolic blood pressure, per 1 mg/dL	−0.18 (1.10)	−0.07 (0.68)	−0.31 (0.89)
Diastolic blood pressure, per 1 mg/dL	−0.08 (0.10)	−0.04(0.13)	−0.10 (0.23)

***p<0.05*. Beta-Coefficients and their corresponding standard error were obtained from linear regression analysis after adjusting for age, (sex), body mass index, physical activity, smoking habits and C-Reactive Protein.

Results from unadjusted analysis regarding the association between the onset of intermediate cardiometabolic conditions within the 10-year follow-up period, separately for men and women are presented in **Table 4.16**. In particular, women in the lowest category of D-AII had about ten points higher likelihood to develop at least one abnormality in metabolic status (i.e. diabetes and/or hypercholesterolemia and/or hypertension) within the decade compared with their counterparts in the highest level of D-AII (*p=0.03*) while in men no significant trend was observed (*p=0.19*). As for the association with each one of the examined metabolic health status related conditions more evident inverse associations were observed in case of diabetes incidence in both men (borderline significant i.e. *p=0.06*) and women (p=0.04) as well as in case of hypertension in women (*p=0.05*).

Table 4.16 Ten-year transition to intermediate cardiometabolic conditions according to dietary inflammatory index tertiles, in apparently healthy men and women (*n*=1,485)

10-year endpoint, %	**Men, dietary anti-inflammatory index tertiles**			*p-value*	**Women, dietary anti-inflammatory index tertiles**			*p-value*
	1st	*2nd*	*3rd*		*1st*	*2nd*	*3rd*	
Metabolically unhealthy status	*42*	*41*	*39*	*0.19*	*41*	*39*	*31*	*0.03*
Diabetes mellitus	*14*	*13*	*11*	*0.06*	*13*	*13*	*10*	*0.04*
Hypercholesterolemia	41	36	39	*0.58*	29	33	28	*0.43*
Hypertension	30	29	29	*0.90*	28	27	24	*0.05*

P-values were obtained using chi-squared test.

The multi-adjusted association between D-AII and the 10-year transition to unhealthy metabolic conditions is presented in **Table 4.17**. In the total sample analysis, a significant inverse association was revealed only in case of transition to unhealthy metabolic status and diabetes and principally for the highest levels of dietary anti-inflammatory load (i.e. 3rd tertile); more specifically, participants assigned to the 3rd D-AII tertile had about 12% and 45% lower likelihood to develop unhealthy metabolic status and diabetes within the decade compared with their 1st tertile counterparts (All *p-values<0.05*). Stratified analysis based on sex revealed that in case of unhealthy metabolic status the association was retained significant only in women (*p for interaction=0.03*). As for the separate conditions that constitute the "metabolically unhealthy status" in case of diabetes the protective effect of increased anti-inflammatory load was retained in both men and women while, interestingly, in case of hypertension only when the association was examined separately for men and women the D-AII reached the level of significance in woman subgroup (*p for interaction =0.002*). As for hypercholesterolemia no significant trends were observed.

Table 4.17 Total and sex-based sensitivity logistic regression analyses to evaluate the association between dietary anti-inflammatory index tertiles and10-year transition to intermediate cardiometabolic conditions (*n*=1,485).

	Total	**Men**	**Women**	
	OR (95%CI)	OR (95%CI)	OR (95%CI)	
10-year **transition to metabolically unhealthy status**	*N, cases*	*N, cases*	*N, cases*	**Model adjusted for**
	1,485/640	*735/300*	*750/340*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)	0.96 (0.94, 0.98)**	1.00 (0.98, 1.04)	0.97 (0.94, 0.99)**	Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, waist circumference, low density lipoprotein.
Model with dietary anti-inflammatory index tertiles				

	1st	*Ref*	*Ref*	*Ref*	
	2nd	0.86 (0.52, 1.17)	0.80 (0.42, 1.53)	0.78 (0.40, 1.03)*	
	3rd	0.88 (0.73, 0.98)**	0.95 (0.55, 1.70)	0.55 (0.26, 0.90)**	
10-year **diabetes mellitus** incidence		*N, cases*	*N, cases*	*N, cases*	
		1,485/191	*735/100*	*750/91*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)		0.94 (0.91, 0.96)**	1.02 (0.98, 1.06)	0.95 (0.91, 0.96)**	
Model with dietary anti-inflammatory index tertiles					Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, waist circumference, low density lipoprotein.
	1st	*Ref*	*Ref*	*Ref*	
	2nd	0.63 (0.31, 0.85)**	0.74 (0.42, 0.91)**	0.56 (0.24, 0.76)**	
	3rd	0.55 (0.29, 0.77)**	0.62 (0.38, 0.93)**	0.41 (0.18, 0.64)**	
10-year **hypercholesterolemia** incidence		*N, cases*	*N, cases*	*N, cases*	
		1,485/578	*735/323*	*750/255*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)		1.00 (0.97, 1.02)	1.01 (0.96, 1.05)	1.00 (0.96, 1.03)	
Model with dietary anti-inflammatory index tertiles					Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, waist circumference, low density lipoprotein.
	1st	*Ref*	*Ref*	*Ref*	
	2nd	1.43 (0.82, 2.45)	0.95 (0.46, 1.97)	1.21 (0.47, 1.88)	

	3rd	0.83 (0.49, 1.41)	0.68 (0.35, 1.31)	0.95 (0.59, 1.60)	
10-year **hypertension** incidence		*N, cases*	*N, cases*	*N, cases*	
		1,485/495	*735/271*	*750/224*	
Model with dietary anti-inflammatory index, per 1 unit (range 10-77)		1.00 (0.97, 1.02)	1.01 (0.97, 1.05)	0.99 (0.96, 1.03)	
Model with dietary anti-inflammatory index tertiles					Age, sex, body mass index, physical activity level, smoking habits, CRP, HOMA-IR, hepatic steatosis index, waist circumference, low density lipoprotein.
	1st	*Ref*	*Ref*	*Ref*	
	2nd	1.18 (0.63, 2.22)	1.02 (0.42, 2.47)	0.84 (0.36, 0.99)**	
	3rd	1.22 (0.66, 2.23)	0.91 (0.41, 2.04)	0.75 (0.20, 0.95)**	

p<0.05*, **p<0.10*. ORs and their corresponding 95%CIs were obtained from logistic regression analysis. Higher values of dietary anti-inflammatory index indicated higher dietary anti-inflammatory load (1st tertile corresponded to lower dietary anti-inflammatory index and 3rd tertile to higher dietary anti-inflammatory index values). ***Abbreviations: Confidence Interval (CI); C-Reactive Protein (CRP); Odds Ratio (OR); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

4.2.6 The sex-specific role of depressive symptomatology on ten-year first and recurrent CVD event: results from ATTICA and GREECS study

4.2.6.1 Scope and research hypothesis

The primary scope was to investigate the sex-specific effect of depressive symptomatology on 10-year first or recurrent CVD event risk in a sample of apparently healthy individuals (i.e., ATTICA study) and a sample of patients with established ACS (i.e., GREECS study). Secondarily, the mediating effect of commonly suggested, as mediators, factors (i.e., socio-demographic, lifestyle factors, biological and clinical factors) in the CVD risk - depressive symptoms association, for healthy as well as CVD population was quantified. Two a priori research hypotheses were performed: **A.** The magnitude of the association between depressive symptomatology and CVD event differs between men and women as well as between healthy and CVD population, and **B.** The mediating effect of the commonly suggested mediators differs between healthy and cardiac population.

4.2.6.2 Methods and analysis

HRs and corresponding 95%CIs for depressive symptomatology in relation to CVD event were evaluated through multivariate Cox-regression analysis in total sample as well as separately for men and women. Then, focusing on women subgroup, mediation analysis was performed; cox-regression analysis was reapplied through adding mediators, either separately or in combination of two or more. PERM was estimated with pooled HRs following the methodology suggested elsewhere (Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration 2014).

4.2.6.3 Findings

Focusing on ATTICA sample used here (n=845 participants with baseline psychological evaluations), n=43 cases were recorded; of these cases 72% were men and 28% were women ($p=0.004$). Regarding ACS patients in GREECS study, the subsequent CVD event rate was 37.3% (n=811) (38.8% in men and 32.9% in women, $p=0.016$).

Both free-of-CVD and ACS women presented higher ZDRS and CES-D score, respectively, compared with men.

In **Figure 4.8** two epidemiological models for first and recurrent CVD event are illustrated. Apparently healthy participants with depressive symptomatology had 2.5 times higher CVD risk compared with their non-depressed counterparts ($p=0.01$). Men-to-women CVD event rate ratio in subgroup with depressive symptoms (n=85) was slightly lower to 1 (0.80) when the respective ratio for the total ATTICA sample (n=845) was consistently higher to 1 (2.30); pointing that depressive symptomatology attenuated the women privilege over CVD onset. Similarly, ACS patients with depressive symptomatology had 35% higher risk to suffer from recurrent events within the decade ($p<0.001$). Men-to-women recurrent CVD event rate ratio for

participants with depressive symptomatology (*n*=1,433) was quite close yet lower to 1 (0.90) with the respective ratio for total sample indicating that men exhibited higher incidence than women (1.18). Interaction analysis revealed that sex interacted with depressive symptoms on first and recurrent CVD event (*p-values for interaction<0.10*). Despite the marginally significant interacting effect of gender on the association between depressive symptomatology and CVD incidence or recurrence, the obtained knowledge and evidence on this issue justifies the stratification of the sample. Stratified analysis revealed that depressive-symptomatology aggravating effect was retained in both men and women, yet it remained significant only for woman.

Depressive symptomatology and 10-year first and recurrent CVD event: a sex-based stratified analysis from ATTICA & GREECS studies

Examined variable	Sex	10-year CVD event	Cases, % (n)	Men-to-women CVD incidence ratio	HR (95%CI)
Depressive symptomatology, (yes vs. no)*	Men	First	12 (3)	-	2.10 (0.95, 10.30)
		Recurrent	38 (414)	-	1.25 (0.94, 1.66)
	Women	First	15 (9)	-	2.72 (1.50, 9.12)
		Recurrent	42 (149)	-	1.35 (1.14, 1.83)
	Total sample	**First**	**14 (12)**	**0.80**	**2.52 (1.40, 9.29)**
		Recurrent	**39 (563)**	**0.90**	**1.31 (1.01, 1.69)**

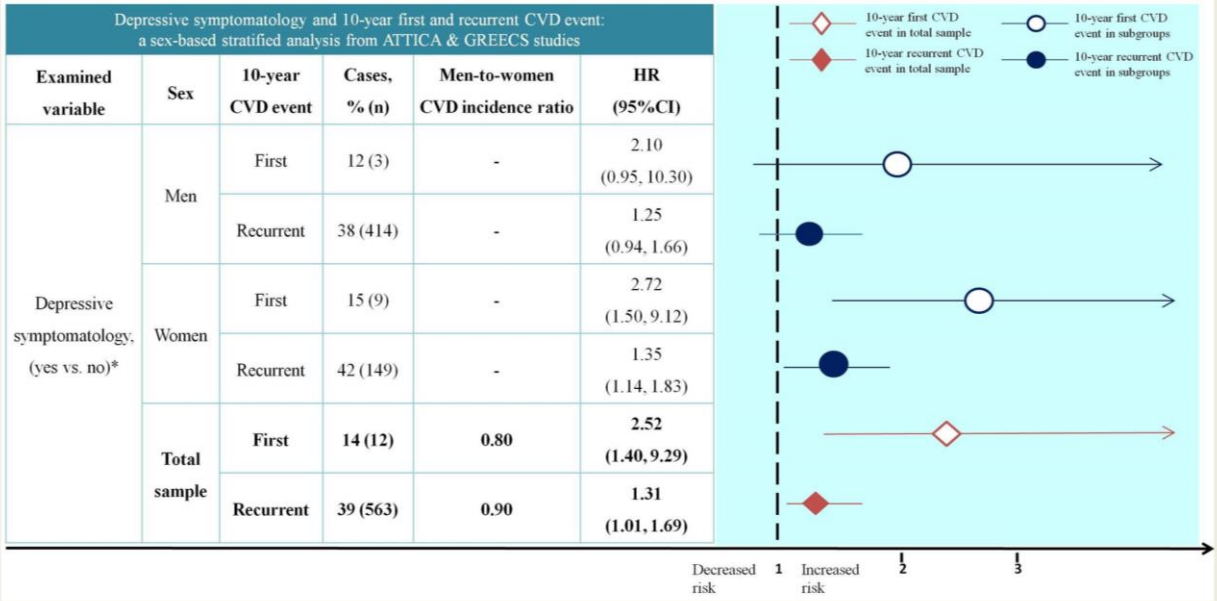

Figure 4.8 Depressive symptomatology and 10-year first and recurrent CVD risk: a sex-based stratified analysis from ATTICA and GREECS studies.

Abbreviations: CVD, Cardiovascular Disease; HR, Hazard Ratio; 95%CI, 95% Confidence Interval. *Depressive symptomatology was defined as Zung Self-Rating Depression Scale (range 20-80)=45-80 in ATTICA and Centre of Epidemiological Studies-Depressive symptoms scale (range 0-60)=7-60 in GREECS. Hazard ratios (dots) and corresponding 95%CIs (horizontal lines) for depressive symptomatology (yes vs. no) were obtained from Cox-regression models adjusted for age, family CVD history, State Anxiety sub-scale of the Spielberger State-Trait Anxiety Inventory, MedDietScore, sleeping duration, physical activity, current smoking, financial status, educational status, diabetes, hypertension, waist-to-hip ratio, high and low density lipoproteins, triglycerides, C-Reactive Protein, Interleukin 6, with 10-year first CVD event as dependent variable and age, family CVD history, MedDietScore, physical activity, current smoking, financial status, educational status, diabetes, hypertension, hypercholesterolemia, body mass index, discharge status, individual CVD history and adherence to medication, with 10-year recurrent CVD event as dependent variable.

Since the independent effect of depressive symptomatology on 10-year first and recurrent CVD event was retained only in women, mediation analysis for the association between these two disease states was performed for this subgroup.

Results for apparently healthy women are illustrated in **Figure 4.9**. In particular, HR of 10-year first CVD event for depressive symptomatology was decreased by 35% (23%, 44%) after adjusting for lifestyle, sociodemographic, clinical, anthropometric factors as well as lipid and inflammatory markers. The factors mostly accounted for the excess depressive-symptomatology

risk on CVD onset, close to the overall, were C-reactive protein (PERM=38% 95%CI 31%, 51%), waist-to-hip ratio (PERM=35% 95%CI 31%, 42%) and diabetes (PERM=32% 95%CI 27%, 36%). Among lipid markers, the biggest mediating effect was observed by high density lipoprotein (PERM=28% 95%CI 25%, 32%) and triglycerides (PERM=26% 95%CI 22%, 33%). As for the non-clinical/biochemical factors, financial status (PERM=23% 95%CI 15%, 31%) presented the biggest mediating effect followed by educational status, adherence to Mediterranean diet and sleep duration, accounting on average for 20% of the examined association.

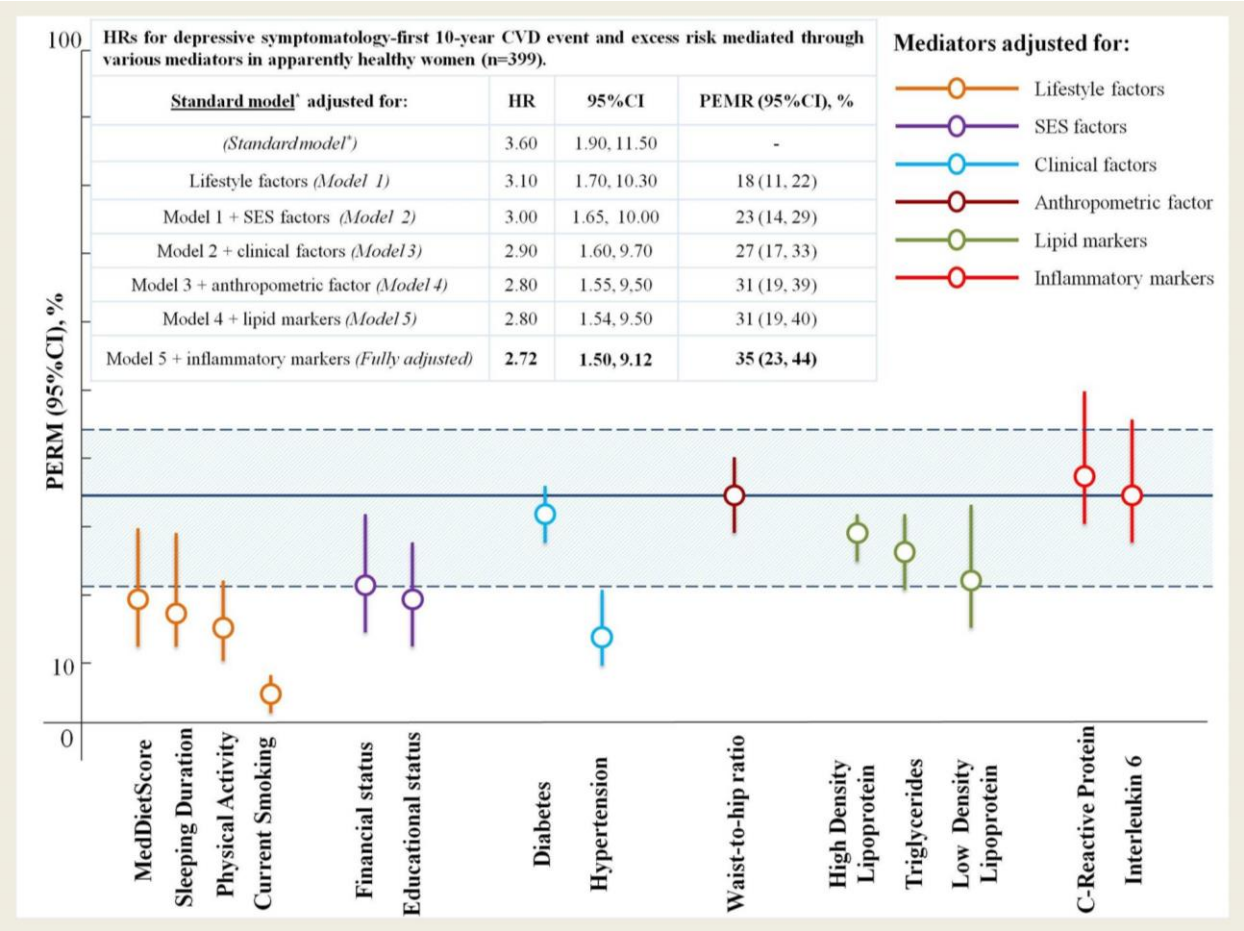

Figure 4.9 Percentage of excess 10-year first CVD event risk for depressive symptomatology mediated by different risk factors in apparently healthy women [*n*=399].

Abbreviations: CVD, Cardiovascular Disease; HR, Hazard Ratio; 95%CI, 95% Confidence Interval, PERM, Percentage of excess mediated risk; SES, Socioeconomic. *Standard model was adjusted for age, family CVD history and State Anxiety sub-scale of the Spielberger State-Trait Anxiety Inventory. PERM and corresponding 95%CIs for depressive symptomatology were evaluated for a. isolated mediators (illustrated as dots and vertical lines) and b. combinations of mediators (presented on Table). Sketched frame represents overall PERM and 95%CI.

Mediation-analysis outcomes for ACS women patients are illustrated in **Figure 4.10**. Diabetes and adherence to medication had the biggest separate mediating effect (PERM=40% 95%CI 27%, 51% and PERM=40% 95%CI 29%, 53%, respectively) followed by hypertension (PERM=38% 95%CI 27%, 48%). Interestingly, disease burden indicators, i.e. patients' CVD history and discharge status, presented a very low mediating effect size which did not exceed, on

average, the 10%. Among the examined lifestyle factors, current smoking was revealed the strongest mediator, accounting for 33% (19%, 39%) of depressive symptomatology aggravating effect on ACS prognosis. Anthropometric parameters in terms of BMI seemed to modestly mediate the examined association (PERM=29% 95%CI 19%, 35%). Overall, 46% (23%, 53%) of the excess 10-year recurrent CVD event depressive symptomatology risk was attributed to all adjustment factors.

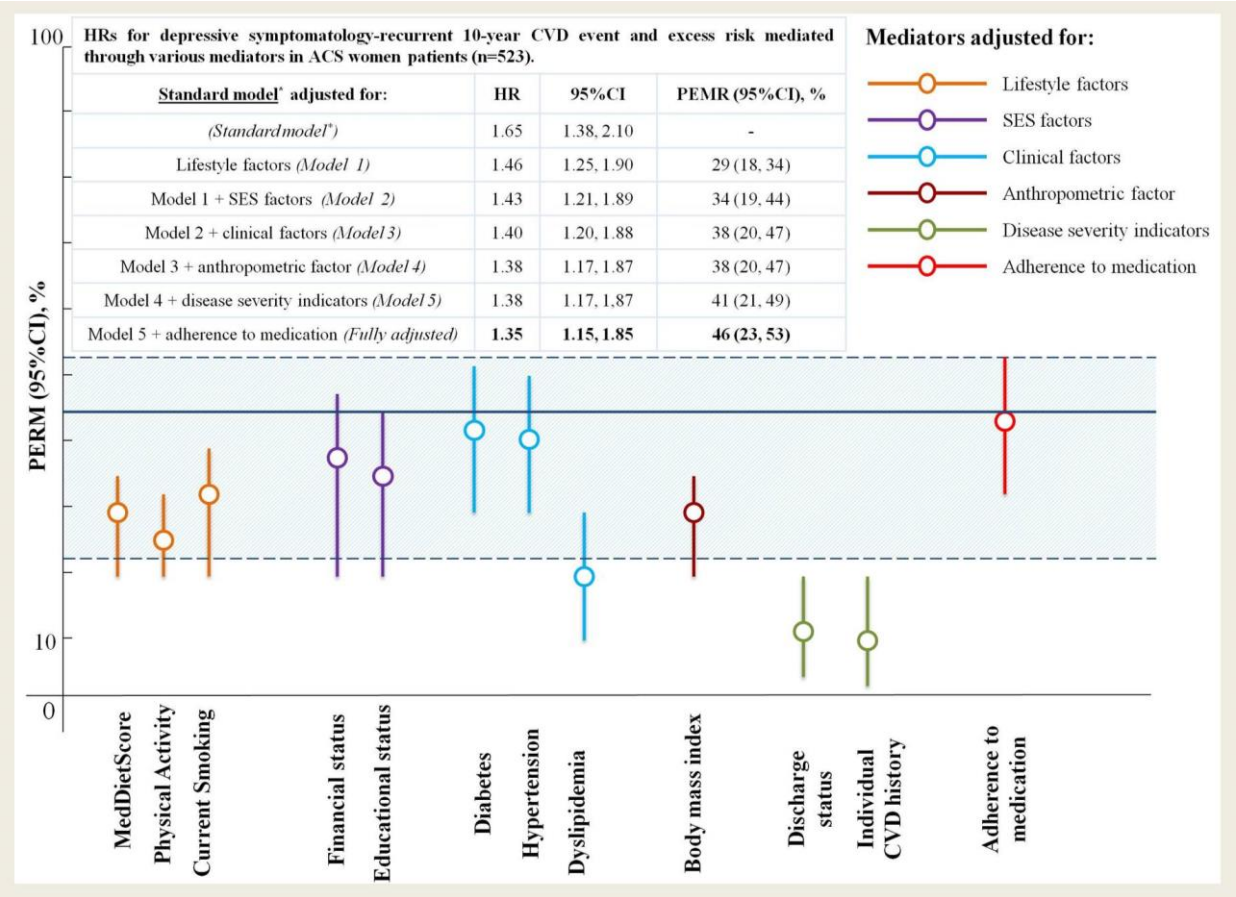

Figure 4.10 Percentage of excess 10-year recurrent CVD event risk for depressive symptomatology mediated by different risk factors in Acute Coronary Syndrome women patients [n=523].

Abbreviations: CVD, Cardiovascular Disease; HR, Hazard Ratio; 95%CI, 95% Confidence Interval, PERM, Percentage of excess mediated risk; SES, Socioeconomic. *Standard model was adjusted for age and family CVD history. PERM and corresponding 95%CIs for depressive symptomatology were evaluated for a. isolated mediators (illustrated as dots and vertical lines) and b. combinations of mediators (presented on Table). Sketched frame represents overall PERM and 95%CI.

4.2.7 Meat consumption, depressive symptomatology and sex in relation on ten-year first CVD event: results from ATTICA study

4.2.7.1 Scope and research hypothesis

The primary scope here was to evaluate the association between meat consumption with depressive symptomatology in apparently healthy individuals. The secondary scope was to estimate the mediating effect of meat intake on the association of depressive symptomatology with 10-year CVD onset. Three a priori research hypotheses were performed; *firstly*, meat

consumption is positively associated with the existence of depressive symptomatology, *secondly*, meat consumption alone mediates the association between depressive symptomatology and CVD onset and *thirdly*, the genetic, metabolic and lifestyle differences between men and women result in sex-specific associations between this dietary factor and the examined endpoints; in particular, it was hypothesized that meat intake would be of high importance for women's psychological health due to crucial micronutrients that are usually deficient in women (e.g., iron).

4.2.7.2 Methods and statistical analysis

'Red meat' was defined as fresh meat from beef, veal, lamb, or pork. 'White meat' was defined as poultry (chicken and turkey) and rabbit. 'Processed meat' was defined as any meat preserved by smoking, curing or salting or addition of chemical preservatives, such as bacon, salami, sausages, hot dogs or luncheon meats. 'Total meat' was defined as the total of these three categories. Frequency of consumption was quantified in terms of servings/month or servings/week a food was consumed. Logistic regression models were fitted to assess the relationship between the level of consumption of total meat and its subtypes i.e. red, white, processed and the prevalence of life-time depressive symptomatology. ORs and their 95%CIs were calculated considering the lowest tertile as the reference category. In addition to this, the potential non-parametrical non-linear association between meat consumption and its subtypes and prevalent depressive symptomatology was calculated with restricted cubic splines. Tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. The results were adjusted for the same potential confounding factors as the main logistic regression analysis. Multi-adjusted Cox-regression analysis was also performed to evaluate the association between 10-year first CVD event and depressive symptomatology score (i.e. per 10 points raise) according to meat consumption tertiles and results are presented as HR and the corresponding 95%CI.

4.2.7.3 Results

The baseline sociodemographic, clinical, anthropometric, biochemical, lifestyle and psychosocial characteristics of men and women from the ATTICA study across the total meat intake tertiles are summarized in **Table 4.18.** Focusing on the depressive symptomatology scales it was revealed that participants of both studies assigned in the 2nd tertile i.e. moderate total meat intake, had the lowest scoring (*all p-values<0.05*). In case of anxiety symptomatology, no significant trends were observed (*p>0.05*).

Table 4.18 Baseline sociodemographic, clinical, anthropometric, biochemical, lifestyle and psychosocial characteristics of apparently healthy men and women from the ATTICA study according to total meat intake categories (*n*=845).

Baseline characteristics	**Total meat intake tertiles**			
	1st tertile	**2nd tertile**	**3rd tertile**	***p for trend***
N	285	288	272	-
Total meat intake, servings/month	<6	6-13	>13	-
Sociodemographic factors				
Age, years	42 (10)	39 (10)	35 (11)	*<0.001*
Male sex, %	45	54	64	*0.001*
Anthropometric factors				
Body mass index, kg/m^2	25.2 (3.9)	26.0 (4.5)	25.9 (4.9)	*0.06*
Waist circumference, cm	86 (14)	90 (16)	100 (15)	*0.01*
Lifestyle factors				
Physical activity, %	49	45	44	*0.01*
MedDietScore, range 0-55	26 (7)	27 (7)	29 (10)	*0.005*
Current smoking, %	45	43	53	*0.12*
Clinical factors				
History of hypertension, %	25	29	22	*0.15*
History of diabetes mellitus, %	3	4	7	*0.16*
History of hypercholesterolemia, %	33	32	25	*0.18*
Family CVD history, %	33	26	24	*0.05*
Inflammation markers				
C-Reactive Protein, mg/L	1.8 (2.3)	1.9 (2.5)	2.0 (2.5)	*0.78*
Interleukin 6, pg/dL	1.4 (0.3)	1.4 (0.3)	1.3 (0.3)	*0.18*
White blood cells, 10^{3}counts	8.0 (2.1)	6.7 (1.9)	6.7 (2.0)	*0.25*
Glucose/insulin homeostasis markers				
HOMA-IR	3.1 (2.5)	3.0(1.8)	3.3 (2.6)	*0.40*
Psychosocial factors				
Zung Depression Scale, range 20-80	39 (8)	26 (7)	45 (8)	*<0.001*
Anxiety symptomatology, STAI scale (range 20-80)	39 (9)	40 (8)	40 (9)	*0.56*

Data are presented as mean ± standard deviation (SD) or median (Interquartile Range) if normality was not met. P-values were obtained using one way ANOVA for the normally distributed variables (age, body mass index), Kruskal Wallis test for the rest quantitative variables and chi-squared test for categorical variables. ***Abbreviations***: Cardiovascular disease (CVD); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Nested logistic regression models to evaluate the association between total meat intake and its subtypes i.e. red meat, white meat and processed meat, with life-time depressive symptomatology prevalence of apparently healthy subjects are presented in **Table 4.19**. In the unadjusted models, it was revealed that participants assigned to the 2nd tertile of total meat intake had about 31% lower likelihood to be detected with depressive symptomatology compared with their 1st tertile counterparts in contrast with participants in 3rd tertile who had 20% higher depression likelihood compared with the aforementioned reference group. In the fully adjusted model where various confounders, including general dietary habits and inflammatory markers, were taken into account, the protective effect of moderate over low meat consumption was retained; participants in the 2nd tertile had about 18% lower likelihood to be depressed. On the other side, increased meat intake in relation to depressive symptomatology prevalence did not reach the level of significance. Similar results were obtained in case of red meat intake with participants in the 2nd tertile having about 21% higher likelihood to be depressed compared with 1st tertile counterparts. Models were repeated after setting participants of 2nd meat intake tertile as reference group to be examined against participants assigned to 3rd tertile; multivariate analysis (on the adjustment basis of Model 4) revealed that participants with the highest reported intake in total meat and red meat had about 50% (OR 1.50, 95%CI (1.07, 2.91)) and 61% (OR 1.61, 95%CI (1.11, 3.14)), respectively, higher likelihood for depressive symptomatology compared with participants in the 2nd tertile (*data not shown*). In case of white meat intake, no significant associations were observed while when the processed meat was examined, ranking from 1st to 3rd tertile a significantly increased likelihood for depressive symptomatology was observed.

Table 4.19 Odds ratios and 95% Confidence Intervals for the association between meat intake and life-time prevalence of depressive symptomatology in apparently healthy men and women (*n*=853).

	Total meat intake, tertiles			
	1st	**2nd**	**3rd**	*p for trend*
Servings/month	<6	6-13	>13	
N	285	288	272	
Life-time prevalence of depressive symptomatology, %	14	9	15	*0.02*
Model 1	1 (ref)	**0.69 (0.38, 0.85)**	**1.20 (1.05, 1.89)**	*0.118*
Model 2	1 (ref)	**0.72 (0.40, 0.93)**	**1.15 (1.03, 1.76)**	*0.323*
Model 3	1 (ref)	**0.80 (0.59, 0.97)**	1.06 (0.98, 1.53)	*0.351*

Model 4	1 (ref)	**0.82 (0.60, 0.97)**	1.01 (0.93, 1.45)	*0.358*
		Red meat intake, tertiles		
	1st	**2nd**	**3rd**	*p for trend*
Servings/month	<4	4-10	>10	
N	285	288	272	
Life-time prevalence of depressive symptomatology, %	14	9	14	*0.003*
Model 1	1 (ref)	**0.66 (0.38, 0.87)**	1.09 (0.43, 2.74)	
Model 2	1 (ref)	**0.70 (0.41, 0.92)**	1.05 (0.35, 2.67)	
Model 3	1 (ref)	**0.75 (0.42, 0.95)**	0.94 (0.36, 2.41)	
Model 4	1 (ref)	**0.79 (0.45, 0.96)**	0.87 (0.34, 2.26)	
		White meat intake, tertiles		
	1st	**2nd**	**3rd**	*p for trend*
Servings/month	<1	1-4	>4	
N	285	288	272	
Life-time prevalence of depressive symptomatology, %	13	12	13	*0.13*
Model 1	1 (ref)	1.97 (0.45, 2.35)	2.19 (0.43, 3.39)	
Model 2	1 (ref)	1.94 (0.44, 2.31)	2.15 (0.40, 3.35)	
Model 3	1 (ref)	1.93 (0.42, 2.29)	2.07 (0.38, 3.31)	
Model 4	1 (ref)	1.87 (0.37, 2.22)	2.01 (0.29, 3.25)	
		Processed meat intake, tertiles		
	1st	**2nd**	**3rd**	*p for trend*
Servings/week	<1	1-2	>2	
N	285	288	272	
Life-time prevalence of depressive symptomatology, %	9	12	14	*<0.001*
Model 1	1 (ref)	**1.25 (1.11, 1.85)**	**1.33 (1.08, 1.75)**	
Model 2	1 (ref)	**1.20 (1.08, 1.82)**	**1.28 (1.06, 1.62)**	
Model 3	1 (ref)	**1.16 (1.05, 1.63)**	**1.22 (1.04, 1.58)**	
Model 4	1 (ref)	**1.15 (1.01, 1.55)**	1.29 (0.95, 1.56)	

Odds Ratios and the corresponding 95% Confidence Intervals were obtained through logistic regression analysis adjusted for sociodemographic, clinical, lifestyle and biochemical factors as follows: *Model 1* (crude model); *Model 2*: Model 1 plus age and

sex; *Model 3*: Model 2 plus body mass index, waist circumference, physical activity, current smoking, MedDietScore, history of hypertension, diabetes mellitus and hypercholesterolemia, family history of cardiovascular disease; *Model 4*: Model 3 plus C-Reactive protein, interleukin 6, white blood cells and Homeostatic Model Assessment of Insulin Resistance. **Bold** indicates statistical significance (*p-value<0.05*).

The hypothesis for potential interaction between meat consumption and sex on the prevalent depressive symptomatology was as well examined and results are presented in **Table 4.20**. Stratified analysis revealed that the aforementioned associations corresponding to total meat and red meat intake were retained particularly in women while in men no significant trends were observed (all *p-values for interaction<0.05*). As for the processed meat intake, this retained its independently aggravating effect on psychological health only in men.

Table 4.20 Sex-based stratified multivariate logistic regression analysis for the association between meat intake and its subtypes on life-time prevalence of depressive symptomatology in apparently healthy individuals (*n*=845).

	Men	**Women**	***p for interaction***
	n=446	*n*=399	
	OR (95%CI)	OR (95%CI)	
Model with total meat tertiles			
1st	Ref	Ref	*0.03*
2nd	1.05 (0.89, 1.40)	**0.65 (0.45, 0.99)**	
3rd	1.15 (0.87, 2.10)	0.95 (0.69, 3.20)	
Model with red meat tertiles			
1st	Ref	Ref	*0.001*
2nd	0.85 (0.53, 1.05)	**0.71 (0.55, 1.00)**	
3rd	1.37 (0.54, 2.31)	0.67 (0.29, 2.90)	
Model with white meat tertiles			
1st	Ref	Ref	*0.25*
2nd	1.55 (0.21, 1.95)	1.95 (0.55, 2.30)	
3rd	1.88 (0.20, 3.10)	2.10 (0.45, 3.51)	
Model with processed meat tertiles			*0.12*
1st	Ref	Ref	

2nd	**1.80 (1.54, 3.30)**	1.01 (0.77, 2.45)
3rd	2.06 (0.76, 3.82)	1.10 (0.90, 1.98)

Odds Ratios (OPs) and the corresponding 95% Confidence Intervals (95%CI) were obtained through logistic regression analysis adjusted for age, body mass index, waist circumference, physical activity, current smoking, MedDietScore, history of hypertension, diabetes mellitus and hypercholesterolemia, family history of cardiovascular disease, C-Reactive protein, interleukin 6, white blood cells and Homeostatic Model Assessment of Insulin Resistance. **Bold** indicates statistical significance (*p-value<0.05*).

To account for non-linear associations between meat consumption and prevalent depressive symptomatology, the restricted cubic spline analysis was applied. A significant suggestion for U-shape trends was revealed in case of total meat (*p=0.02*) and red meat consumption (*p=0.001*) as suggested in logistic regression analyses. Results are illustrated in **Figure 4.11 (A)** (for total meat) and **Figure 4.11 (B)** (for red meat). Moderate consumption of total meat (around 10-12 serving/month) and red meat (around 6-8 servings/months) was inversely associated with depressive symptomatology prevalence while a steep increase in odds of depressive symptoms was revealed in higher levels of consumption (i.e. >15 servings/month of total meat and >10 servings/month of red meat).

The mediating effect of meat consumption on the association between depressive symptomatology and CVD incidence was also evaluated in the total sample as well as separately for men and women. In the formal analysis of interaction, a significant interacting effect of meat intake on the association between depressive symptomatology prevalence and 10-year first CVD event was observed (*p for interaction=0.03*). Hence, the association between depressive symptomatology and 10-year first CVD incidence was examined per meat intake and meat-subtypes consumption tertile and results are presented in **Table 4.21**. Stratified analysis revealed that the independent aggravating effect of depressive symptomatology on 10-year risk to develop CVD was retained only in participants at the lowest level of consumption in terms of total meat and red meat intake (*all p-values<0.05*) yet significance was lost when it came to higher level of consumption. Further sensitivity analysis with sex as strata revealed that the aforementioned trends retained their significance only in woman subgroup. As for the processed meat intake, the aggravating effect of depressive symptomatology was retained only in the context of consumption levels on a weekly basis i.e. 2nd and 3rd tertile (*all p-values<0.05*). These associations were retained significant in both sexes.

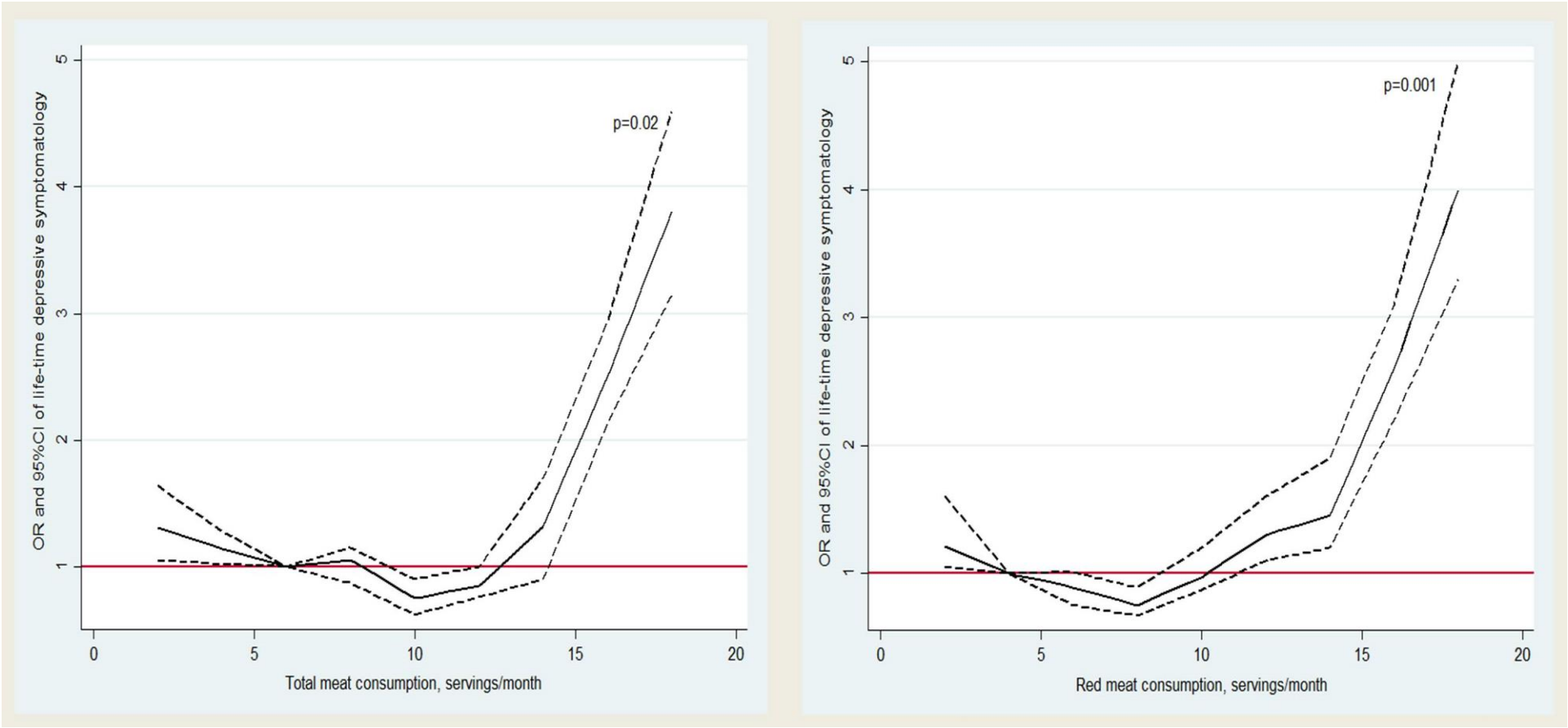

Figure 4.11 **A.** Spline regression model of the odds ratio of depressive symptomatology according to total meat consumption (dash lines represent 95% Confidence Interval). **B.** Spline regression model of the odds ratio of depressive symptomatology according to red meat consumption (dash lines represent 95% Confidence Interval).

Total meat intake tertiles were defined as follows: <6, 6-13, >13 servings/month. Red meat intake tertiles were defined as follows: <4, 4-10, >10 servings/month.

Table 4.21 Multi-adjusted Cox regression analysis models to evaluate the association of depressive symptomatology score with 10-year cardiovascular disease incidence, according to meat intake, in apparently healthy men and women (*n*=845).

	Total meat intake, tertiles		
	1st	**2nd**	**3rd**
Zung depression scale, *per 10 points raise*	*n*=285 HR (95%CI)	*n*=288 HR (95%CI)	*n*=272 HR (95%CI)
Total sample, *n*=845	**1.28 (1.05, 2.83)**	1.38 (0.66, 2.59)	2.02 (0.38, 2.51)
Men, , *n*=446	1.34 (0.34, 4.80)	1.21 (0.38, 3.39)	2.15 (0.43, 4.08)
Women, *n*=399	**2.20 (1.66, 5.10)**	1.34 (0.38, 4.80)	1.48 (0.73, 5.19)
	Red meat intake, tertiles		
	1st	**2nd**	**3rd**
Zung depression scale, *per 10 points raise*	*n*=285 HR (95%CI)	*n*=288 HR (95%CI)	*n*=272 HR (95%CI)
Total sample, *n*=845	**1.21 (1.03, 2.77)**	1.34 (0.66, 2.83)	1.96 (0.38, 3.31)
Men, , *n*=446	1.42 (0.51, 4.72)	1.31 (0.48, 3.25)	2.19 (0.41, 4.18)
Women, , *n*=399	**2.31 (1.75, 4.94)**	1.07 (0.52, 4.02)	1.23 (0.61, 4.95)
	White meat intake, tertiles		
	1st	**2nd**	**3rd**
Zung depression scale, *per 10 points raise*	*n*=285 HR (95%CI)	*n*=288 HR (95%CI)	*n*=272 HR (95%CI)
Total sample, *n*=845	1.25 (0.66, 2.63)	1.39 (0.73, 2.76)	1.88 (0.42, 3.29)
Men, , *n*=446	1.31 (0.47, 4.61)	1.29 (0.39, 3.62)	2.15 (0.51, 4.20)
Women, , *n*=399	2.22 (0.89, 4.01)	1.11 (0.62, 4.12)	1.24 (0.62, 4.90)
	Processed meat intake, tertiles		
	1st	**2nd**	**3rd**
Zung depression scale, *per 10 points raise*	*n*=285 HR (95%CI)	*n*=288 HR (95%CI)	*n*=272 HR (95%CI)
Total sample, *n*=845	**0.70 (0.35, 0.97)**	1.22 (0.57, 2.12)	**2.01 (1.20, 3.38)**
Men, , *n*=446	**0.61 (0.42, 0.99)**	1.12 (0.45, 3.14)	**2.20 (1.22, 4.10)**
Women, , *n*=399	0.88 (0.24, 1.10)	1.33 (0.64, 3.99)	**1.89 (1.34, 3.95)**

Hazard ratios (HR) and their corresponding 95% Confidence Intervals (95%CI) were obtained from Cox regression analysis adjusted for age, (sex), body mass index, waist circumference, physical activity, current smoking, MedDietScore, history of hypertension, diabetes mellitus and hypercholesterolemia, family history of cardiovascular disease, C-Reactive protein, interleukin 6, white blood cells and Homeostatic Model Assessment of Insulin Resistance. **Bold** indicates statistical significance (*p-value<0.05*).

4.3 The sex-specific role of body composition in relation to ten-year first and recurrent CVD incidence

4.3.1 The sex-specific role of predicted lean and fat mass in relation to ten-year first and recurrent CVD incidence

4.3.1.1 Scope and research hypothesis

The scope here was to examine the association of predicted lean and fat mass on ten-year first and recurrent CVD incidence separately in men and women of ATTICA and GREECS study samples. Two a priori research hypotheses were performed; *firstly*, the inverse association of predicted lean mass, highly supported in cardiac patients at advanced age, will be replicated for middle-aged apparently healthy subjects as well and *secondly*, the anatomic, biological and lifestyle differences between men and women will result in sex-mediated associations between body composition estimations and CVD onset or recurrence.

4.3.1.2 Methods ans analysis

Due to the lack of imaging data for body composition i.e. lean and fat mass (kg), sex-specific population-based equations were used to predict body composition, recently validated by the investigators of the National Health and Nutrition Examination Survey study (Lee et al 2017). For the *ATTICA* participants, the equations adjusted for weight, height and waist circumference were used, since these were suggested to have the best predictive and discrimination ability against CVD risk factors. For the *GREECS study*, because of the lack of waist circumference measurements, lean and fat mass were calculated based on weight- and height- adjusted equations. The population-based equations used here can be found elsewhere (Lee et al 2017). Then, the generated body composition estimations for lean and fat mass (kg) was standardized through dividing by height (in meters squared), to create LMI and FMI (kg/m^2); increased values in these indexes corresponded to increased lean and fat mass, respectively. Participants were separated according to sex-specific LMI and FMI tertiles.

HRs and their corresponding 95%CIs of LMI and FMI tertiles in relation to CVD endpoints were evaluated through multivariable Cox-regression analysis. The concordance statistics, i.e., C-statistic, was used to evaluate the predictive accuracy of multivariate models adjusted for various lipid markers against CVD event. C-indexes and the corresponding 95%CIs were equal to the areas under the curve obtained from ROC analysis. Curves were constructed by plotting sensitivity against (1-specifity). Significance of the changes in C-index was tested by differences in 2 log likelihood of regression models with and without anthropometric measurements.

4.3.1.3 Findings

The baseline characteristics of ATTICA and GREECS men and women participants according to their BMI status can be found in **Table 4.22**.

Table 4.22 Baseline sociodemographic, anthropometric and clinical characteristics of men and women from the ATTICA (*n*=2,020) and GREECS (*n*=2,172) study according to body mass index status, respectively.

	ATTICA study				GREECS study			
Men	**Normalweight**	**Overweight**	**Obese**	***p***	**Normalweight**	**Overweight**	**Obese**	***p***
N	*267*	*537*	*202*		*400*	*875*	*374*	
Age, years	42 (14)	46 (11)	48 (11)	<0.001	67 (13)	64 (12)	61 (12)	*<0.001*
Body fat mass index, kg/m^2	5.5 (1.2)	7.7 (1.2)	10.9 (2.0)	<0.001	6.1 (0.7)	8.2 (0.7)	11.0 (1.4)	*<0.01*
Body lean mass index, kg/m^2	17.1 (1.1)	18.9 (1.2)	21.5 (1.9)	<0.001	16.4 (0.7)	18.3 (0.7)	21.0 (1.3)	*<0.001*
Waist circumference, cm	87.5 (9.0)	97.9 (8.9)	111.7 (12.2)	<0.001	-	-	-	-
Current smoking, %	48	46	48	0.12	36	38	37	*0.94*
History of hypertension, %	26	39	56	<0.001	41	48	52	*0.01*
History of diabetes mellitus, %	5	8	15	0.003	25	32	27	*0.03*
History of hypercholesterolemia, %	38	49	51	0.007	35	48	48	*<0.001*
Family CVD history, %	24	27	29	0.64	33	36	41	*0.10*
Baseline CVD history, %	0	0	0	-	40	42	43	*0.75*
Women	**Normalweight**	**Overweight**	**Obese**	***p***	**Normalweight**	**Overweight**	**Obese**	***p***
N	*548*	*312*	*154*		*145*	*246*	*132*	

Age, years	40 (13)	49 (13)	51 (13)	*<0.001*	72 (12)	70 (10)	68 (12)	*0.02*
Body fat mass index, kg/m^2	7.6 (1.1)	11.0 (0.9)	15.3 (2.1)	*<0.001*	8.6 (1.1)	11.6 (0.9)	15.6 (2.2)	*<0.001*
Body lean mass index, kg/m^2	14.0 (0.5)	15.6 (0.5)	17.8 (1.1)	*<0.001*	13.5 (0.7)	15.1 (0.5)	17.4 (1.2)	*<0.001*
Waist circumference, cm	76.3 (9.7)	87.9 (8.3)	101.1 (12.6)	*<0.001*	-	-	-	-
Current smoking, %	44	37	42	*<0.001*	19	11	9	*0.03*
History of hypertension, %	14	32	47	*<0.001*	64	70	76	*0.13*
History of diabetes mellitus, %	3	6	14	*<0.001*	34	35	40	*0.57*
History of hypercholesterolemia, %	31	49	46	*<0.001*	41	51	50	*0.24*
Family CVD history, %	30	35	21	*<0.001*	33	30	43	*0.03*
Baseline CVD history, %	0	0	0	-	62	63	64	*0.94*

Data are presented as mean ± standard deviation (SD) or median (Interquartile Range) if normality was not met. P-values were obtained using one way ANOVA for normally distributed variables (age, body mass index), Kruskal Wallis test for the rest quantitative variables and chi-squared test for categorical variables. Body fat mass index (FMI) was created to reflect total body fat mass and body lean mass index (LMI) to reflect total body lean mass (indirectly calculated through population formulas). ***Abbreviations***: Cardiovascular disease (CVD)

In

Table 4.23 ten-year CVD event rates are presented separately, in apparently healthy individuals (ATTICA study) and in ACS patients (GREECS study), overall, as well as according to BMI and body composition estimations. Apparently healthy men with FMI in 3rd tertile were about 3 times more likely to suffer from a cardiac episode with a similar trend observed in women. As for ACS patients, women in the 2nd FMI tertile exhibited the lowest CVD recurrence rate with men of the same tertile having about 45% higher likelihood to suffer from a new episode. In the ATTICA sample, men with low LMI were about 2.38 times more likely to develop CVD compared with women of the same tertile. When it came to 2nd and 3rd tertile the consistent exceeding of men on CVD event rate over women was significantly alleviated. In the patients sample of GREECS study, different trends were observed with women in the 2nd LMI tertile presenting the best ACS prognosis revealing a U-shape trend which was not retained in case of their men counterparts. In the ATTICA sample, even in high FMI men with LMI within the 3rd tertile had closer-to-women 10-year CVD event rate (i.e. 1.49). As for the ACS patients, even if in the overall sample men's and women's CVD recurrence rate was quite similar in the context of high lean yet low fat mass, women presented even better ACS prognosis.

Table 4.23 Unadjusted cardiovascular disease incidence rate in men and women from the ATTICA and GREECS study according to their body mass and body composition indexes.

ATTICA study (Outcome: First fatal/non-fatal CVD event)

CVD incidence rate per 100 participants	**Total sample**	**Men**	**Women**	*Men-to-women CVD incidence rate ratio*
	n=2,020	*n*=1,005	*n*=1,015	
Overall	15.7	19.7	11.7	*1.66*
BMI categories				
Normalweight	9.6	14.4	7.2	*2.00*

Overweight	18.6	20.2	15.8	*1.27*
Obese	23.6	26.0	20.6	*1.26*
Body fat mass index tertiles				
1st	9.4	13.8	5.2	*2.65*
2nd	16.1	21.1	11.0	*1.91*
3rd	19.9	19.9	16.3	*1.02*
Body lean mass index tertiles				
1st	17.1	23.8	12.0	*2.38*
2nd	15.0	16.9	13.0	*1.30*
3rd	11.9	18.3	9.7	*1.88*
Body fat and lean mass status				
Low lean/low fat mass	9.9	15.1	5.7	*2.64*
High lean/low fat mass	8.4	20.2	6.9	*2.92*
Low lean/high fat mass	27.9	11.9	19.2	*1.75*
High lean/high fat mass	15.7	19.0	12.7	*1.49*
GREECS study (Outcome: Recurrent fatal/non-fatal CVD event)				
CVD incidence rate per 100 participants	**Total sample**	**Men**	**Women**	*Men-to-women CVD incidence rate ratio*
	n=2,172	*n*=1,649	*n*=523	
Overall	37.3	38.8	32.9	*1.17*

BMI categories				
Normalweight	36.7	38.7	33.8	*1.14*
Overweight	35.8	39.2	26.9	*1.45*
Obese	38.0	37.2	35.2	*1.05*
Body fat mass index tertiles				
1st	36.2	37.5	33.1	*1.13*
2nd	38.0	38.9	27.6	*1.40*
3rd	37.3	39.6	36.4	*1.08*
Body lean mass index tertiles				
1st	38.8	39.5	34.0	*1.16*
2nd	35.8	40.2	28.5	*1.41*
3rd	36.7	36.2	34.7	*1.04*
Body fat and lean mass status				
Low lean/low fat mass	37.5	39.7	30.8	*1.28*
High lean/low fat mass	41.8	44.6	44.4	*1.01*
Low lean/high fat mass	31.0	32.8	23.5	*1.39*
High lean/high fat mass	36.5	36.7	35.9	*1.02*

Unadjusted 10-year CVD rates were obtained through chi-squared test. Body fat mass index (FMI) was created to reflect total body fat mass and body lean mass index (LMI) to reflect total body lean mass (indirectly calculated through population formulas); high fat mass corresponded to 3rd FMI sex-specific tertile and high lean mass corresponded to 3rd LMI sex-specific tertile while low fat mass corresponded to 1st merged with 2nd FMI sex-specific tertile and low lean mass corresponded to 1st merged with 2nd LMI sex-specific tertile. ***Abbreviations***: Cardiovascular disease (CVD)

Nested Cox regression models to evaluate the role of BMI and body composition estimations on 10-year first CVD event rate are presented in **Table 2**. In the fully adjusted model, obesity as well as FMI in the 3rd tertile were associated with about 40% higher CVD risk compared with their reference groups. As for the lean mass, participants with the highest LMI had about 10% lower risk to develop a cardiac episode within the decade ($p<0.05$). When the combined effect of lean and fat mass was examined, participants with high fat and low lean mass presented the highest CVD risk compared with the reference group (i.e. low lean and low fat mass) (HR=2.50 95%CI (1.26, 4.40)) while this trend was alleviated in the context of high fat yet high lean mass (HR=1.68 95%CI (1.15, 2.44)).

Table 4.24 Multi-adjusted analysis to evaluate the association between body composition status and 10-year cardiovascular disease incidence in apparently healthy individuals of the ATTICA study (*n*=2,020).

	Model 1 HR (95%CI)	**Model 2** HR (95%CI)	**Model 3** HR (95%CI)	**Model 4** HR (95%CI)
Model for body mass index				
Normalweight	Ref	Ref	Ref	Ref
Overweight	**2.17 (1.63, 2.91)**	**1.27 (1.00, 1.77)**	0.91 (0.57, 1.44)	0.80 (0.48, 1.31)
Obese	**2.94 (2.11, 4.11)**	**1.69 (1.16, 2.44)**	**1.44 (1.05, 2.27)**	**1.41 (1.00, 2.20)**
Model for body fat mass index				
1st	Ref	Ref	Ref	Ref
2nd	**1.81 (1.27, 2.58)**	1.14 (0.77, 1.69)	0.83 (0.49, 1.41)	0.75 (0.42, 1.30)
3rd	**3.20 (2.29, 4.47)**	**1.55 (1.17, 2.35)**	**1.45 (1.10, 2.18)**	**1.39 (1.04, 2.12)**
Model for body lean mass index				
1st	Ref	Ref	Ref	Ref
2nd	**0.81 (0.40, 0.92)**	0.96 (0.52, 1.07)	1.05 (0.67, 1.22)	1.09 (0.75, 1.35)
3rd	**0.65 (0.31, 0.75)**	**0.83 (0.64, 0.91)**	**0.89 (0.71, 0.93)**	**0.91 (0.74, 0.95)**
Model for body fat and lean mass status				
Low lean/low fat mass	Ref	Ref	Ref	Ref
High lean/low fat mass	**0.62 (0.22, 0.73)**	**0.79 (0.27, 0.86)**	**0.82 (0.46, 0.97)**	**0.82 (0.46, 0.97)**

Low lean/high fat mass	**3.02 (2.14, 4.80)**	**2.71 (1.33, 4.66)**	**2.50 (1.26, 4.40)**	**2.50 (1.26, 4.40)**
High lean/high fat mass	**1.97 (1.35, 2.69)**	**1.82 (1.28, 2.52)**	**1.68 (1.15, 2.44)**	**1.68 (1.15, 2.44)**

HRs and their corresponding 95%CIs were obtained from Cox regression analysis; *Model 1:* crude model; *Model 2:* age and sex; *Model 3:* Model 2 plus current smoking, hypertension, diabetes mellitus, MedDietScore, physical activity status, years of school, family history of cardiovascular disease; *Model 4*: Model 3 plus C-reactive protein, alanine transaminase, aspartate transaminase, creatinine clearance. Body fat mass index (FMI) was created to reflect total body fat mass and body lean mass index (LMI) to reflect total body lean mass (indirectly calculated through population formulas); high fat mass corresponded to 3rd FMI sex-specific tertile and high lean mass corresponded to 3rd LMI sex-specific tertile while low fat mass corresponded to 1st merged with 2nd FMI sex-specific tertile and low lean mass corresponded to 1st merged with 2nd LMI sex-specific tertile. ***Abbreviations:*** Hazard Ratio (HR), 95% Confidence Interval (95%CI). **Bold** indicates statistically significant outcomes i.e. $p<0.05$

Nested Cox regression models were constructed to evaluate the role of BMI and body composition estimations on recurrent CVD event rate and presented in **Table 4.25**. Focusing on BMI, in the fully adjusted model a U-shape trend was observed with overweight patients having about 50% lower risk to suffer from a new cardiac episode over their normalweight counterparts ($p<0.05$). As for FMI, even if unadjusted model revealed a significantly increased risk in patients within the 3rd FMI tertile, this trend was lost in the fully adjusted model. Focusing on lean mass, patients in 2nd LMI tertile had about 30% lower risk for CVD recurrence yet higher LMI did not reach the level of significance. In the analysis with the combined effect of lean and fat mass, participants with low fat and high lean mass presented the lowest CVD risk compared with the reference group (i.e. low lean and low-fat mass) (HR=0.61 95%CI (0.38, 0.95)). The protective role of lean mass was lost in the context of high fat mass (HR=1.57 95%CI (1.04, 2.17)) even if it seemed to provide a prognostic advantage compared with the respective HR for patients with high fat yet low lean mass (HR=2.19 95%CI (1.17, 3.05)).

Table 4.25 Multi-adjusted analysis to evaluate the association between body composition status and 10-year cardiovascular disease incidence in patients with established Acute Coronary Syndrome of the GREECS study (*n*=2,172).

	Model 1	**Model 2**	**Model 3**	**Model 4**
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Model for <u>body mass index</u>				

Normalweight	Ref	Ref	Ref	Ref
Overweight	**0.34 (0.22, 0.78)**	**0.48 (0.36, 0.89)**	**0.55 (0.33, 0.97)**	**0.55 (0.33, 0.97)**
Obese	**2.53 (1.41, 3.41)**	2.01 (0.96, 2.89)	1.77 (0.95, 2.78)	1.78 (0.95, 2.76)
Model for <u>body fat mass index</u>				
1st	Ref	Ref	Ref	Ref
2nd	**0.89 (0.41, 0.94)**	**0.94 (0.50, 0.99)**	1.08 (0.72, 1.20)	1.09 (0.72, 1.23)
3rd	2.15 (1.12, 3.05)	**2.04 (1.10, 2.79)**	**1.85 (1.03, 2.48)**	1.67 (0.92, 2.20)
Model for <u>body lean mass index</u>				
1st	Ref	Ref	Ref	Ref
2nd	**0.63 (0.41, 0.96)**	**0.68 (0.46, 0.94)**	**0.69 (0.46, 0.95)**	**0.77 (0.58, 0.98)**
3rd	1.31 (0.85, 2.02)	1.28 (0.82, 2.01)	1.30 (0.73, 2.29)	1.19 (0.69, 2.10)
Model for <u>body fat and lean mass status</u>				
Low lean/low fat mass	Ref	Ref	Ref	Ref
High lean/low fat mass	**0.41 (0.11, 0.65)**	**0.56 (0.25, 0.79)**	**0.60 (0.38, 0.94)**	**0.61 (0.38, 0.95)**
Low lean/high fat mass	**2.43 (1.34, 3.80)**	**2.23 (1.20, 3.47)**	**2.18 (1.15, 3.10)**	**2.19 (1.17, 3.05)**
High lean/high fat mass	**1.85 (1.24, 2.67)**	**1.64 (1.18, 2.32)**	**1.57 (1.05, 2.16)**	**1.57 (1.04, 2.17)**

HRs and their corresponding 95%CIs were obtained from Cox regression analysis; *Model 1:* crude model; *Model 2:* age and sex; *Model 3:* Model 2 plus current smoking, hypertension, diabetes mellitus, MedDietScore, physical activity status, years of school, family history of cardiovascular disease; *Model 4*: Model 3 plus baseline cardiovascular disease history, discharge status (i.e. acute myocardial infarction or unstable angina at baseline) and adherence to medication. Body fat mass index (FMI) was created to reflect total body fat mass and body lean mass index (LMI) to reflect total body lean mass (indirectly calculated through population formulas); high fat mass corresponded to 3rd FMI sex-specific tertile and high lean mass corresponded to 3rd LMI sex-specific tertile while low fat mass corresponded to 1st merged with 2nd FMI sex-specific tertile and low lean mass corresponded to 1st merged with 2nd LMI sex-specific tertile. ***Abbreviations:*** Hazard Ratio (HR), 95% Confidence Interval (95%CI). **Bold** indicates statistically significant outcomes i.e. $p<0.05$

Table 4.26 Sex-based stratified multivariate analysis to evaluate the association between body composition status and 10-year cardiovascular disease incidence in men and women of the ATTICA (*n*=2,020) and GREECS study (*n*=2,172).

ATTICA study (Outcome: First fatal/non-fatal cardiovascular disease event)

	Men	**Women**
	HR (95%CI)	HR (95%CI)
Model for body mass index		
Normalweight	Ref	Ref
Overweight	1.04 (0.57, 1.88)	0.76 (0.35, 1.65)
Obese	**1.85 (1.28, 3.68)**	1.10 (0.59, 2.47)
p for interaction=0.07		
Model for body fat mass index		
1st	Ref	Ref
2nd	0.82 (0.41, 1.63)	1.32 (0.82, 2.13)
3rd	1.24 (0.64, 2.39)	**1.66 (1.05, 2.62)**
p for interaction=0.01		
Model for body lean mass index		
1st	Ref	Ref
2nd	0.96 (0.84, 1.17)	1.10 (0.62, 1.27)
3rd	**0.77 (0.58, 0.89)**	0.95 (0.81, 1.13)
p for interaction=0.04		
Model for body fat and lean mass status		
Low lean/low fat mass	Ref	Ref
High lean/low fat mass	**0.75 (0.37, 0.95)**	0.61 (0.23, 2.16)
Low lean/high fat mass	**2.91 (1.81, 4.44)**	**2.83 (1.65, 4.84)**
High lean/high fat mass	1.31 (0.81, 2.10)	**2.41 (1.28, 4.53)**
p for interaction=0.08		

GREECS study (Outcome: Recurrent fatal/non-fatal cardiovascular disease event)

	Men	Women
	HR (95%CI)	HR (95%CI)
Model for <u>body mass index</u>		
Normalweight	Ref	Ref
Overweight	0.65 (0.40, 1.10)	**0.47 (0.29, 0.92)**
Obese	**1.82 (1.00, 2.94)**	1.64 (0.90, 2.66)
p for interaction=0.01		
Model for <u>body fat mass index</u>		
1st	Ref	Ref
2nd	1.12 (0.75, 1.25)	0.95 (0.61, 1.11)
3rd	**1.75 (1.10, 2.10)**	1.46 (0.85, 1.89)
p for interaction =0.05		
Model for <u>body lean mass index</u>		
1st	Ref	Ref
2nd	0.84 (0.38, 1.10)	**0.63 (0.31, 0.99)**
3rd	1.85 (0.82, 2.38)	0.91 (0.36, 2.30)
p for interaction=0.02		
Model for <u>body fat and lean mass status</u>		
Low lean/low fat mass	Ref	Ref
High lean/low fat mass	**0.75 (0.55, 0.99)**	**0.44 (0.20, 0.87)**
Low lean/high fat mass	**2.41 (1.36, 3.24)**	**1.87 (1.10, 2.79)**
High lean/high fat mass	**1.64 (1.18, 2.25)**	1.42 (0.90, 1.99)
p for interaction=0.02		

HRs and their corresponding 95%CIs were obtained from Cox regression analysis; for ATTICA study, model was adjusted for: age, current smoking, hypertension, diabetes mellitus, MedDietScore, physical activity status, years of school, family history of cardiovascular disease, C-reactive protein, alanine transaminase, aspartate transaminase, creatinine clearance / for GREECS study, model was adjusted for age, current smoking, hypertension, diabetes mellitus, MedDietScore, physical activity status, years of school, family history of cardiovascular disease, baseline cardiovascular disease history, discharge status (i.e. acute myocardial infarction or unstable angina at baseline) and adherence to medication. Body fat mass index (FMI) was created to reflect total body fat mass and body lean mass index (LMI) to reflect total body lean mass (indirectly calculated through population formulas); high fat mass corresponded to 3rd FMI sex-specific tertile and high lean mass corresponded to 3rd LMI sex-specific tertile while low fat mass corresponded to 1st merged with 2nd FMI sex-specific tertile and low lean mass

corresponded to 1st merged with 2nd LMI sex-specific tertile. ***Abbreviations:*** Hazard Ratio (HR), 95% Confidence Interval (95%CI). **Bold** indicates statistically significant outcomes i.e. $p<0.05$

In the formal analysis of interaction, significant heterogeneities were produced in relation to sex and body weight or body composition estimations in both ATTICA and GREECS study (all *p-values for sex interaction<0.10*). Thereby, stratified analyses were performed using sex as strata and the respective results are presented in **Table 4.26**.

Obesity was independently associated with first CVD event only in men (HR=1.85 95%CI 1.28, 3.68) while FMI within the 3rd tertile range only in women (HR=1.66 95%CI 1.05, 2.62). Men in 3rd LMI tertile were protected against CVD onset (HR=0.77 95%CI 0.58, 0.89); this trend was retained in women yet without being significant. Men with high fat yet low lean mass had significantly higher CVD risk compared with the reference group (HR=2.91 95%CI 1.81, 4.44). In the context of high fat mass yet LMI on the 3rd tertile the aforementioned high CVD risk was retained significant only in women.

Obesity was independently associated with recurrent CVD event only in men (HR=1.82 95%CI 1.00, 2.94). Overweight women were protected against CVD recurrence (HR=0.47 95%CI 0.29, 0.92). As for FMI, only men in the 3rd tertile had increased CVD risk (HR=1.75 95%CI 1.10, 2.10). In women patients, a U-shape LMI-related trend was observed with those in the 2nd tertile having 40% lower risk to develop a new cardiac episode. Both men and women with high fat yet low lean mass had significantly higher CVD risk compared with the reference group. However, in the context of high fat mass yet LMI on the 3rd tertile the aforementioned high CVD risk was retained significant only in men. Lastly, a status with high lean yet low fat mass was protective for both sexes.

The discrimination ability of epidemiological models adjusted for BMI or body composition estimations was evaluated separately for men and women and results are presented in **Table 4.27**. Overall, the discrimination ability (expressed through C-index) of the examined models was better in ATTICA study sample. In men, both FMI and LMI significantly contributed to principle endpoint yet with the result being more evident for LMI (*p for C-index difference=0.001*). As for women, only FMI-adjusted model had an added discrimination ability (*p for C-index difference=0.003*). As for the results corresponding to *GREECS study*, in men, FMI seemed to significantly increase the base-model discrimination ability against CVD recurrence (*p for C-index difference=0.04*) while in women patients, LMI-adjusted model discriminated better the primary endpoint (*p for C-index difference=0.002*).

Table 4.27 C-index of multivariate models containing different anthropometric measurements to evaluate the discriminative ability against 10-year cardiovascular disease event in men and women of the ATTICA (*n*=2,020) and GREECS study (*n*=2,172).

ATTICA study (Outcome: First fatal/non-fatal cardiovascular disease event)

	C-index (95%CI)	***p-value***	**C-index changes (95%CI)**	***p-value***
Men				
Base model	0.700 (0.678, 0.723)	*<0.001*	-	-
Base model + BMI	0.705 (0.683, 0.728)	*<0.001*	0.005 (-0.001, 0.007)	*0.12*
Base model + FMI	0.711 (0.689, 0.734)	*<0.001*	0.011 (0.008, 0.018)	*0.01*
Base model + LMI	0.731 (0.709, 0.753)	*<0.001*	0.031 (0.025, 0.039)	*0.001*
Base model + body composition status	0.732 (0.711, 0.754)	*<0.001*	0.032 (0.026, 0.040)	*0.001*
Women				
Base model	0.751 (0.718, 0.784)	*<0.001*	-	
Base model + BMI	0.759 (0.728, 0.791)	*<0.001*	0.008 (-0.003, 0.014)	*0.31*
Base model + FMI	0.774 (0.742, 0.806)	*<0.001*	0.017 (0.010, 0.025)	*0.003*
Base model + LMI	0.758 (0.725, 0.790)	*<0.001*	0.007 (-0.003, 0.009)	*0.25*
Base model + body composition status	0.774 (0.742, 0.806)	*<0.001*	0.008 (-0.003, 0.014)	*0.003*

GREECS study (Outcome: Recurrent fatal/non-fatal cardiovascular disease event)

	C-index (95%CI)	***p-value***	**C-index changes (95%CI)**	***p-value***
Men				
Base model	0.615 (0.578, 0.643)	*<0.001*	-	-
Base model + BMI	0.619 (0.580, 0.642)	*<0.001*	0.004 (-0.002, 0.007)	*0.15*
Base model + FMI	0.630 (0.586, 0.700)	*<0.001*	0.015 (0.006, 0.021)	*0.04*
Base model + LMI	0.620 (0.581, 0.643)	*<0.001*	0.005 (-0.003, 0.008)	*0.10*

Base model + body composition status	0.632 (0.587, 0.701)	*<0.001*	0.017 (0.008, 0.024)	*0.05*
Women				
Base model	0.665 (0.618, 0.699)	*<0.001*	-	-
Base model + BMI	0.669 (0.620, 0.701)	*<0.001*	0.004 (-0.003, 0.005)	*0.26*
Base model + FMI	0.668 (0.619, 0.700)	*<0.001*	0.003 (-0.002, 0.005)	*0.12*
Base model + LMI	0.677 (0.627, 0.710)	*<0.001*	0.012 (0.007, 0.019)	*0.002*
Base model + body composition status	0.680 (0.671, 0.712)	*<0.001*	0.015 (0.010, 0.023)	*0.002*

Base model was adjusted for conventional cardiovascular disease risk factors i.e. age, hypercholesterolemia, diabetes mellitus, hypertension, currents smoking and family history of cardiovascular disease. C-index and the corresponding confidence interval was evaluated through the area under the curve obtained from the Receiver operating Characteristics (ROC) analysis. ROC analysis was performed using the probabilities for 10-year first fatal/non-fatal cardiovascular disease event, corresponding to each study participant, separately for men and women, calculated from Cox regression analysis using the multivariate models described. Significance of the changes in C-index was tested by differences in 2 log likelihood of regression models with and without anthropometric measurements. FMI was created to reflect total body fat mass and LMI to reflect total body lean mass (indirectly calculated through population formulas). Body composition status was defined as the combined lean and fat mass status as follows; high fat mass corresponded to 3rd FMI sex-specific tertile and high lean mass corresponded to 3rd LMI sex-specific tertile while low fat mass corresponded to 1st merged with 2nd FMI sex-specific tertile and low lean mass corresponded to 1st merged with 2nd LMI sex-specific tertile ***Abbreviations:*** body fat mass index (FMI), body mass index (BMI), body lean mass index (LMI), 95% Confidence Interval (95%CI).

4.4 The sex-specific role of metabolic syndrome and non-alcoholic fatty liver disease in relation to ten-year first CVD incidence

4.4.1 The sex-specific role of metabolically healthy obesity in relation to ten-year first CVD incidence

4.4.1.1 Scope and research hypothesis

The scope here was to evaluate **a.** the prevalence of strictly defined MHO status in a sample of apparently healthy men and women from Greece, **b.** the transition of MHO to MUO status within a 10-year follow-up period and **c.** the 10-year combined CVD risk of MHO individuals over various reference groups. Two a priori research hypotheses were posed: **A.** The 10-year combined CVD event risk corresponding to MHO participants will be intermediate to the respective risk of their MHN and MUO counterparts; **B.** Metabolically healthy obesity is not a stable condition; a significant portion of MHO subjects will transient to metabolically unhealthy status within the decade, with this transition increasing their CVD risk.

4.4.1.2 Methods ans analysis

For the scope of the present work participants were divided in four groups as follows; **a.** MHN defined as BMI<30kg/m^2 and healthy metabolic status; **b.** MHO defined as BMI≥30kg/m^2 and healthy metabolic status; **c.** MUN defined as BMI<30kg/m^2 and unhealthy metabolic status; **d.** MUO defined as BMI≥30kg/m^2 and unhealthy metabolic status.

Associations between normally distributed variables and the combined obesity and metabolic status were evaluated through one-way analysis of variance or Student's t-test for independent samples. Whether these variables were normally distributed was tested through P-P plot and equality of variances through Levene's test. For non-normally distributed variables, Kruskal-Wallis and Mann-Whitney tests were used. Associations between categorical variables and the combined obesity and metabolic status were tested with the chi-squared test. HRs and their corresponding 95% CIs for the combined obesity and metabolic status in relation to 10-year CVD event were evaluated through multivariable Cox-regression analysis in the total sample, as well as in each of the respective subgroups. Proportional hazards' assumption was graphically tested. Interactions between groups of participants were tested, and when significant the analyses were further stratified.

4.4.1.3 Findings

Table 4.28 depicts the baseline sociodemographic, lifestyle, clinical and biochemical characteristics of study participants according to their combined obesity and metabolic status. MHO participants were almost one decade older compared with their MUO counterparts (*p<0.001*). As for the lifestyle factors, MHO and MUO subjects presented a similar pattern of unhealthy lifestyle habits, including smoking and sedentary physical activity, yet better than their non-obese metabolically healthy or unhealthy counterparts (*p<0.001*). However, when it came to the level of adherence to the Mediterranean diet, an inverse association was observed with MHO participants presenting the lowest MedDietScore values (*p<0.001*). Additionally, regarding insulin resistance, an increasing trend of HOMA-IR was observed passing from MHN to MUO while regarding CRP levels obese participants presented the highest values irrespective of their metabolic status (*all p-values<0.001*).

Unadjusted models revealed that MHO participants presented 2.66 times higher CVD event rate compared with their non-obese counterparts i.e. MHN (*p<0.001*). On the other side, MHO had from 1.25 to 1.56 lower times likelihood to suffer from CVD within the decade compared with their metabolically unhealthy counterparts, irrespective of their weight status (*p<0.001*).

Table 4.28 Baseline sociodemographic, lifestyle, clinical and biochemical factors and 10-year cardiovascular

disease (CVD) event of apparently healthy participants according to combined obesity and metabolic status (*n*=1,890).

	Combined obesity and metabolic status				
Baseline factors	**MHN**	**MHO**	**MUN**	**MUO**	*p-value*
	n=686	*n=107*	*n=672*	*n=425*	
Age, years	38 (12)	45 (12)	50 (13)	52 (12)	*<0.001*
Men, %	40	50	54	58	*<0.001*
Years of school	13 (3)	11 (4)	12 (4)	11 (4)	*<0.001*
Body mass index, kg/m^2	23.9 (3.06)	32.8 (3.89)	25.5 (2.67)	33.5 (3.17)	*<0.001*
Waist circumference, cm	82.1 (12.0)	102.1 (14.1)	90.2 (12.2)	108.3 (12.1)	*<0.001*
Current smoking, %	47	38	42	40	*0.024*
Physical activity, %	45	27	40	29	*<0.001*
MedDietScore (range 0-55)	28. 2 (6.8)	21.8 (6.1)	24.9 (5.6)	23.8 (4.6)	*<0.001*
Systolic blood pressure, mmHg	112 (11)	118 (10)	128 (19)	137 (19)	*<0.001*
Low Density Lipoprotein, mg/dL	98 (22)	104 (18)	140 (35)	136 (36)	*<0.001*
High Density Lipoprotein, mg/dL	55 (14)	52 (12)	46 (15)	44 (12)	*<0.001*
Triglycerides, mg/dL	81 (30)	122 (28)	138 (107)	158 (91)	*<0.001*
Fasting glucose, mg/dL	86 (12)	87 (12)	95 (25)	104 (35)	*<0.001*
HOMA-IR	2.59 (0.61)	2.98 (1.55)	3.29 (1.97)	3.87 (3.22)	*<0.001*
CRP, mg/L	1.51 (2.25)	2.88 (2.84)	1.84 (2.07)	3.21 (3.20)	*<0.001*
Alanine transaminase, U/L	18.62 (11.54)	28.00 (14.78)	20.08 (11.25)	23.35 (14.34)	*<0.001*
Aspartate transaminase, U/L	23.77 (11.06)	31.62 (16.38)	24.91 (11.44)	24.74 (10.93)	*0.02*
Creatinine clearance, mL/min/1.73m^2	93 (24)	127 (34)	88 (26)	113 (34)	*<0.001*
Family history of CVD, %	26	25	31	28	*0.192*
10-year follow-up					
First combined CVD event, %	6	16	20	25	*<0.001*

Data are presented as mean ± standard deviation (SD) (i.e. mean (SD)). P-values were obtained using One-way analysis of variance for the normally distributed variables (age, MedDietScore, body mass index), Kruskal-Wallis Test for the rest quantitate variables (years of school, waist circumference, systolic blood pressure, fasting glucose, triglycerides, high density lipoprotein, low density lipoprotein, C-Reactive Protein (CRP), Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)), alanine transaminase, aspartate transaminase, creatinine clearance and chi-squared test for categorical variables. Metabolically healthy non-obese (MHN): BMI<30kg/m^2 with metabolically health status; Metabolically healthy obese (MHO): BMI≥30kg/m^2 without metabolically healthy status; Metabolically unhealthy non-obese (MUN): BMI<30kg/m^2 without metabolically health status; Metabolically unhealthy obese (MUO): BMI≥30kg/m^2 without metabolically healthy status. Metabolically healthy status was defined as the absence of 4 metabolic syndrome components i.e. elevated triglycerides, reduced high density lipoprotein, elevated blood pressure and elevated fasting glucose including the drug treatment for all these conditions.

The transition of MHO participants to metabolically unhealthy states within the follow up is shown in **Table 4.29**. In the 5-year follow-up period, transition to metabolically unhealthy status was observed for 33% of MHO participants. Within the decade, almost half of obese

participants who were initially metabolically benign resulted in presenting with MUO. In particular, among this group, 24% achieved the highest disease risk burden, including abnormal glycaemic, lipidemic, and blood pressure profiles.

Table 4.29 Metabolic status and transition to metabolically unhealthy status in terms of isolated or combined metabolic syndrome (MetS) components in metabolically healthy obese at 5-year and 10-year follow up periods (*n*=107).

	5-year follow up	**10-year follow up**
Across follow-up, transition to:		
Diabetes/prediabetes, %	11	24
Hypertension, %	23	45
Dyslipidaemia, %	33	52
Metabolically healthy, %	67	48
≥1 MetS component, %	33	52
≥2 MetS components, %	20	30
All 3 MetS components, %	14	24

Metabolically healthy status was defined as the absence of 4 MetS components i.e. elevated triglycerides, reduced high density lipoprotein, elevated blood pressure and elevated fasting glucose including the drug treatment for all these conditions. The examined MetS components within the follow-up periods were : a. diabetes/prediabetes status; b. hypertension and c. dyslipidaemia (elevated triglycerides and high-density lipoprotein were assessed under this term). To evaluate the prevalence of transition to the aforementioned conditions within the follow-up periods chi-squared tests were performed.

Multivariable Cox regression analysis revealed that the 10-year CVD event HR (95%CI) for obesity (yes vs. no) (not adjusted for metabolic status) was 1.65 (1.00, 2.92). The unadjusted-for-obesity HR (95% CI) corresponding to metabolic status (healthy vs. unhealthy) was 0.44 (0.18, 0.99). In mediation analysis where both obesity and metabolic status were included in the model, the independent effect on 10-year CVD event was retained only for metabolic status (HR=0.43, 95%CI 0.17, 0.99), yet not for obesity (HR=1.61 95%CI 0.89, 2.52) (data not shown).

Nested Cox regression models to evaluate the association of the combined obesity and metabolic status on 10-year CVD event are presented in **Table 4.30**. In the unadjusted models, MHO participants presented almost 89% significantly higher risk for developing 10-year CVD events, as compared to their MHN counterparts ($p<0.001$). In the age- and gender- adjusted model this association was attenuated yet remained significant. However, after adjusting for lifestyle, clinical, and biochemical markers, MHO status retained its aggravating effect, yet it did not reach the level of significance. On the other hand, non-persistent MHO status (i.e. transition from MHO to MUO) was independently associated with elevated CVD risk compared with the MHN counterpart even in the fully adjusted model. Moreover, sensitivity analysis excluding

overweight subjects was performed; even in this case all the aforementioned trends were sustained (data not shown).

Table 4.30 Nested Cox-regression analysis models to evaluate the association of combined obesity and metabolic status with 10-year cardiovascular disease (CVD) event (*n*=1 890).

	Model 1	Model 2	Model 3	Model 4	Model 5
Combined obesity and metabolic status					
MHN	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
MHO	**1.89 (1.24, 2.87)**	**1.37 (1.00, 2.17)**	**1.26 (0.99, 2.03)**	**1.07 (0.98, 2.37)**	**0.95 (0.37, 2.08)**
MHO to MUO	**2.76 (2.01, 3.40)**	**1.81 (1.22, 2.55)**	**1.82 (1.25, 2.63)**	**1.83 (1.24, 2.69)**	**1.43 (1.02, 2.01)**
MUN	2.39 (1.67, 3.10)	1.81 (1.26, 2.60)	1.73 (1.16, 2.86)	1.72 (1.03, 2.87)	1.45 (0.85, 2.50)
MUO	2.93 (2.05, 3.37)	2.73 (1.85, 3.22)	2.54 (1.55, 3.10)	2.41 (1.42, 3.08)	2.04 (1.15, 2.89)
Age, per 1 year	-	1.08 (1.07, 1.09)	1.08 (1.06, 1.09)	1.07 (1.05, 1.09)	1.07 (1.05, 1.09)
Male gender	-	1.86 (1.41, 2.46)	1.82 (1.36, 2.45)	1.81 (1.17, 2.76)	1.66 (1.07, 2.61)
Years of school, per 1 year	-	-	0.96 (0.92, 0.99)	0.97 (0.92, 1.02)	0.95 (0.90, 1.01)
MedDietScore (range 0-55), per 1/55	-	-	0.98 (0.96, 0.99)	0.98 (0.94, 0.99)	0.97 (0.94, 1.01)
Physical activity, yes vs. no	-	-	0.94 (0.70, 1.25)	1.32 (0.88, 1.98)	1.43 (0.94, 2.17)
Current smoking, yes vs. no	-	-	1.27 (0.94, 1.71)	1.50 (1.00, 2.28)	1.45 (0.94, 2.23)
LDL, per 1 mg/dL	-	-	-	1.01 (1.00, 1.03)	1.00 (0.99, 1.01)
Family history of CVD, yes vs. no	-	-	-	1.37 (0.90, 2.08)	1.39 (0.89, 2.17)
ALT, per 1 U/L	-	-	-	1.01 (0.98, 1.04)	1.00 (0.97, 1.04)
AST, per 1 U/L	-	-	-	0.99 (0.95, 1.02)	0.98 (0.94, 1.01)
$C_{(CR)}$, per 1 mL/min/1.73m^2	-	-	-	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)
Waist circumference, per 1 cm	-	-	-	-	1.00 (0.98, 1.02)
HOMA-IR, per 1 unit	-	-	-	-	1.06 (0.98, 1.16)
CRP, per 1 mg/L	-	-	-	-	1.06 (0.98, 1.15)

MHN: BMI<30kg/m^2 with metabolically health status; MHO: BMI≥30kg/m^2 without metabolically healthy status; MUN: BMI<30kg/m^2 without metabolically health status; MUO: BMI≥30kg/m^2 without

metabolically healthy status. Metabolically healthy status was defined as the absence of 4 metabolic syndrome components i.e. elevated triglycerides, reduced high density lipoprotein, elevated blood pressure and elevated fasting glucose including the drug treatment for all these conditions. Abbreviations: Alanine transaminase (ALT); Aspartate transaminase (AST); C-Reactive Protein (CRP); Creatinine clearance ($C_{(CR)}$); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR); Low Density Lipoprotein (LDL); Metabolically healthy non-obese (MHN); Metabolically healthy obese (MHO); Metabolically unhealthy non-obese (MUN); Metabolically unhealthy obese (MUO)

In the formal interaction analysis, little evidence of significant heterogeneity was observed (*all p-values for interaction>0.10*) – among others in relation to sex. Thereby, stratified analysis was performed using sex as strata and results are presented in **Table 4.31**. It was revealed that MHO status was positively associated with 10-year CVD event only in women (*p<0.05*).

Table 4.31 Sex-based stratified analysis to evaluate the association of combined metabolic- and obesity- related status with 10-year cardiovascular disease event (*n*=1,890).

	Combined Obesity- and Metabolic- status	**Hazard ratio**	**95% Confidence Interval**
Sex (n/cases)			
Men (941/189)	MHN	1.00	Ref
	MHO	1.09	0.35, 3.36
	MUN	**1.92**	**1.06, 3.48**
	MUO	**2.64**	**1.42, 4.91**
Women (949/110)	MHN	1.00	Ref
	MHO	**2.01**	**1.34, 3.49**
	MUN	1.42	0.50, 4.04
	MUO	**2.07**	**1.06, 3.79**
p for interaction=0.05			

All models were adjusted for age, educational status, physical activity, current smoking, low density lipoprotein levels, family history of cardiovascular disease, alanine transaminase, aspartate transaminase and creatinine clearance. **Bold** indicates estimates that are significantly different from the reference group at *p*<0.05. Abbreviations: Metabolically healthy non-obese (MHN); Metabolically healthy obese (MHO); Metabolically unhealthy non-obese (MUN); Metabolically unhealthy obese (MUO)

4.4.2 NAFLD, metabolically healthy obesity and ten-year first CVD incidence: sex-specific remarks

4.4.2.1 Scope and research hypothesis

The scope here was to evaluate **a.** the association between NAFLD and MHO vs MUO, i.e. the combined obesity- and metabolic- status, at baseline, **b.** the role of NAFLD on influencing the transition from MHO to MUO over a 10-year period and **c.** the 10-year risk of MHO individuals to develop fatal/non-fatal cardiovascular disease (CVD) and how this may be affected by their NAFLD status at baseline, providing sex-specific conclusions. Three a priori research hypotheses were posed and specified to men and women: **A.** the presence of liver fat (NAFLD), which is currently not part of the criteria to differentiate between MHO vs. MUO, in certain individuals with the MHO phenotype is related with increased risk to transition to unhealthy metabolic status over time; **B.** the presence of NAFLD at baseline interacts with MHO status in relation to the incidence of CVD over a ten year period of observation; **C.** NAFLD increases the ability of metabolic status (MHO vs MUO) to predict future CVD risk.

4.4.2.2 Methods ans statistical analysis

NAFLD in terms of liver steatosis was evaluated using HSI, FLI and NAFLD-FLS index (Vilar-Gomez et al 2018).

ORs and their corresponding 95%CIs were evaluated through multivariable logistic-regression analysis. HRs and their corresponding 95%CIs were evaluated through multivariable Cox-regression analysis and proportional hazards' assumption was graphically tested. The concordance statistics i.e. C-statistics was used to evaluate the predictive accuracy of models adjusted for metabolic status, and central obesity, and/or NAFLD against 10-year CVD event. C-indexes and the corresponding 95%CIs were equal to the areas under the curve obtained from the ROC analysis.

4.4.2.3 Results

At baseline, the prevalence of MHO status was 9.8% (*n*=277) (9.9% in men and 9.7% in women, *p*=0.25). Among obese adults, 31.6% presented metabolically healthy status.

Multivariable analysis revealed that the predicted NAFLD OR (95%CI) for obesity (yes vs. no) (not adjusted for metabolic status) was 1.80 (1.10, 3.09). The unadjusted-for-obesity OR (95%CI) corresponding to metabolic status (healthy vs. unhealthy) was 0.55 (0.20, 1.00). In mediation analysis where both obesity and metabolic status were included in the model, the independent association between NAFLD status and obesity was retained irrespective of the adjustment for metabolic status (OR=1.70 95%CI (1.02, 2.91)). In this case, any significance of metabolic status was lost (OR=0.69 95%CI (0.34, 1.20) (*data not shown in tables*) indicating that NAFLD per se may be a better criterion for classifying MUO vs MHO in the future.

Logistic regression models to evaluate the association of the combined obesity and metabolic status on predicted NAFLD are presented in **Table 4.32**. MUO participants had about twice as high likelihood for liver steatosis, yet significance was lost in the fully adjusted model adjusting for many variables, with central obesity, MedDietScore and HOMA-IR having the strongest moderating effect. In MHO participants, no significant associations were found.

About 54.8% of MHO participants transitioned to MUO status within the decade. Hence, the above analyses were repeated focusing on this MHO subgroup. Unadjusted analysis revealed that the MHO subgroup that eventually transitioned to MUO had about two times higher likelihood to suffer from NAFLD compared with their MHN counterparts; obese participants with unhealthy metabolic status (MUO) even during the recruitment phase had similar likelihood to suffer from NAFLD. This association was attenuated yet remained significant even after adjusting for various factors.

Table 4.32 Logistic regression analysis models to evaluate the association of combined obesity and metabolic status with predicted NAFLD (*n*=2,817).

	N	NAFLD Prevalence, %	Crude model	Age- and sex-adjusted model	Multi-adjusted models			
					Model 1 *(adjusted for waist circumference)*	Model 2 *(adjusted for MedDietScore)*	Model 3 *(adjusted for insulin resistance)*	Model 4 (*fully adjusted model*)
NAFLD-FLS defined steatosis, yes/no			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Combined obesity and metabolic status								
MHN	942	26	*(ref)*	*(ref)*	*(ref)*	*(ref)*	*(ref)*	*(ref)*
MHO	277	31	1.27 (0.62, 2.59)	1.23 (0.60, 2.52)	0.99 (0.45, 2.14)	0.99 (0.45, 2.14)	1.01 (0.51, 2.17)	1.07 (0.53, 3.54)
MHO throughout[1]	125	21	1.25 (0.58, 2.50)	1.19 (0.51, 2.42)	1.05 (0.39, 2.09)	1.07 (0.40, 2.11)	1.05 (0.38, 2.10)	1.05 (0.38, 2.10)
MHO to MUO[2]	152	40	2.17 (1.52, 3.19)	2.10 (1.48, 3.14)	1.80 (1.05, 2.67)	1.95 (1.29, 2.80)	1.81 (1.07, 2.69)	1.90 (1.20, 2.68)
MUN	1,001	27	1.02 (0.66, 1.57)	0.91 (0.56, 1.46)	0.82 (0.50, 1.34)	0.81 (0.49, 1.30)	0.82 (0.50, 1.34)	0.78 (0.38, 1.58)
MUO	597	44	2.16 (1.25, 3.62)	1.95 (1.13, 3.39)	1.49 (0.80, 2.85)	1.47 (0.77, 2.79)	1.51 (0.82, 2.94)	1.77 (0.98, 3.25)
			p for trend=0.01	*p for trend=0.02*	*p for trend=0.15*	*p for trend=0.20*	*p for trend=0.17*	*p for trend=0.22*

	N/cases	NAFLD Prevalence, %	Crude model	Age- and sex-adjusted model	Multi-adjusted models			
					Model 1 *(adjusted for waist circumference)*	Model 2 *(adjusted for MedDietScore)*	Model 3 *(adjusted for insulin resistance)*	Model 4 (*fully adjusted model*)
HSI defined steatosis, yes/no Combined obesity and metabolic status			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
MHN	942	34	*(ref)*	*(ref)*	*(ref)*	*(ref)*	*(ref)*	*(ref)*
MHO	277	39	1.23 (0.62, 2.44)	1.16 (0.58, 2.30)	1.52 (0.70, 3.31)	1.49 (0.68, 3.29)	1.50 (0.69, 3.28)	1.67 (0.65, 4.26)
MHO throughout	125	30	1.22 (0.64, 2.41)	1.15 (0.57, 2.31)	1.50 (0.69, 3.30)	1.44 (0.60, 3.21)	1.48 (0.65, 3.21)	1.65 (0.59, 4.20)
MHO to MUO	152	46	2.20 (1.53, 3.30)	2.19 (1.52, 3.30)	1.95 (1.21, 2.59)	2.12 (1.40, 3.02)	1.94 (1.20, 2.55)	2.05 (1.33, 3.00)
MUN	1,001	28	0.75 (0.50, 1.14)	0.60 (0.38, 0.95)	0.72 (0.47, 1.12)	0.57 (0.33, 0.99)	0.72 (0.48, 1.13)	0.52 (0.26. 1.03)
MUO	597	41	2.34 (1.81, 3.22)	2.10 (1.64, 2.89)	1.41 (0.72, 2.73)	1.41 (0.72, 2.73)	1.40 (0.72, 2.71)	1.20 (0.51, 2.80)
			p for trend=0.03	*p for trend=0.04*	*p for trend=0.12*	*p for trend=0.05*	*p for trend=0.12*	*p for trend=0.03*

	N/cases	NAFLD Prevalence, %	Crude model	Age- and sex-adjusted model	Multi-adjusted models			
					Model 1 *(adjusted for*	Model 2 *(adjusted for*	Model 3 *(adjusted for*	Model 4

					waist circumference)	*MedDietScore)*	*insulin resistance)*	(*fully adjusted model*)
FLI defined steatosis, yes/no			OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Combined obesity and metabolic status								
MHN	942	21	*(ref)*	*(ref)*	*(ref)*	*(ref)*	*(ref)*	*(ref)*
MHO	277	29	0.79 (0.32, 1.92)	0.71 (0.28, 1.75)	0.74 (0.28, 1.93)	0.88 (0.30, 3.25)	0.75 (0.25, 1.92)	0.76 (0.29, 3.18)
MHO throughout	125	24	0.81 (0.35, 2.01)	0.78 (0.30, 1.90)	0.72 (0.21, 1.89)	0.75 (0.24, 3.20)	0.73 (0.22, 1.90)	0.73 (0.21, 3.08)
MHO to MUO	152	33	1.87 (1.15, 2.81)	1.85 (1.14, 2.78)	1.62 (1.08, 2.12)	1.79 (1.08, 2.20)	1.62 (1.08, 2.11)	1.72 (1.10, 2.21)
MUN	1,001	23	1.09 (0.67, 1.76)	0.78 (0.46, 1.33)	0.77 (0.44, 1.32)	0.79 (0.39, 1.70)	0.74 (0.41, 1.32)	0.70 (0.30, 1.64)
MUO	597	32	2.74 (1.91, 3.98)	2.31 (1.71, 3.40)	1.33 (0.65, 2.73)	1.87 (0.64, 3.99)	1.30 (0.63, 2.70)	1.42 (0.53, 3.81)
			p for trend=0.01	*p for trend=0.05*	*p for trend=0.30*	*p for trend=0.26*	*p for trend=0.28*	*p for trend=0.39*

ORs and their 95%CIs were obtained though logistic regression analysis. Model 1 was adjusted for age, sex, and waist circumference. Model 2 was adjusted for age, sex, and MedDietScore. Model 3 adjusted for age, sex, and Homeostatic Model Assessment of Insulin Resistance. Model 4 was adjusted for age, sex, waist circumference, MedDietScore, Homeostatic Model Assessment of Insulin Resistance, educational status, physical activity, current smoking, low density lipoprotein levels and C-reactive protein and. Bold indicates estimates that are significantly different from the reference group at $p<0.05$. *Abbreviations:* Metabolically healthy non-obese (MHN); Metabolically healthy obese (MHO); Metabolically unhealthy non-obese (MUN); Metabolically unhealthy obese (MUO); Fatty liver index (FLI); Hepatic steatosis index (HSI); Non-alcoholic fatty liver disease (NAFLD); Non-alcoholic fatty liver disease fatty liver score (NAFLD-FLS); Odds ratio (OR); 95% Confidence Interval (95%CI).

[1]MHO throughout was defined as the subgroup of MHO individuals that retained their metabolically healthy status within the decade.

[2]MHO to MUO was defined as the subgroup of MHO individuals that lost their metabolically healthy status within the decade and became MUO.

In the formal interaction, little evidence of significant heterogeneity was produced (*all p-values for interaction>0.10*) apart from – among others – sex (*p=0.002*). Subsequently, stratified analyses were performed, and the respective results are presented in **Table 4.33**. It was revealed that MHO status was significantly associated with NAFLD primarily in women (*p<0.05*).

Table 4.33 Sex-specific stratified analysis to evaluate the association of combined obesity and metabolic status with predicted NAFLD (*n*=2,817).

n/NAFLD predicted cases[1]	**Combined Obesity- and Metabolic- status**	**OR**	**95%CI**
Sex (n/cases)			
Men (1,402/539)	MHN	*1.00*	*Ref*
	MHO	1.01	0.37, 3.74
	MUN	0.62	0.34, 1.13
	MUO	2.83	1.41, 3.70
Women (1,415/414)	MHN	*1.00*	*Ref*
	MHO	1.35	1.08, 2.28
	MUN	0.47	0.22, 1.03
	MUO	1.67	1.03, 3.82
p for interaction=0.002			

ORs and their 95%CIs were obtained though logistic regression analysis. All models were adjusted for age, educational status, physical activity, current smoking and low-density lipoprotein levels. Bold indicates estimates that are significantly different from the reference group at $p<0.05$. Abbreviations: Homeostatic Model Assessment of Insulin Resistance (HOMA-IR); Metabolically healthy non-obese (MHN); Metabolically healthy obese (MHO); Metabolically unhealthy non-obese (MUN); Metabolically unhealthy obese (MUO); Odds ratio (OR); 95% Confidence Interval (95%CI).

[1]Participants were assigned to NAFLD subgroup in case they met the criteria of at least one of here following scores: Fatty liver index (FLI); Hepatic steatosis index (HSI); Non-alcoholic fatty liver disease fatty liver score (NAFLD-FLS)

The associations between NAFLD and risk to develop unhealthy metabolic status within the decade are summarized in **Table 4.34**. Irrespective to NAFLD score, unadjusted analysis revealed a 2.1 to 2.4 higher odds to develop unhealthy metabolic status within the decade in the presence of NAFLD compared with no presence of NAFLD. This association remained significant after adjusting for various factors yet was attenuated or even lost its significance when adjusting for lifestyle factors, obesity, visceral adiposity and various biomarkers related to central obesity. Among them, obesity and visceral adiposity seemed to have the strongest moderating effect on the examined association followed by HOMA-IR and CRP as well as adiponectin.

Multivariable analysis to evaluate the interaction between NAFLD and the combined obesity and metabolic status defined category on 10-year risk to develop CVD was performed and results are presented in **Table 4.35**. MHO participants had 2.66 times higher CVD event rate compared with their non-obese counterparts i.e. MHN (*p<0.001*). When the sample was stratified according to NAFLD status it was revealed that MHO with NAFLD had about 1.5 times higher risk to develop CVD compared to their non-NAFLD MHO counterparts. Interaction analysis revealed a significant interaction effect of NAFLD on this association (*p for interaction=0.002*). Subgroup analysis revealed an independent aggravating effect of MHO status on CVD incidence

only when NAFLD was present; MHO participants with NAFLD had about 3 times higher CVD risk compared with their MHN counterparts even in multi-adjusted models.

Table 4.34 Logistic-regression analysis models to evaluate the association of NAFLD with 10-year transition to metabolically unhealthy status in the subset of metabolically healthy participants at baseline (*n*=1,219).

	Crude model	**Basic model**	**Basic model adjusted for lifestyle factors**	**Basic model adjusted for anthropometric factors**	**Basic model adjusted for biomarkers**	***Basic model adjusted for adiponectin**	**Fully adjusted model without adiponectin**	***Fully adjusted model with adiponectin**
	OR (95%CI)	*OR (95%CI)*	*OR (95%CI)*	*OR (95%CI)*	*OR (95%CI)*	*OR (95%CI)*	*OR (95%CI)*	*OR (95%CI)*
NAFLD-FLS defined steatosis, yes/no	2.12 (1.45, 4.50)	2.02 (1.22, 4.19)	1.98 (1.10, 3.69)	1.68 (0.78, 2.99)	1.79 (0.85, 3.08)	1.84 (0.91, 3.12)	1.69 (0.70, 2.99)	1.69 (0.70, 2.99)
Age, per 1 year	-	1.11 (1.08, 1.15)	1.09 (1.04, 1.13)	1.08 (1.03, 1.13)	1.09 (1.03, 1.14)	1.09 (1.03, 1.14)	1.09 (1.03, 1.14)	1.09 (1.03, 1.14)
Male gender	-	1.87 (1.40, 2.44)	1.82 (1.36, 2.45)	1.81 (1.17, 2.76)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)
Years of school, per 1 year	-	1.15 (1.00, 1.33)	1.15 (1.00, 1.33)	1.14 (0.98, 1.32)	1.14 (0.98, 1.32)	1.14 (0.98, 1.32)	1.14 (0.98, 1.32)	1.14 (0.98, 1.32)
MedDietScore (range 0-55), per 1/55	-	-	0.92 (0.80, 1.07)	-	-	-	0.97 (0.84, 1.11)	0.97 (0.84, 1.11)
Physical activity, yes vs. no	-	-	0.34 (0.13, 0.88)	-	-	-	0.38 (0.14, 1.02)	0.38 (0.14, 1.02)
Current smoking, yes vs. no	-	-	1.33 (0.53, 3.29)	-	-	-	1.36 (0.51, 3.59)	1.36 (0.51, 3.59)
Obesity,	-	-	-	1.95 (1.20, 4.50)	-	-	1.95 (1.20, 4.50)	1.95 (1.20, 4.50)

yes vs. no								
Waist circumference, per 1 cm	-	-	-	1.03 (0.99, 1.08)	-	-	1.03 (0.99, 1.08)	1.03 (0.99, 1.08)
HOMA-IR, per 1 unit	-	-	-	-	1.04 (0.52, 2.07)	-	1.04 (0.52, 2.07)	1.04 (0.52, 2.07)
CRP, per 1 mg/L	-	-	-	-	0.98 (0.80, 1.21)	-	0.98 (0.80, 1.21)	0.98 (0.80, 1.21)
LDL, per 1 mg/dL	-	-	-	-	1.36 (0.51, 3.59)	-	1.36 (0.51, 3.59)	1.36 (0.51, 3.59)
Adiponectin, per 1 μg/L	-	-	-	-	-	0.79 (0.65, 0.97)	-	0.79 (0.65, 0.97)
HSI defined steatosis, yes/no	2.34 (1.74, 3.41)	1.81 (1.31, 3.08)	1.80 (1.39, 2.65)	1.65 (0.91, 2.93)	1.74 (0.97, 2.10)	1.78 (1.04, 2.40)	1.62 (0.97, 2.10)	1.62 (0.97, 2.10)
Age, per 1 year	-	1.11 (1.07, 1.15)	1.09 (1.04, 1.14)	1.08 (1.04, 1.14)	1.08 (1.03, 1.14)	1.08 (1.03, 1.14)	1.08 (1.03, 1.14)	1.08 (1.03, 1.14)
Male gender	-	1.87 (1.40, 2.44)	1.82 (1.36, 2.45)	1.81 (1.17, 2.76)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)
Years of school, per 1 year	-	1.15 (1.00, 1.33)	1.15 (1.00, 1.33)	1.15 (0.99, 1.32)	1.14 (0.99, 1.32)	1.14 (0.99, 1.32)	1.14 (0.99, 1.32)	1.14 (0.99, 1.32)
MedDietScore (range 0-55), per 1/55	-	-	0.93 (0.81, 1.07)	-	-	-	0.97 (0.85, 1.11)	0.97 (0.85, 1.11)
Physical activity,	-	-	0.32	-	-	-	0.35	0.35

yes vs. no			(1.12, 0.82)				(0.13, 0.95)	(0.13, 0.95)
Current smoking, yes vs. no	-	-	1.38 (0.55, 3.42)	-	-	-	1.60 (0.59, 4.28)	1.60 (0.59, 4.28)
Obesity, yes vs. no	-	-	-	1.95 (1.20, 4.50)	-	-	1.95 (1.20, 4.50)	1.95 (1.20, 4.50)
Waist circumference, per 1 cm	-	-	-	1.02 (0.98, 1.07)	-	-	1.03 (0.98, 1.08)	1.03 (0.98, 1.08)
HOMA-IR, per 1 unit	-	-	-	-	1.14 (0.56, 2.33)	-	1.14 (0.56, 2.33)	1.14 (0.56, 2.33)
CRP, per 1 mg/L	-	-	-	-	0.99 (0.80, 1.23)	-	0.99 (0.80, 1.23)	0.99 (0.80, 1.23)
LDL, per 1 mg/dL	-	-	-	-	1.03 (1.01, 1.05)	-	1.03 (1.01, 1.05)	1.03 (1.01, 1.05)
Adiponectin, per 1 μg/L	-	-	-	-	-	0.87 (0.75, 0.99)	-	0.87 (0.75, 1.05)
FLI defined steatosis, yes/no	2.44 (1.72, 3.90)	1.97 (1.28, 3.35)	1.65 (1.20, 3.15)	0.85 (0.20, 2.95)	1.20 (0.55, 3.30)	1.31 (0.67, 3.40)	0.80 (0.21, 3.04)	0.80 (0.21, 3.04)
Age, per 1 year	-	1.12 (1.09, 1.16)	1.10 (1.05, 1.16)	1.10 (1.05, 1.16)	1.11 (1.05, 1.17)	1.11 (1.05, 1.17)	1.11 (1.05, 1.17)	1.11 (1.05, 1.17)
Male gender	-	1.87 (1.40, 2.44)	1.82 (1.36, 2.45)	1.81 (1.17, 2.76)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)
Years of school,	-	1.21 (1.03, 1.42)	1.21	1.19	1.20	1.20	1.20	1.20

per 1 year			(1.03, 1.42)	(1.02, 1.40)	(1.02, 1.42)	(1.02, 1.42)	(1.02, 1.42)	(1.02, 1.42)
MedDietScore (range 0-55), per 1/55	-	-	0.95 (0.82, 1.09)	-	-	-	0.99 (0.86, 1.13)	0.99 (0.86, 1.13)
Physical activity, yes vs. no	-	-	0.39 (0.14, 1.09)	-	-	-	0.44 (0.15, 1.32)	0.44 (0.15, 1.32)
Current smoking, yes vs. no	-	-	1.22 (0.45, 3.26)	-	-	-	1.32 (0.44, 3.91)	1.32 (0.44, 3.91)
Obesity, yes vs. no	-	-	-	1.95 (1.20, 4.50)	-	-	1.95 (1.20, 4.50)	1.95 (1.20, 4.50)
Waist circumference, per 1 cm	-	-	-	1.02 (0.98, 1.08)	-	-	1.04 (0.98, 1.10)	1.04 (0.98, 1.10)
HOMA-IR, per 1 unit	-	-	-	-	1.01 (0.48, 2.13)	-	1.01 (0.48, 2.13)	1.01 (0.48, 2.13)
CRP, per 1 mg/L	-	-	-	-	0.92 (0.73, 1.15)	-	0.92 (0.73, 1.15)	0.92 (0.73, 1.15)
LDL, per 1 mg/dL	-	-	-	-	1.02 (1.01, 1.04)	-	1.02 (1.01, 1.04)	1.02 (1.01, 1.04)
Adiponectin, per 1 μg/L	-	-	-	-	-	0.75 (0.63, 0.99)	-	0.75 (0.63, 0.99)

ORs and their 95%CIs were obtained though logistic regression analysis. Metabolically healthy status was defined as the absence of 4 metabolic syndrome components i.e. elevated triglycerides, reduced high density lipoprotein, elevated blood pressure and elevated fasting glucose including the drug treatment for all these conditions. Abbreviations: C-Reactive Protein (CRP); Fatty liver index (FLI); Hepatic steatosis index (HSI); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR); Low Density Lipoprotein (LDL); Non-alcoholic fatty liver disease fatty liver score (NAFLD-FLS); Odds ratio (OR); 95% Confidence Interval (95%CI).

*Models with adiponectin were run in *n*=90 obese subjects for whom adiponectin metrics were available.

Table 4.35 Nested Cox regression analysis models to evaluate the association of combined obesity and metabolic status with 10-year cardiovascular disease onset according to predicted NAFLD (*n*=1,890).

	N	CVD incidence, %	Crude model	Age- and sex-adjusted model	Multi-adjusted model	
					Model 1	Model 2
Total sample			HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Combined obesity and metabolic status						
MHN	686	6	*(ref)*	*(ref)*	*(ref)*	*(ref)*
MHO	107	16	1.89 (1.24, 2.87)	1.37 (1.00, 2.17)	1.07 (0.98, 2.37)	0.95 (0.37, 2.08)
MHO throughout[1]	51	12	1.75 (1.15, 2.69)	1.22 (0.92, 2.05)	1.01 (0.84, 1.97)	0.91 (0.30, 1.89)
MHO to MUO[2]	56	21	2.76 (2.01, 3.40)	1.81 (1.22, 2.55)	1.83 (1.24, 2.69)	1.43 (1.02, 2.01)
MUN	672	20	2.39 (1.67, 3.10)	1.81 (1.26, 2.60)	1.72 (1.03, 2.87)	1.45 (0.85, 2.50)
MUO	425	25	2.93 (2.05, 3.37)	2.73 (1.85, 3.22)	2.41 (1.42, 3.08)	2.04 (1.15, 2.89)
			p for trend=0.001	*p for trend=0.001*	*p for trend=0.02*	*p for trend=0.03*

p for interaction between NAFLD and combined obesity and metabolic status on 10-year CVD incidence=0.002

	N/cases	CVD incidence, %	Crude model	Age- and sex-adjusted model	Multi-adjusted model	
					Model 1	Model 2

NAFLF			HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Combined obesity and metabolic status						
MHN	148	4	*(ref)*	*(ref)*	*(ref)*	*(ref)*
MHO	19	20	3.11 (1.65, 5.86)	3.02 (1.50, 5.79)	3.02 (1.50, 5.79)	2.90 (1.35, 5.40)
MUN	155	23	3.55 (0.72, 5.30)	3.30 (0.61, 5.05)	3.30 (0.61, 5.05)	2.95 (0.40, 4.90)
MUO	138	24	4.32 (2.02, 6.23)	4.25 (1.90, 6.13)	4.25 (1.90, 6.13)	4.22 (1.85, 6.10)
			p for trend=0.01	*p for trend=0.01*	*p for trend=0.04*	*p for trend=0.04*
	N/cases	CVD incidence, %	Crude model	Age- and sex-adjusted model	Multi-adjusted model	
					Model 1	Model 2
Non-NAFLD			HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Combined obesity and metabolic status						
MHN	538	5	*(ref)*	*(ref)*	*(ref)*	*(ref)*
MHO	88	13	2.87 (0.93, 6.88)	2.80 (0.85, 6.75)	2.80 (0.85, 6.75)	2.60 (0.55, 6.65)
MUN	517	17	3.03 (1.81, 5.08)	2.95 (1.70, 4.99)	2.95 (1.70, 4.99)	2.51 (1.64, 4.84)
MUO	287	18	4.01 (1.90, 6.46)	3.90 (1.85, 6.40)	3.90 (1.85, 6.40)	3.47 (1.55, 6.30)
			p for trend=0.02	*p for trend=0.01*	*p for trend=0.03*	*p for trend=0.03*

HRs and their 95%CIs were obtained though Cox regression analysis. Participants were assigned to NAFLD subgroup in case they met the criteria of at least one of here following scores: Fatty liver index (FLI); Hepatic steatosis index (HSI); Non-alcoholic fatty liver disease fatty liver score (NAFLD-FLS). Model 1 was adjusted for age, sex, educational status, physical activity, current smoking, MedDietScore, low density lipoprotein levels, family history of cardiovascular disease. Model 2 was adjusted for Model 1 plus waist circumference, C-reactive protein and Homeostatic Model Assessment

of Insulin Resistance. Bold indicates estimates that are significantly different from the reference group at $p<0.05$. *Abbreviations:* Hazard ratio (HR); Metabolically healthy non-obese (MHN); Metabolically healthy obese (MHO); Metabolically unhealthy non-obese (MUN); Metabolically unhealthy obese (MUO); Non-alcoholic fatty liver disease (NAFLD); 95% Confidence Interval (95%CI).

[1] MHO throughout was defined as the subgroup of MHO individuals that retained their metabolically healthy status within the decade.

[2] MHO to MUO was defined as the subgroup of MHO individuals that lost their metabolically healthy status within the decade and became MUO.

The discriminatory ability of several epidemiological models adjusted for metabolic status was evaluated in the entire sample as well as separately for men and women and results are presented in **Table 4.36**. As base model it was considered a model adjusted for conventional CVD risk factors and total metabolic status. Overall, this base model had a good discriminative ability against CVD in both men and women. The 1st phase of adjustment was obesity and visceral adiposity. This significantly increased the discriminative ability of the epidemiological model (*p=0.01*); the added value of obesity and visceral adiposity was more evident in men (C-index for men=0.715 vs. C-index for women=0.702). The 2nd phase was constructing a basic model adjusted for NAFLD. This model discriminated significantly better the primary endpoint of developing CVD (*p=0.002*). This was slightly more evident in women (C-index for women=0.719 vs. C-index for men=0.711). The 3rd phase was a model where in addition to metabolic status obesity, visceral adiposity and NAFLD were included. This model had the best – and similar between men and women– discriminating ability towards CVD (*p=0.002*).

Table 4.36 C-index of multiadjusted models to evaluate the discriminative ability of healthy vs. unhealthy metabolic status against 10-year cardiovascular disease event in men and women of the ATTICA (*n*=1890) study.

	C-index (95%CI)	*p-value*	C-index changes (95%CI)	*p-value*
Total sample				
Base model	0.699 (0.659, 0.711)	*<0.001*	-	-
Base model plus obesity and waist circumference	0.710 (0.679, 0.720)	*<0.001*	0.011 (0.009, 0.020)	*0.01*
Base model plus NAFLD status	0.711 (0.689, 0.734)	*<0.001*	0.011 (0.008, 0.018)	*0.002*
Fully-adjusted model	0.718 (0.681, 0.723)	*<0.001*	0.019 (0.012, 0.022)	*0.002*
Men				
Base model	0.700 (0.678, 0.723)	*<0.001*	-	-
Base model plus obesity and waist circumference	0.715 (0.690, 0.730)	*<0.001*	0.015 (0.007, 0.012)	*0.02*
Base model plus NAFLD status	0.711 (0.689, 0.734)	*<0.001*	0.011 (0.008, 0.018)	*0.01*

Fully adjusted model	0.717 (0.691, 0.732)	*<0.001*	0.017 (0.009, 0.013)	*0.02*
Women				
Base model	0.690 (0.618, 0.700)	*<0.001*	-	
Base model plus obesity and waist circumference	0.702 (0.627, 0.714)	*<0.001*	0.012 (0.009, 0.014)	*0.04*
Base model plus NAFLD status	0.719 (0.640, 0.731)	*<0.001*	0.029 (0.022, 0.031)	*0.003*
Fully adjusted model	0.719 (0.641, 0.731)	*<0.001*	0.029 (0.023, 0.031)	*0.003*

Base model was adjusted for age, (sex), metabolic status (healthy vs. unhealthy), current smoking and family history of cardiovascular disease. C-index and the corresponding confidence interval was evaluated through the area under the curve obtained from the Receiver operating Characteristics (ROC) analysis. ROC analysis was performed using the probabilities for 10-year first fatal/non-fatal cardiovascular disease event, corresponding to each study participant, separately for men and women, calculated from Cox regression analysis using the multiadjusted models described. Significance of the changes in C-index was tested by differences in 2 log likelihood of regression models with and without cardiometabolic measurements. Participants were assigned to NAFLD subgroup in case they met the criteria of at least one of here following scores: Fatty liver index (FLI); Hepatic steatosis index (HSI); Non-alcoholic fatty liver disease fatty liver score (NAFLD-FLS). *Abbreviations:* Non-alcoholic fatty liver disease (NAFLD); 95% Confidence Interval (95%CI).

4.4.3 Mediterranean diet, NAFLD and cardiometabolic risk factors: a sex-based analysis

4.4.3.1 Scope and research hypothesis

The scope here was **a)** to examine the association between Mediterranean diet and liver steatosis or fibrosis, diagnosed through non-invasive methods, **b)** to study the potentially protective effect of Mediterranean diet on diabetes onset and first fatal/non-fatal CVD event over a period of ten-years in a cohort of apparently healthy men and women with and without NAFLD and **c)** to explore underlying mechanisms. Three a priori research hypotheses were tested; *first*, high adherence to Mediterranean diet is independently associated with better liver health, *second*, the protective role of the examined dietary pattern on liver health is mediated primarily by adiponectin and secondarily several biomarkers related with inflammation, insulin resistance (IR) and redox stress that are downstream of adiponectin and *third*, that the Mediterranean diet protects against CVD and diabetes among patients with NAFLD. Research hypotheses were specified to men and women.

4.4.3.2 Methods ans statistical analysis

Participants' total daily energy and macronutrient (i.e. total carbohydrate, total fat, animal- and plant-based protein) intake was derived through their responses to the FFQ based on standard food databases. As already reported, adherence to Mediterranean diet was evaluated on the basis

of the MedDietScore (range 0-55, with higher values for greater adherence). The tertiles of the score were as follows; low (<25/55), moderate (26-35/55), and high (>35/55) level of adherence. Additionally, to avoid external splits of the sample in several types of analyses, due to sample size limitations, the median value of MedDietScore was used i.e. 27/55. MedDietScore<27 corresponded to low adherence to Mediterranean diet while MedDietScore≥27 corresponded to moderate/high level of adherence. This cut-off value had the best discriminative ability against 10-year CVD incidence. Liver steatosis was evaluated using TyG index.

Odds ratios (OR) and their corresponding 95% confidence intervals (95%CI) were evaluated through multivariable logistic regression analysis. Hazard ratios (HR) and their corresponding 95%CI were obtained through Cox regression analysis. Multi-adjusted linear regression models were applied to test the association between MedDietScore (per 1/55 unit) and various biomarkers (per 1 unit).

4.4.3.3 Findings

Nested Cox regression models to evaluate the association between adherence to Mediterranean diet and TyG-defined liver steatosis are presented in **Table 4.37**. In the sample used for the primary research hypothesis (*n*=3,042), *n*=1,263 cases of liver steatosis were defined through the TyG index (TyG cut-off of 8·5 was used to categorize liver steatosis). In the crude model, participants assigned in 2nd and 3rd MedDietScore tertile presented about 45% and 87% lower likelihood of having liver steatosis; sex-based stratified analysis revealed that this association reached significance in both sexes with the man-to-woman rate ratio being close to -1- in the 2nd tertile and -1·38- within 3rd tertile (*p for sex interaction=0.03*). On the basis of *Model 5*, where various factors including obesity and waist-to-hip ratio were included as confounders, this association was retained only for participants assigned in the 3rd tertile and only in total sample (*p for trend=0.002*) and men (*p for trend=0.01*). Adjusting for total energy intake did not alter these observations.

Table 4.37 Nested total and sex-based sensitivity logistic regression analysis to evaluate the association of level of adherence to Mediterranean diet (defined through MedDietScore) with liver steatosis presence (defined through TyG cut-off point) (*n*=3,042).

Liver Steatosis (yes vs. no)		**Total**	**Men**	**Women**	
		OR (95%CI)	OR (95%CI)	OR (95%CI)	
N/cases		3,042/1,263	1514/793	1528/470	**Model adjusted for**
MedDietScore tertiles					
	1st	*Ref*	*Ref*	*Ref*	**Model 1**: crude model
	2nd	0.55 (0.45, 0.67)*	0.56 (0.44, 0.71)*	0.57 (0.40, 0.82)*	
	3rd	0.13 (0.10, 0.16)*	0.18 (0.12, 0.28)*	0.13 (0.09, 0.18)*	
p for trend		*<0.001*	*<0.001*	*<0.001*	
MedDietScore tertiles					
	1st	*Ref*	*Ref*	*Ref*	**Model 2:** age, (gender)
	2nd	0.77 (0.62, 0.95)**	0.75 (0.57, 0.98)***	0.72 (0.50, 1.05)	
	3rd	0.29 (0.21, 0.39)*	0.32 (0.20, 0.52)*	0.28 (0.18, 0.44)*	
p for trend		*<0.001*	*<0.001*	*<0.001*	
MedDietScore tertiles					
	1st	*Ref*	*Ref*	*Ref*	**Model 3**: Model 2 plus educational status, smoking habits, physical activity
	2nd	0.76 (0.61, 0.94)**	0.75 (0.57, 0.99)***	0.70 (0.48, 1.01)	
	3rd	0.29 (0.21, 0.40)*	0.34 (0.21, 0.55)*	0.27 (0.18, 0.42)*	
p for trend		*<0.001*	*<0.001*	*<0.001*	
MedDietScore tertiles					
	1st	*Ref*	*Ref*	*Ref*	**Model 4**: Model 3 plus hypertension, hypercholesterolemia, diabetes mellitus, creatinine clearance
	2nd	0.74 (0.58, 0.94)**	0.42 (0.53, 0.99)***	0.72 (0.47, 1.09)	

	3rd	0.32 (0.23, 0.46)*	0.38 (0.22, 0.65)*	0.31 (0.19, 0.50)*	
p for trend		*<0.001*	*<0.001*	*0.002*	
MedDietScore tertiles					
	1st	*Ref*	*Ref*	*Ref*	**Model 5:** Model 4 plus obesity, waist-to-hip ratio
	2nd	0.98 (0.74, 1.29)	0.90 (0.63, 1.01)	1.05 (0.68, 1.78)	
	3rd	0.56 (0.37, 0.84)**	0.55 (0.40, 0.85)***	0.71 (0.38, 1.33)	
p for trend		*0.002*	*0.01*	*0.24*	
MedDietScore tertiles					
	1st	*Ref*	*Ref*	*Ref*	**Model 6:** Model 5 plus total daily energy intake
	2nd	1.07 (0.73, 1.58)	0.93 (0.65, 1.02)	1.05 (0.68, 1.79)	
	3rd	0.53 (0.29, 0.95)***	0.54 (0.41, 0.85)***	0.73 (0.39, 1.32)	
p for trend		*0.02*	*0.02*	*0.30*	

ORs and their corresponding 95%CIs were obtained from logistic regression analysis. ***Abbreviations:*** Confidence Interval (CI); Odds Ratio (HR); Triglycerides-glucose (TyG). **p<0.001*, ***p<0.01*, ****p<0.05*.

Prospective interactions between nutrition and liver steatosis in the entire cohort, or stratifying by sex, on 10-year onset of diabetes and CVD incidence were also examined and results are presented in **Table 4.38**. Focusing on diabetes, in crude models, participants with NAFLD had about four times higher risk to develop diabetes compared with their non-NAFLD counterparts. After adjusting for various factors including obesity and metabolic syndrome parameters, this association was slightly alleviated by one point. Sensitivity analysis revealed that this aggravating effect was alleviated for MedDietScore above median; while participants with NAFLD and MedDietScore below median had about three times higher risk to develop diabetes those who adhere to Mediterranean diet were protected from an abnormal glycaemic profile. These associations were more evident in women (*p for sex interaction*=0·02).

Table 4.38 HRs and 95%Cs of TyG-defined liver steatosis in relation to 10-year diabetes incidence according to the reported dietary habits (*n*=1,485) and in relation to 10-year first fatal/non-fatal cardiovascular disease incidence according to the reported dietary habits (*n*=2,020).

Total sample			
	Total sample	**Men**	**Women**
N, cases	*1,485/191*	*726/97*	*759/94*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	3.66 (2.55, 5.25)*	2.98 (1.73, 5.13)*	4.88 (2.77, 6.02)*
Multi-adjusted model	2.95 (2.01, 4.32)*	2.74 (1.56, 4.81)*	3.38 (2.00, 5.70)*
Multi-adjusted model plus MedDietScore	2.95 (2.01, 4.31)*	1.62 (1.01, 2.63)*	3.35 (1.98, 5.66)*
Sample stratified according to level of adherence to Mediterranean diet			
	Total sample	**Men**	**Women**
MedDietScore<27			
N, cases	*924/159*	*624/94*	*300/65*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	2.92 (1.90, 4.46)*	2.87 (1.63, 5.06)*	3.46 (1.79, 6.71)*
Multi-adjusted model	3.05 (1.96, 4.72)*	2.94 (1.63, 5.30)*	3.38 (1.74, 6.57)*
MedDietScore≥27			
N, cases	*561/32*	*102/3*	*459/29*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	2.34 (1.01, 5.43)**	3.10 (0.57, 4.53)	3.23 (0.98, 7.71)
Multi-adjusted model	1.87 (0.75, 4.61)	2.26 (0.40, 4.84)	2.29 (0.90, 5.80)
Total sample			
	Total sample	**Men**	**Women**
N, cases	*2,020/317*	*1,006/198*	*1,014/119*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	3.01 (2.28, 3.95)*	2.70 (1.84, 3.95)*	2.83 (1.86, 4.30)*
Multi-adjusted model	1.37 (1.10, 2.10)**	1.61 (1.01, 2.57)*	1.11 (0.66, 1.88)
Multi-adjusted model plus MedDietScore	1.36 (0.96, 1.94)	1.62 (1.01, 2.63)**	1.08 (0.63, 1.85)
Sample stratified according to level of adherence to Mediterranean diet			
	Total sample	**Men**	**Women**
MedDietScore<27			
N, cases	*1,223/280*	*854/188*	*369/92*

Liver steatosis (yes vs. no)		**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
	Crude model	1.92 (1.41, 2.62)*	2.30 (1.54, 3.42)*	1.49 (0.89, 2.50)
	Multi-adjusted model	1.40 (1.01, 2.03)**	1.65 (1.02, 2.69)**	1.09 (0.60, 1.98)
MedDietScore≥27				
N, cases		*797/37*	*152/10*	*645/27*
Liver steatosis (yes vs. no)		**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
	Crude model	2.05 (0.94, 4.50)	3.11 (0.66, 4.55)	1.72 (0.66, 4.48)
	Multi-adjusted model	1.00 (0.38, 2.63)	1.26 (0.20, 5.64)	0.83 (0.24, 2.84)

HRs and corresponding CIs were obtained through Cox regression analysis. Multi-adjusted model was adjusted for age, (gender), hypertension, hypercholesterolemia, current smoking, physical activity, body mass index, family history of cardiovascular disease. ***Abbreviations:*** Confidence Interval (CI); Hazard ratio (HR); Triglycerides-glucose (TyG). *p<0.001*, ***p<0.05*.

Regarding the association between NAFLD and CVD as well as the interacting effect of Mediterranean diet, in crude models, NAFLD led to a threefold increase in CVD. However, after adjusting for various factors including obesity and metabolic syndrome parameters, this association was alleviated. These findings indicate that the Mediterranean diet may affect CVD through its effects on obesity, metabolic syndrome etc. confounders studied herein, yet the association retained its significance indicating an independent effect; in particular, participants with liver steatosis had about 37% higher risk to develop CVD compared with their liver steatosis-free counterparts. Sensitivity analysis revealed that this aggravating effect was alleviated for MedDietScore above median; while participants with NAFLD and MedDietScore below median had about 40% higher risk to develop CVD those who adhere to Mediterranean diet were protected from CVD. These associations were more evident in men (*p for sex interaction*=0·001)

The combined role of level of adherence to Mediterranean diet and total carbohydrate intake on the likelihood of liver steatosis is presented in the **Table 4.40**. Multi-adjusted analysis revealed that high level of adherence to Mediterranean diet was associated with about 41-48% lower likelihood of liver steatosis irrespective to the carbohydrate intake status, compared with the reference group (low adherence to Mediterranean diet and high carbohydrate intake).

Table 4.39 HRs and 95%Cs of TyG-defined liver steatosis in relation to 10-year diabetes incidence according to the reported dietary habits (*n*=1,485) and in relation to 10-year first fatal/non-fatal cardiovascular disease incidence according to the reported dietary habits (*n*=2,020).

Total sample			
	Total sample	**Men**	**Women**
N, cases	*1,485/191*	*726/97*	*759/94*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	3.66 (2.55, 5.25)*	2.98 (1.73, 5.13)*	4.88 (2.77, 6.02)*
Multi-adjusted model	2.95 (2.01, 4.32)*	2.74 (1.56, 4.81)*	3.38 (2.00, 5.70)*
Multi-adjusted model plus MedDietScore	2.95 (2.01, 4.31)*	1.62 (1.01, 2.63)*	3.35 (1.98, 5.66)*
Sample stratified according to level of adherence to Mediterranean diet			
	Total sample	**Men**	**Women**
MedDietScore<27			
N, cases	*924/159*	*624/94*	*300/65*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	2.92 (1.90, 4.46)*	2.87 (1.63, 5.06)*	3.46 (1.79, 6.71)*
Multi-adjusted model	3.05 (1.96, 4.72)*	2.94 (1.63, 5.30)*	3.38 (1.74, 6.57)*
MedDietScore≥27			
N, cases	*561/32*	*102/3*	*459/29*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	2.34 (1.01, 5.43)**	3.10 (0.57, 4.53)	3.23 (0.98, 7.71)
Multi-adjusted model	1.87 (0.75, 4.61)	2.26 (0.40, 4.84)	2.29 (0.90, 5.80)
Total sample			
	Total sample	**Men**	**Women**

N, cases	*2,020/317*	*1,006/198*	*1,014/119*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	3.01 (2.28, 3.95)*	2.70 (1.84, 3.95)*	2.83 (1.86, 4.30)*
Multi-adjusted model	1.37 (1.10, 2.10)**	1.61 (1.01, 2.57)*	1.11 (0.66, 1.88)
Multi-adjusted model plus MedDietScore	1.36 (0.96, 1.94)	1.62 (1.01, 2.63)**	1.08 (0.63, 1.85)
Sample stratified according to level of adherence to Mediterranean diet			
	Total sample	**Men**	**Women**
MedDietScore<27			
N, cases	*1,223/280*	*854/188*	*369/92*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	1.92 (1.41, 2.62)*	2.30 (1.54, 3.42)*	1.49 (0.89, 2.50)
Multi-adjusted model	1.40 (1.01, 2.03)**	1.65 (1.02, 2.69)**	1.09 (0.60, 1.98)
MedDietScore≥27			
N, cases	*797/37*	*152/10*	*645/27*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	2.05 (0.94, 4.50)	3.11 (0.66, 4.55)	1.72 (0.66, 4.48)
Multi-adjusted model	1.00 (0.38, 2.63)	1.26 (0.20, 5.64)	0.83 (0.24, 2.84)

HRs and corresponding CIs were obtained through Cox regression analysis. Multi-adjusted model was adjusted for age, (gender), hypertension, hypercholesterolemia, current smoking, physical activity, body mass index, family history of cardiovascular disease. ***Abbreviations:*** Confidence Interval (CI); Hazard ratio (HR); Triglycerides-glucose (TyG). *$p<0.001$, **$p<0.05$.*

Table 4.40 Nested total and sex-based sensitivity logistic regression analysis to evaluate the combined association of level of adherence to Mediterranean diet (defined through MedDietScore) and carbohydrate content of diet with liver steatosis presence(defined through TyG cut-off point) (*n*=3,042).

Liver Steatosis (yes vs. no)	**Total**	**Men**	**Women**	
	OR (95%CI)	OR (95%CI)	OR (95%CI)	
N/cases	3,042/1,263	1514/793	1528/470	**Model adjusted for**
MedDietScore/CHO content				
Low MedDietScore/High CHO	*Ref*	*Ref*	*Ref*	
Low MedDietScore/Low CHO	1.07 (0.73, 1.57)	1.04 (0.67, 1.60)	1.08 (0.48, 2.41)	**Model 1**: crude model
High MedDietScore/High CHO	0.37 (0.18, 0.76)*	0.20 (0.10, 0.56)*	0.24 (0.10, 0.59)*	
High MedDietScore/Low CHO	0.41 (0.39, 0.83)*	0.33 (0.18, 0.64)*	0.26 (0.12, 0.56)*	
p for trend	*<0.001*	*<0.001*	*<0.001*	
MedDietScore/CHO content				
Low MedDietScore/High CHO	*Ref*	*Ref*	*Ref*	
Low MedDietScore/Low CHO	1.16 (0.78, 1.71)	1.18 (0.73, 1.80)	1.16 (0.50, 2.67)	**Model 2:** age, (gender)
High MedDietScore/High CHO	0.42 (0.22, 0.81)**	0.34 (0.10, 0.91)**	0.36 (0.16, 1.00)***	
High MedDietScore/Low CHO	0.51 (0.30, 0.85)**	0.60 (0.31, 1.02)	0.39 (0.15, 0.93)**	
p for trend	*<0.001*	*<0.001*	*<0.001*	
MedDietScore/CHO content				
Low MedDietScore/High CHO	*Ref*	*Ref*	*Ref*	
Low MedDietScore/Low CHO	1.16 (0.78, 1.71)	1.18 (0.73, 1.80)	1.16 (0.50, 2.67)	**Model 3**: Model 2 plus educational status, smoking habits, physical activity
High MedDietScore/High CHO	0.42 (0.22, 0.81)**	0.34 (0.10, 0.91)**	0.36 (0.16, 1.00)***	
High MedDietScore/Low CHO	0.51 (0.30, 0.85)**	0.60 (0.31, 1.02)	0.39 (0.15, 0.93)**	
p for trend	*<0.001*	*<0.001*	*<0.001*	
MedDietScore/CHO content				**Model 4**: Model 3 plus

Low MedDietScore/High CHO	*Ref*	*Ref*	*Ref*	hypertension, hypercholesterolemia, diabetes mellitus
Low MedDietScore/Low CHO	1.16 (0.78, 1.71)	1.18 (0.73, 1.80)	1.16 (0.50, 2.67)	
High MedDietScore/High CHO	0.42 (0.22, 0.81)**	0.34 (0.10, 0.91)**	0.36 (0.16, 1.00)***	
High MedDietScore/Low CHO	0.51 (0.30, 0.85)**	0.60 (0.31, 1.02)	0.39 (0.15, 0.93)**	
p for trend	*<0.001*	*<0.001*	*0.002*	
MedDietScore/CHO content				
Low MedDietScore/High CHO	*Ref*	*Ref*	*Ref*	**Model 5:** Model 4 plus obesity, waist-to-hip ratio
Low MedDietScore/Low CHO	1.16 (0.78, 1.72)	1.16 (0.74, 1.82)	1.18 (0.52, 2.68)	
High MedDietScore/High CHO	0.44 (0.23, 0.84)***	0.40 (0.13, 0.99)***	0.38 (0.15, 1.01)	
High MedDietScore/Low CHO	0.52 (0.31, 0.88)***	0.65 (0.32, 1.04)	0.41 (0.17, 0.95)***	
p for trend	*0.002*	*0.01*	*0.24*	
MedDietScore/CHO content				
Low MedDietScore/High CHO	*Ref*	*Ref*	*Ref*	**Model 6:**Model 5 plus total daily energy intake
Low MedDietScore/Low CHO	1.12 (0.73, 1.72)	1.15 (0.73, 1.80)	1.21 (0.53, 2.74)	
High MedDietScore/High CHO	0.52 (0.26, 0.99)***	0.42 (0.14, 1.00)***	0.40 (0.15, 1.10)	
High MedDietScore/Low CHO	0.59 (0.33, 0.95)***	0.68 (0.33, 1.08)	0.42 (0.18, 0.98)***	
p for trend	*0.04*	*0.25*	*0.05*	

ORs and their corresponding 95%CIs were obtained from logistic regression analysis. Low MedDietScore was defined as MedDietScore below the median value i.e. MedDietScore<27. Low daily total CHO intake was defined as CHO<35% of the total daily energy intake. ***Abbreviations:*** Carbohydrates (CHO); Confidence Interval (CI); Odds Ratio (HR); Triglycerides-glucose (TyG). *$p<0.001$, **$p<0.01$, ***$p<0.05$.

Independent associations between Mediterranean diet and adipokines and biomarkers of IR and inflammation in the total study sample and in subgroups defined according to the presence or absence of liver steatosis are summarized in **Table 4.41** indicating significant positive associations of

Mediterranean diet with the endogenous insulin sensitizer adiponectin and negative associations with leptin, a marker of overall obesity, IR and circulating inflammatory markers.

Table 4.41 Results from multi-adjusted linear regression analysis regarding the association between MedDietScore (per 1/55 unit) and inflammation-, insulin resistance- and adipokines-related markers according to participants' TyG-defined liver steatosis status (*n*=3,042).

TOTAL	**Total sample**	**Men**	**Women**
N	3,042	1,598	1,444
	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)
C-Reactive Protein, per 1 mg/L	−0.09 (0.07)*	−0.03 (0.09)	−0.11 (0.10)*
Interleukin 6, per 1 pg/dL	−0.19 (0.43)*	−0.18 (0.53)*	−0.22 (0.66)*
HOMA-IR, per 1 unit	−0.12 (0.21)*	−0.10 (0.30)*	−0.11 (0.41)*
Leptin, per 1 μg/L	−0.13 (0.12)*	−0.18 (0.16)*	−0.13 (0.11)**
Adiponectin, per 1 μg/L	+0.15 (0.12)*	+0.19(0.20)*	+0.03 (0.19)
Adiponectin-to-leptin ratio, per 1 unit	+0.13 (0.27)*	+0.22 (0.34)*	+0.11 (0.45)
TyG-defined liver steatosis, no	**Total sample**	**Men**	**Women**
N	1,799	805	974
	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)
C-Reactive Protein, per 1 mg/L	−0.10 (0.08)*	−0.02 (0.10)	−0.14 (0.11)*
Interleukin 6, per 1 pg/dL	−0.22 (0.48)*	−0.15 (0.54)*	−0.23 (0.68)*
HOMA-IR, per 1 unit	−0.13 (0.28)*	−0.06 (0.31)**	−0.01 (0.48)
Leptin, per 1 μg/L	−0.08 (0.10)	−0.15 (0.15)*	−0.14 (0.13)**
Adiponectin, per 1 μg/L	+0.09 (0.16)	+0.16 (0.18)**	+0.04 (0.23)
Adiponectin-to-leptin ratio, per 1 unit	+0.04 (0.31)	+0.19 (0.39)	+0.11 (0.41)

TyG-defined liver steatosis, yes	**Total sample**	**Men**	**Women**
N	1,263	793	470
	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)	Beta-Coefficient (standard error)
C-Reactive Protein, per 1 mg/L	−0.06 (0.08)**	−0.06 (0.10)**	−0.09 (0.13)**
Interleukin 6, per 1 pg/dL	−0.14 (0.47)*	−0.10 (0.56)*	−0.20 (0.83)*
HOMA-IR, per 1 unit	−0.13 (0.06)*	−0.10 (0.13)*	−0.13 (0.13)*
Leptin, per 1 μg/L	−0.16 (0.16)*	−0.27 (0.19)*	−0.12 (0.33)
Adiponectin, per 1 μg/L	+0.23 (0.30)*	+0.27 (0.35)*	+0.01 (0.66)
Adiponectin-to-leptin ratio, per 1 unit	+0.27 (0.34)*	+0.36 (0.36)*	+0.11 (0.75)

Beta-Coefficients and their corresponding standard error were obtained from linear regression analysis after adjusting for age, (gender), body mass index, current smoking, physical activity, hypertension, diabetes mellitus and hypercholesterolemia. ***Abbreviations:*** Homeostatic Model Assessment of Insulin Resistance (HOMA-IR); Triglycerides-glucose (TyG). ***p<0.05*, ***p<0.10*.

4.5 The sex-specific role of markers of lipidemic profile in relation to ten-year first CVD incidence

4.5.1 The sex-specific role of lipoproteins and apolipoproteins in relation to ten-year first CVD incidence

4.5.1.1 Scope and research hypothesis

The scope here was to evaluate the sex-specific effect of conventional lipid-related markers (i.e., TC, LDL-C, HDL-C, TAG)—on the basis of existing thresholds of blood lipid concentrations—non-conventional lipid-related markers (i.e., TC/HDL-C, non-HDL-C, non-HDL-C/HDL-C), as well as their apolipoproteins on 10-year first fatal/non-fatal CVD incidence, in apparently healthy men and women. The primary research hypothesis was that, considering the biological and lifestyle discrepancies between men and women, sex-specific associations of the examined lipid-related biomarkers against CVD onset exist.

4.5.1.2 Methods ans analysis

Cut-off values of LDL-C, TAG, and non-HDL-C were defined according to the most updated guidelines for dyslipidaemias (Mach et al 2020). In the case of HDL-C, the sex-specific cut-off values suggested in NCEP ATP III (revised) criteria for MetS were used. For TC/HDL and non-HDL-C/HDL-C variables, participants were categorized according to the generated tertiles owing to the lack of national or European thresholds; this categorization contributed to the best discriminative ability against the outcome of interest, that is, the 10-year CVD event.

HRs and their corresponding 95% CIs for lipid-related markers in relation to 10-year CVD event were evaluated through multivariable Cox-regression analysis in the total sample, as well as in subgroups. Total or CVD case-related correct classification rate was also obtained from the aforementioned multivariate analyses. The concordance statistics, that is, C-statistics, was used to evaluate the predictive accuracy of multivariate models adjusted for various lipid markers against the 10-year CVD event. C-indexes and the corresponding 95% CIs were equal to the areas under the curve obtained from the ROC analysis.

4.5.1.3 Findings

The findings from nested Cox regression models that evaluated the association between conventional lipid markers (i.e., TC, LDL-C, HDL-C, and TAG) and CVD incidence in free-of-CVD men and women of the ATTICA study are presented in **Table 4.42**. In the unadjusted models, a positive association was observed between all lipid-related factors and CVD in both men and women (all p-values < 0.05). However, in the age-adjusted models, several sex-specific associations were revealed. In particular, TC lost its independent aggravating effect in both men and women. In the case of men only, HDL-C in terms of continuous variable was inversely associated with CVD onset; a 10 mg/dL HDL-C increase was associated with 20% lower CVD

risk within the decade. However, when adjusting for other clinical and lifestyle factors, this association remained, yet without reaching the level of significance. As for women, the age-adjusted models revealed significant associations only in the case of HDL-C and TAG. This was retained even in multi-adjusted models. Specifically, per 10 mg/dL increase in HDL-C, 10% lower CVD risk was observed, while women with HDL-C>45 mg/dL had about 27% lower risk of developing CVD within the decade. Similarly, per 10 mg/dL rise in TAG, 10% lower CVD risk was revealed, with the risk for CVD onset being about 31% higher in women with TAG>150 mg/dL, in the multi-adjusted model.

Table 4.42 Cox regression analysis to evaluate the association between conventional lipid markers and 10-year first fatal/non-fatal cardiovascular disease incidence in apparently healthy men and women (*n*=2020).

Men (*n*=1006/*n*=198 CVD cases)

	Unadjusted (Crude) Model	**Age-Adjusted model**	**Fully Adjusted Model**
Model for TC			
TC, per 10 mg/dL increase	**1.10 (1.00, 1.21)**	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)
TC (>200 vs. ≤200 mg/dL))	**1.75 (1.28, 2.40)**	1.24 (0.87, 1.75)	1.21 (0.85, 1.73)
Model for LDL			
LDL, per 10 mg/dL increase	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)
LDL status (>100 vs. ≤100 mg/dL)	**1.57 (1.00, 2.50)**	0.90 (0.54, 1.52)	1.10 (0.57, 2.13)
Model for HDL			
HDL, per 10 mg/dL increase	**0.81 (0.66, 0.90)**	**0.81 (0.66, 0.90)**	0.81 (0.66, 1.21)
HDL status (<50 vs. ≥50 mg/dL)	1.42 (0.91, 2.21)	1.44 (0.87, 2.46)	1.36 (0.81, 2.31)
Model for TAG			
TAG, per 10 mg/dL increase	1.10 (1.00, 1.21)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)
TAG status (>150 vs. ≤150 mg/dL)	**2.29 (1.39, 3.77)**	1.10 (0.63, 1.92)	1.60 (0.24, 1.49)

Women (*n*=1014/*n*=119 CVD cases)

	Unadjusted (Crude) Model	**Age-Adjusted Model**	**Multi-Adjusted Model**
Model for TC			
TC, per 10 mg/dL increase	**1.10 (1.00, 1.21)**	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)
TC (>200 vs. ≤200 mg/dL))	**2.19 (1.49, 3.23)**	0.96 (0.42, 1.69)	0.91 (0.58, 1.43)

Model for LDL			
LDL, per 10 mg/dL increase	**1.10 (1.00, 1.21)**	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)
LDL status (>100 vs. ≤100 mg/dL)	**2.66 (1.50, 4.72)**	1.19 (0.63, 2.25)	2.10 (0.72, 2.57)
Model for HDL			
HDL, per 10 mg/dL increase	**0.73 (0.66, 0.90)**	**0.73 (0.66, 0.90)**	**0.73 (0.53, 1.00)**
HDL status (<40 vs. ≥40 mg/dL)	**1.53 (1.07, 2.17)**	**1.65 (1.12, 2.43)**	**1.44 (1.17, 2.14)**
Model for TAG			
TAG, per 10 mg/dL increase	**1.10 (1.00, 1.21)**	**1.10 (1.00, 1.21)**	**1.10 (1.00, 1.21)**
TAG status (>150 vs. ≤150 mg/dL)	**2.14 (1.52, 3.03)**	**1.60 (1.09, 2.34)**	**1.31 (1.01, 2.12)**

HRs and their corresponding 95% CIs were obtained through Cox regression analysis. Multi-adjusted model was adjusted for age, body mass index, current smoking, MedDietScore, hypertension, diabetes mellitus, lipid-lowering treatment, and family history of cardiovascular disease. Bold indicates statistically significant outcomes (p-value < 0.05). Abbreviations: cardiovascular disease (CVD); hazard ratio (HR); high density lipoprotein cholesterol (HDL-C); low density lipoprotein cholesterol (LDL-C); total cholesterol (TC); triglycerides (TAG); 95% confidence interval (95% CI).

The association between apolipoproteins and 10-year CVD incidence was also evaluated in free-of-CVD men and women of the ATTICA study through nested Cox regression analysis, and the results are summarized in **Table 4.43**. It was revealed that, in the case of women, ApoA1 was independently associated with 10-year CVD onset; particularly, per 10 mg/dL increase in ApoA1, the risk of developing CVD was 19% lower. In the case of men, besides the significant trends observed principally for ApoB100, indicating an independent aggravating effect in age-adjusted models, this was not the case after taking into account potential confounders.

Table 4.43 Cox regression analysis to evaluate the association between apolipoproteins and 10-year first fatal/non-fatal cardiovascular disease incidence in apparently healthy men and women (n = 2020).

Men (*n*=1006/*n*=198 CVD cases)

	Unadjusted (Crude) Model	**Age-Adjusted Model**	**Multi-Adjusted Model**
Model for ApoB100			
ApoB100, per 10 mg/dL increase	**1.21 (1.10, 1.34)**	**1.10 (1.00, 1.21)**	**1.10 (1.00, 1.21)**
Model for ApoA1			

ApoA1, per 10 mg/dL increase	**0.81 (0.66, 0.90)**	**0.81 (0.66, 0.90)**	0.81 (0.66, 1.21)
Model for ApoB100/ApoA1			
ApoB100/ApoA1, per 1-unit increase	**1.63 (1.03, 2.57)**	1.18 (0.73, 1.89)	0.93 (0.56, 1.54)
Women (*n*=1014/*n*=119 CVD cases)			
	Unadjusted (Crude) Model	**Age-Adjusted Model**	**Multi-Adjusted Model**
Model for ApoB100			
ApoB100, per 10 mg/dL increase	**1.10 (1.00, 1.21)**	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)
Model for ApoA1			
ApoA1, per 10 mg/dL increase	**0.81 (0.66, 0.90)**	**0.90 (0.81, 0.99)**	**0.90 (0.81, 0.99)**
Model for ApoB100/ApoA1			
ApoB100/ApoA1, per 1-unit increase	1.40 (0.89, 2.22)	0.83 (0.34, 2.00)	0.69 (0.25, 1.88)

HRs and their corresponding 95% CIs were obtained through Cox regression analysis. Multi-adjusted model was adjusted for age, body mass index, current smoking, MedDietScore, hypertension, diabetes mellitus, lipid-lowering treatment, and family history of cardiovascular disease. Bold indicates statistically significant outcomes (p-value < 0.05). Abbreviations: apolipoprotein A1 (ApoA1); apolipoprotein B100 (ApoB100); cardiovascular disease (CVD); hazard ratio (HR); 95% Confidence Interval (95% CI).

Among the principle aims of the present work was to evaluate the effect of non-conventional lipid-related markers—highly discussed—in the recent literature in relation to primary CVD incidence. The results are presented in **Table 4.44**. In particular, the non-HDL-C variable was independently associated with 10-year CVD incidence in the total sample; a 10 mg/dL increase in this variable was associated with about a 10% rise in CVD risk. Interestingly, this effect was retained only in women. When the thresholds suggested by the European Society of Cardiology were used, a steep increase in HR was observed for values >185 mg/dL in the total sample as well as in men, while in the case of women, a significant rise in CVD risk was observed even from lower values, that is, >145 mg/dL. As for the non-HDL-C/HDL-C ratio, similar trends were observed; per 1 unit rise in this ratio, about a 13%–18% increase in CVD risk was observed in the total sample as well as in the men and woman subsamples. Interestingly, when the analysis was repeated in relation to non-HDL-C/HDL-C tertiles, significant positive associations with CVD risk were observed only in the total sample and women, yet only in the case of values >3.75. In the case of TC/HDL-C, similar trends were revealed.

Table 4.44 Cox regression analysis to evaluate the association between novel lipid markers and 10-year first fatal/non-fatal cardiovascular disease incidence in apparently healthy men and women (*n*=2020).

Total (*N*/cases) *2020/317*			**Men (*N*/cases)** *1006/198*			**Women (*N*/cases)** *1014/119*		
Non-HDL-C	**CVD incidence, %**	**HR (95% CI)**	**Non-HDL-C**	**CVD incidence, %**	**HR (95%CI)**	**Non-HDL-C**	**CVD incidence, %**	**HR (95% CI)**
per 10 mg/dL	-	**1.10 (1.00, 1.21)**	per 10 mg/dL	-	1.00 (0.90, 1.10)	per 10 mg/dL	-	**1.10 (1.00, 1.21)**
<100 mg/dL	5.3	*ref*	<100 mg/dL	7.6	*ref*	<100 mg/dL	4.2	*ref*
100–<145 mg/dL	13.3	1.18 (0.61, 2.27)	100–<145 mg/dL	18.2	2.69 (0.91, 6.50)	100–<145 mg/dL	9.4	2.32 (0.89, 5.34)
145–<185 mg/dL	18.0	1.16 (0.60, 2.24)	145–<185 mg/dL	20.4	3.12 (0.89, 4.50)	145–<185 mg/dL	14.8	**3.45 (1.09, 7.43)**
185–<220 mg/dL	20.0	**2.10 (1.54, 3.26)**	185–<220 mg/dL	21.6	**3.04 (1.32, 4.44)**	185–<220 mg/dL	17.3	**3.64 (1.10, 5.96)**
>220 mg/dL	28.9	**2.95 (1.24, 4.32)**	>220 mg/dL	33.9	**3.14 (1.26, 5.10)**	>220 mg/dL	20.6	**3.79 (1.20, 6.20)**
Non-HDL-C/HDL-C	**CVD incidence, %**	**HR (95% CI)**	**Non-HDL-C/HDL-C**	**CVD incidence, %**	**HR (95% CI)**	**Non-HDL-C/HDL-C**	**CVD incidence, %**	**HR (95% CI)**
per 1 unit	-	**1.15 (1.06, 1.26)**	per 1 unit	-	**1.13 (1.02, 1.26)**	per 1 unit	-	**1.18 (1.02, 1.36)**
<2.49	8.4	*ref*	<2.49	13.2	*ref*	<2.49	6.5	*ref*
2.49–3.71	15.2	1.27 (0.85, 1.90)	2.49–3.71	17.7	0.95 (0.53, 1.71)	2.49–3.71	12.4	1.25 (0.70, 2.21)
>3.71	22.0	**1.96 (1.34, 2.86)**	>3.71	24.4	1.35 (0.78, 2.33)	>3.71	18.9	**1.72 (1.10, 3.07)**
TC/HDL-C	**CVD incidence, %**	**HR (95% CI)**	**TC/HDL-C**	**CVD incidence, %**	**HR (95% CI)**	**TC/HDL-C**	**CVD incidence, %**	**HR (95% CI)**
per 1 unit	-	**1.15**	per 1 unit	-	**1.13**	per 1 unit	-	**1.18**

		(1.06, 1.26)			**(1.02, 1.26)**			**(1.02, 1.36)**
<3.49	8.4	*ref*	<3.49	13.2	*ref*	<3.49	6.5	*ref*
3.49–4.71	15.2	1.10 (0.73, 1.66)	3.49–4.71	17.7	0.95 (0.53, 1.71)	3.49–4.71	12.4	1.25 (0.70, 2.21)
>4.71	22.0	**1.54 (1.04, 2.29)**	>4.71	23.4	1.35 (0.78, 2.33)	>4.71	18.9	**1.72 (1.01, 3.07)**

HRs and their corresponding 95% CIs were obtained through Cox regression analysis adjusted for age, body mass index, current smoking, MedDietScore, hypertension, diabetes mellitus, lipid-lowering treatment, and family history of cardiovascular disease. Bold indicates statistically significant outcomes (p-value < 0.05). Abbreviations: Cardiovascular disease (CVD); hazard ratio (HR); high density lipoprotein cholesterol (HDL-C); total cholesterol (TC); 95% confidence interval (95% CI).

The discrimination ability of multi-adjusted epidemiological models adjusted for different combinations of lipid-related biomarkers was evaluated separately for men and women, and the results are summarized in **Table 4.45**. Overall, the discrimination ability (expressed through C-index) of the examined multi-adjusted models adjusted for HDL-C and TAG or non-HDL-C or non-HDL-C/HDL-C or TC/HDL-C was better in the case of women. The correct classification rate for cases in women was more than twice as high in Models 3 and 5–7 compared with Model 2 (i.e., LDL-C adjusted model) (23.3%–26.4% vs. 10.9%). On the other side, in the case of men, models adjusted for LDL-C and ApoB100/ApoA1 had a better discriminative ability against CVD. This observation was followed by the highest correct classification rates for CVD cases in models adjusted for LDL-C (i.e., Models 1 and 2).

Table 4.45 Discrimination-ability parameters of multivariate models adjusted for different combinations of lipid markers over the 10-year first fatal/non-fatal cardiovascular disease event (n = 2020).

Models	Model Adjustment Description	C-Index (95% CI)	Correct Classification Rate, % (Total)	Correct Classification Rate, % (Cases)
Model 1	Standard model* adjusted for **conventional lipid markers**[l]	**Men** 0.772 (0.713, 0.831)	83.6	33.3
		Women 0.831 (0.777, 0.886)	89.6	19.6

Model 2	Standard model* adjusted for **LDL-C**	**Men** 0.830 (0.789, 0.872)	88.6	21.9
		Women 0.772 (0.728, 0.816)	82.8	10.9
Model 3	Standard model* adjusted for **HDL-C & TAG**	**Men** 0.784 (0.741, 0.827)	83.0	18.3
		Women 0.829 (0.795, 0.877)	89.3	24.4
Model 4	Standard model* adjusted for **ApoB100/ApoA1**	**Men** 0.833 (0.792, 0.874)	88.6	23.9
		Women 0.776 (0.734, 0.818)	82.4	13.3
Model 5	Standard model* adjusted for **non-HDL-C**	**Men** 0.769 (0.729, 0.809)	82.4	13.0
		Women 0.836 (0.790, 0.869)	89.1	23.3
Model 6	Standard model* adjusted for **non-HDL-C/HDL-C**	**Men** 0.772 (0.732, 0.812)	82.2	15.0
		Women 0.833 (0.793, 0.873)	89.1	26.2
Model 7	Standard model* adjusted for **TC/HDL-C**	**Men** 0.772 (0.732, 0.812)	82.2	15.0
		Women 0.836 (0.793, 0.873)	89.1	26.2

*Standard model was adjusted for age, body mass index, current smoking, MedDietScore, hypertension, diabetes mellitus, and family history of cardiovascular disease. ǂConventional lipid markers examined were low density lipoprotein cholesterol, high density lipoprotein cholesterol, and triglycerides. C-index and the corresponding confidence interval were evaluated through the area under the curve obtained from the receiver operating characteristics (ROC) analysis. ROC analysis was performed using the probabilities for 10-year first fatal/non-fatal cardiovascular disease event, corresponding to each study participant, separately for men and women, calculated from Cox regression analysis using the multivariate models described. Correct classification rate was obtained from the Cox regression analysis performed using the described models, separately for men and women. Abbreviations: apolipoprotein A1 (ApoA1); apolipoprotein B100 (ApoB100); high density lipoprotein cholesterol (HDL-C); low density lipoprotein cholesterol (LDL-C); total cholesterol (TC); triglycerides (TAG).

4.5.2 The sex-specific role of Lp(a) in relation to ten-year first CVD incidence

4.5.2.1 Scope and research hypothesis

The primary scope here was to evaluate the association between Lp(a) levels and 10-year first fatal/non-fatal CVD risk, the potential mediating effect of sex on the such an association as well as the contribution of Lp(a) to the predictive ability of an epidemiological model with traditional CVD risk factors. The secondary scope was to assess the results from sensitivity analyses based on lipidemic profile and dietary habits of the participants. Three a priori research hypotheses were posed: Lp(a) will be independently associated with 10-year combined CVD risk; Lp(a) will interact with sex regarding CVD onset; the contribution of Lp(a) to CVD risk stratification will be significant.

4.5.2.2 Methods and analysis

The clinically recommended – by the European Society of Atherosclerosis – threshold of 50 mg/dL was used to define Lp(a) status as normal i.e. Lp(a) <50 mg/dL *vs* abnormal Lp(a) ≥50 mg/dL (Catapano et al 2016); in the sample of ATTICA study, this cut-off point had the best discriminative ability against the outcome of interest i.e. 10-year CVD event, through the ROC analysis and the obtained – separately for men and women – optimal point. Besides this, the cut-off point of 30 mg/dL suggested in the literature, was also examined (Tsimikas et al 2017). Lp(a)-corrected LDL-C, that is not taken into account by the Friedewald equation, was derived using the formula Lp(a)-cholesterol=Lp(a) mass * 0.3.

HR and their corresponding 95%CI for the Lp(a) status in relation to 10-year CVD event were evaluated through multivariable Cox-regression analysis in the total sample, as well as in subgroups. Total or CVD-case related correct classification rate was also obtained from the aforementioned multivariate analyses. Interactions between groups of participants were tested, and when significant, analyses were further stratified. The concordance statistics i.e. C-statistics was used to evaluate the predictive accuracy of multivariate models adjusted for various lipid markers against 10-year CVD event. C-indexes and the corresponding 95%CIs were equal to the areas under the curve obtained from the ROC analysis. Curves were constructed by plotting sensitivity against 1-specifity.

4.5.2.3 Findings

At baseline, the mean Lp(a) value in the total sample of the ATTICA study was 19±23 mg/dL (17±21 mg/dL in men and 21±24 mg/dL in women, $p=0.02$). The prevalence of Lp(a) values over the clinically recommended threshold of 50 mg/dL was 8.5% (7.5% in men *vs* 9.5% in women, $p=0.21$). 19.2% of men and 19.2% women exceeded the threshold of 30mg/dL. For the purposes of the present analysis, only n=1,890 participants with complete CVD evaluation metrics at 10-year follow-up were retained for further analyses. In this subsample, the 10-year

CVD event rate was 15.4% (*n*=291) [19.5% (*n*=184) in men and 11.3% (*n*=107) in women, *p<0.001*]. Nested Cox regression models to evaluate the association between Lp(a) categories and 10-year CVD event are presented in **Table 4.46** (Lp(a) cut-off point of 50 mg/dL) and **Table 4.47** (Lp(a) cut-off point of 30 mg/dL). Starting with **Table 4.46**, in the unadjusted model, participants with Lp(a) ≥50 mg/dL presented almost 2.65 times higher risk for developing 10-year CVD events, compared with their lower Lp(a) values counterparts (*p<0.001*). Multi-adjusted models where demographic and clinical factors were taken into account revealed that Lp(a) ≥50 mg/dL retained its significantly aggravating effect (*Model 2 and 3*). In *Model 4* the association was controlled for other lipid markers as well as for the use of statins; even in this case participants with Lp(a) ≥50 mg/dL presented about 2 times higher risk for incident CVD within the 10-year follow-up period (*p<0.05*). In the fully adjusted model where participants' kidney and liver function as well as systemic inflammation were included in the analysis the level of significance was retained (*Model 5*, *p<0.05*). The same multi-adjusted models were performed in case of the Lp(a) threshold of 30 mg/dL, yet no significant outcomes were observed as presented in (**Table 4.47**).

Table 4.46 Cox-regression models to evaluate the association of abnormal Lp(a) levels (cut-off point of 50 mg/dL) with 10-year cardiovascular disease risk (*n*=1,890).

	Model 1 HR (95%CI)	**Model 2** HR (95%CI)	**Model 3** HR (95%CI)	**Model 4** HR (95%CI)	**Model 5** HR (95%CI)
Lp(a), ≥50 mg/dL *vs* <50 mg/dL	2.65 (2.01, 4.02)***	2.43 (1.93, 3.94)**	2.34 (1.80, 3.80)**	2.21 (1.16, 4.21)*	2.18 (1.11, 4.28)*
Age, per 1 year	-	1.06 (1.05, 1.07)	1.06 (1.05, 1.07)	1.04 (1.03, 1.06)	1.04 (1.02, 1.07)
Male *vs* female sex	-	1.75 (1.38, 2.22)	1.65 (1.29, 2.12)	1.51 (1.04, 2.21)	1.63 (0.98, 2.70)
Body mass index, per 1 kg/m^2	-	-	1.03 (1.01, 1.06)	1.04 (1.01, 1.08)	1.01 (0.96, 1.06)
Current smoking, yes *vs* no	-	-	1.35 (1.04, 1.76)	1.05 (0.72, 1.53)	1.09 (0.69, 1.72)
Diabetes mellitus, yes *vs* no	-	-	1.52 (1.10, 2.09)	1.77 (1.11, 2.80)	1.77 (0.95, 3.34)
Hypertension, yes	-	-	1.05	1.04	1.06

vs no			(0.81, 1.35)	(0.72, 1.48)	(0.81, 1.97)
MedDietScore, <27 *vs* ≥27	-	-	0.89 (0.46, 1.34)	0.92 (0.47, 1.36)	0.92 (0.47, 1.36)
Lp(a)-corrected LDL-C, per 1 mg/dL	-	-	-	1.00 (0.99, 1.01)	1.01 (1.00, 1.02)
HDL-C, per 1 mg/dL	-	-	-	0.98 (0.97, 1.00)	0.98 (0.97, 1.00)
Triglycerides, per 1 mg/dL	-	-	-	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)
Use of statins, yes *vs* no	-	-	-	2.20 (1.45, 3.34)	2.53 (1.44, 4.00)
Family history of cardiovascular disease, yes *vs* no	-	-	-	-	1.00 (0.61, 1.63)
CRP, per 1 mg/L	-	-	-	-	1.09 (1.02, 1.18)
ALT, per 1 U/L	-	-	-	-	1.00 (0.97, 1.04)
AST, per 1 U/L	-	-	-	-	0.98 (0.94, 1.01)
$C_{(CR)}$, per 1 mL/min/1.73m^2	-	-	-	-	0.99 (0.98, 1.01)

****p-value<0.001*; ***p-value<0.01*; **p-value<0.05*

LDL-C was corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. ***Abbreviations:*** Alanine transaminase (ALT); Aspartate transaminase (AST); 95% Confidence Interval (95%CI); C-Reactive Protein (CRP); Creatinine clearance ($C_{(CR)}$); Hazard Ratio (HR); High density lipoprotein cholesterol (HDL-C); Lipoprotein(a) (Lp(a)); Low density lipoprotein cholesterol (LDL-C)

Table 4.47 Cox-regression models to evaluate the association of abnormal Lp(a) levels (cut-off point of 30mg/dL) with 10-year cardiovascular disease risk (n=1,890).

	Model 1 HR (95%CI)	**Model 2** HR (95%CI)	**Model 3** HR (95%CI)	**Model 4** HR (95%CI)	**Model 5** HR (95%CI)

Lp(a), ≥30 mg/dL *vs* <30 mg/dL	1.31 (0.81, 2.12)	1.22 (0.90, 1.66)	1.20 (0.85, 1.68)	1.18 (0.82, 1.60)	1.18 (0.82, 1.60)
Age, per 1 year	-	1.06 (1.04, 1.08)	1.06 (1.05, 1.07)	1.04 (1.03, 1.06)	1.04 (1.02, 1.07)
Male *vs* female sex	-	1.76 (1.39, 2.20)	1.64 (1.27, 2.13)	1.51 (1.04, 2.21)	1.63 (0.98, 2.70)
Body mass index, per 1 kg/m^2	-	-	1.02 (1.01, 1.06)	1.05 (1.01, 1.09)	1.01 (0.95, 1.06)
Current smoking, yes *vs* no	-	-	1.37 (1.05, 1.78)	1.06 (0.72, 1.54)	1.09 (0.69, 1.72)
Diabetes mellitus, yes *vs* no	-	-	1.52 (1.10, 2.09)	1.77 (1.11, 2.80)	1.77 (0.95, 3.34)
Hypertension, yes *vs* no	-	-	1.05 (0.81, 1.35)	1.04 (0.72, 1.48)	1.06 (0.81, 1.97)
MedDietScore, <27 *vs* ≥27	-	-	0.87 (0.44, 1.33)	0.90 (0.46, 1.37)	0.93 (0.46, 1.36)
Lp(a)-corrected LDL-C, per 1 mg/dL	-	-	-	1.00 (0.99, 1.01)	1.01 (1.00, 1.02)
HDL-C, per 1 mg/dL	-	-	-	0.98 (0.97, 1.00)	0.98 (0.97, 1.00)
Triglycerides, per 1mg/dL	-	-	-	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)
Use of statins, yes *vs* no	-	-	-	2.20 (1.45, 3.34)	2.53 (1.44, 4.00)
Family history of cardiovascular disease, yes *vs* no	-	-	-	-	1.01 (0.62, 1.61)
CRP, per 1 mg/L	-	-	-	-	1.10 (1.01, 1.17)
ALT, per 1 U/L	-	-	-	-	1.00 (0.97, 1.04)
AST, per 1 U/L	-	-	-	-	0.98 (0.94, 1.01)
$C_{(CR)}$, per 1	-	-	-	-	0.98

mL/min/1.73m^2	(0.97, 1.01)

****p <0.001; **p <0.01; *p <0.05*

LDL-C was corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. ***Abbreviations:*** Alanine transaminase (ALT); Aspartate transaminase (AST); 95% Confidence Interval (95%CI); C-reactive protein (CRP); Creatinine clearance ($C_{(CR)}$); Hazard Ratio (HR); High density lipoprotein cholesterol (HDL-C); Lipoprotein(a) (Lp(a)); Low density lipoprotein cholesterol (LDL-C)

Considering the hypotheses regarding the potential differences on the association between Lp(a) and incident CVD between men and women, among the aims of the present work was to evaluate the interacting effect of sex on the examined association as well as the sex-based effect size of Lp(a)-status (i.e. 50 mg/dL threshold) aggravating effect on the risk to develop a cardiac episode within the decade. A significant interaction was observed between sex and Lp(a) status on 10-year CVD event (p for interaction=0.01). Hence, nested Cox regression models were developed separately for men and women and results are shown in **Figure 4.12**. Age-standardized models showed that the men-to-woman hazard rate ratio was 1.24 revealing that a male with abnormal Lp(a) status is at higher risk to develop a cardiac episode within the decade compared with a female at the same age and Lp(a) status (Model 2).

In the subgroup of men, further adjustment with clinical factors, lipid markers as well as biomarkers related with systemic inflammation, liver and kidney function resulted in a progressively reduction of the effect size of the examined association yet without losing its significance (Model 5, $p<0.05$). On the other hand, in case of women, when conventional lipid markers were taken into account i.e. Lp(a)-corrected LDL-C, HDL-C, triglycerides as well as the statin use, the independent effect of Lp(a) status was lost; in the fully adjusted model i.e. Model 5, Lp(a) ≥50 mg/dL retained its aggravating effect on 10-year CVD incidence, yet without reaching the level of significance ($p>0.05$).

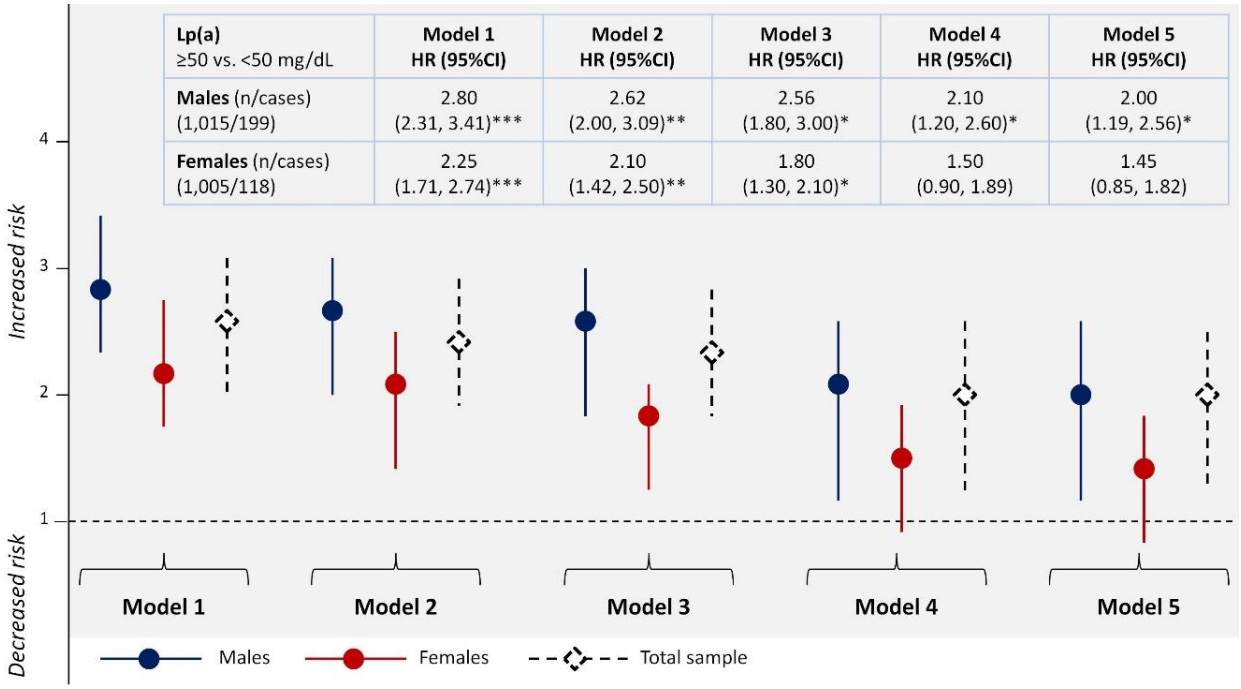

Figure 4.12 Nested Cox-regression analysis to evaluate the association between Lipoprotein (a) status (cut-off point of 50 mg/dL) and the 10-year cardiovascular disease event, separately for men and women.

HRs (dots) and their corresponding 95%CIs (vertical lines) for Lipoprotein (a) ≥50 mg/dL vs <50 mg/dL were obtained through Cox regression analysis. Model 1: crude model; Model 2: age; Model 3: Model 2 plus body mass index, current smoking, hypertension, diabetes mellitus, MedDietScore; Model 4: Model 3 plus lipoprotein(a)-corrected low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, use of statins; Model 5: Model 4 plus C-reactive protein, alanine transaminase, aspartate transaminase, creatinine clearance, family history of cardiovascular disease. Abbreviations: Hazard ratio (HR), 95% Confidence Interval (95%CI). ***p <0.001; **p <0.01; *p <0.05

Due to the observed interacting effect of sex on the association between Lp(a) status and 10-year CVD risk, the discrimination ability of multivariate epidemiological models adjusted for Lp(a) and other lipid markers was evaluated separately for men and women and results are presented in **Table 4.48**. Overall, the discrimination ability (expressed through C-index) of the examined multiadjusted models was better in case of women. However, the level to which Lp(a) contributed to the discrimination ability of the model was higher in the subgroup of men; in particular the difference between the estimated C-index in the model adjusted for both conventional lipid markers and Lp(a) and the C-index corresponding to the model adjusted only for conventional lipid markers was >0.01. This was not observed in case of women. The total correct classification rate was higher in case of *Model 1* (i.e. adjusted only for conventional lipid markers) in women (89.6%) with the correct classification rate for CVD cases being more than twice as high in *Model 1 vs Model 2* (adjusted only for Lp(a)) (19.6 *vs* 8.5%). As for the subgroup of men, the Lp(a)-adjusted model presented about 4 times higher correct classification rate for CVD cases (24.8%) compared with the respective number in women while their fully adjusted model (i.e. *Model 3*, all lipid markers) corresponded to the highest total as well as case-related correct classification rate.

Table 4.48 Discrimination-ability parameters of multivariate models adjusted for conventional lipid markers or Lipoprotein (a) or the combination of them over the 10-year first fatal/non-fatal cardiovascular disease event (*n*=1,890).

Models	Model adjustment description	C-index (95%CI)	Correct classification rate, % (total)	Correct classification rate, % (cases)
			Men	
Model 1	Standard model* adjusted for **conventional lipid markers**[l]	0.772 (0.713, 0.831)	83.6	33.3
			Women	
		0.831 (0.777, 0.886)	89.6	19.6
			Men	
Model 2	Standard model* adjusted for **Lipoprotein (a)**	0.769 (0.709, 0.828)	81.9	24.8
			Women	
		0.820 (0.772, 0.880)	87.9	8.5
			Men	
Model 3	Standard model* adjusted for **all lipid markers**	0.784 (0.725, 0.839)	96.5	32.2
			Women	
		0.829 (0.774, 0.883)	88.7	15.7

*Standard model was adjusted for age, body mass index, current smoking, MedDietScore, hypertension, diabetes mellitus, family history of cardiovascular disease.

[l]Conventional lipid markers examined were lipoprotein (a) corrected low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides.

C-index and the corresponding confidence interval was evaluated through the area under the curve obtained from the Receiver operating Characteristics (ROC) analysis. ROC analysis was performed using the probabilities for 10-year first fatal/non-fatal cardiovascular disease event, corresponding to each study participant, separately for men and women, calculated from Cox regression analysis using the multivariate models described. Correct classification rate was obtained from the Cox regression analysis performed using the described models, separately for men and women.

An formal interaction analysis was also performed. Participants' lipidemic status in terms of TC seemed to interact on the examined association (*p for interaction<0.05*). Hence, it was decided to perform an extensive sensitivity analysis within the different clusters of lipidemic profile from the standpoint of TC, LDL-C, HDL-C and their apolipoproteins as well as the level of TAG. Additionally, formal analysis of interaction revealed that the level of adherence to Mediterranean diet had a significant interacting effect on the examined association. Results from stratified analyses with the Lp(a) status defined through the cut-off point of 50mg/dL and the aforementioned markers as strata are presented in **Table 4.49**.

Table 4.49 Sensitivity analyses to evaluate the association of Lp(a) status (cut-off point of 50 mg/dL) with 10-year cardiovascular disease risk in specific subgroups (n=1,890).

	Lp(a) status	Hazard ratio	95% Confidence Interval
Lp(a)-corrected total cholesterol (n/cases)			
<200 mg/dL (1,057/117)	≥50 *vs* <50 mg/dL	1.42	0.62, 3.29
≥200 mg/dL (833/174)		**1.91**	**1.10, 4.00**
p for interaction=0.005			
Lp(a)-corrected LDL-C (n/cases)			
LDL-C <100 mg/dL (624/30)	≥50 *vs* <50 mg/dL	2.05	0.81, 5.22
LDL-C ≥100 mg/dL (1,266/261)		1.72	0.92, 3.21
p for interaction=0.87			
HDL-C (n/cases)			
HDL-C ≥40 mg/dL in men and ≥50 mg/dL in women (1,076/128)	≥50 *vs* <50 mg/dL	1.24	0.55, 2.76
HDL-C <40 mg/dL in men and <50 mg/dL in women (814/163)		**1.89**	**1.01, 3.55**
p for interaction=0.01			
Triglycerides (n/cases)			
Triglycerides <150 mg/dL (1,451/113)	≥50 *vs* <50 mg/dL	1.39	0.75, 2.57
Triglycerides ≥150 mg/dL (439/178)		**2.15**	**1.37, 5.26**
p for interaction=0.005			
ApoB (n/cases)			
ApoB <100 mg/dL (805/74)	≥50 *vs* <50 mg/dL	1.82	0.47, 4.01
ApoB ≥100 mg/dL (1,085/217)		1.75	0.93, 2.98
p for interaction=0.12			
ApoA1 (n/cases)			
ApoA1 ≥120 mg/dL for men and ≥140 mg/dL for women (1,621/246)	≥50 *vs* <50 mg/dL	1.28	0.79, 5.31
ApoA1 <120 mg/dL for men and <140 mg/dL for women (269/45)		**1.86**	**1.12, 3.10**
p for interaction=0.002			
MedDietScore (n/cases)			

MedDietScore <27 (892/259)	≥50 *vs* <50 mg/dL	**1.90**	**1.08, 3.33**
MedDietScore ≥27 (707/32)		1.11	0.55, 5.12
p for interaction=0.001			

LDL-C was corrected for the Lp(a) contribution by subtracting 30% of total Lp(a) mass. Cut-off values of the lipids-oriented strata were set according to the "2016 ESC/EAS Guidelines for the Management of Dyslipidaemias". All models were adjusted for age, sex, current smoking, family history of cardiovascular disease, C-reactive protein, alanine transaminase, aspartate transaminase and creatinine clearance. **Bold** indicates estimates that are significantly different from the reference group at $p<0.05$. ***Abbreviations:*** Apolipoprotein A1 (ApoA1); Apolipoprotein B100 (ApoB); High density lipoprotein cholesterol (HDL-C); Lipoprotein(a) (Lp(a)); Low density lipoprotein cholesterol (LDL-C).

As it was revealed, from the clusters of lipidemic profile only Lp(a)-corrected total cholesterol, HDL-C, ApoA1 and TAG presented a significant interacting effect on the examined association (all p values for interaction <0.05); to this effect, the independent association between Lp(a) status and 10-year CVD event was retained only in participants with hypercholesterolemia accompanied by abnormal HDL-C, ApoA1 and TAG levels (all *p values <0.05*). As for the level of adherence to Mediterranean diet, only in the context of MedDietScore below the median value participants with Lp(a)≥50 mg/dL had an independently increased CVD risk within the decade compared with their counterparts with lower Lp(a) values (*p=0.02*); in participants with moderate to high level of adherence to this dietary pattern, the level of significance was lost. Further interaction analysis taking into account the sex, a triple significant interaction between sex, Lp(a) and specific lipid markers i.e. HDL-C, ApoA1 and TAG was observed (all *p values for interaction<0.05*); however additional stratification was not performed for statistical power limitations. The triple interaction between sex, MedDietScore and Lp(a) was also examined yet this did not reach the level of significance (*p for interaction=0.52*). In all the aforementioned cases, the analyses were re-performed with the Lp(a) status being defined in terms of 30 mg/dL as cut-off point; no significant outcomes were observed (*data not presented on table*).

4.6 Other biomarkers and ten-year first CVD incidence: sex-specific results

4.6.1 The sex-specific role of SUA in relation to ten-year first CVD incidence

4.6.1.1 Scope and research hypothesis

The scope here was to evaluate the association between SUA levels and 10-year CVD incidence in the ATTICA cohort study, as well as the potential synergistic effects of sex and metabolic health status on this association. SUA cut-off values predicting CVD incidence were also identified separately for men and women.

4.6.1.2 Methods and analysis

In the present work, participants were categorized according to sex-specific SUA tertiles.

HRs and their corresponding 95%CIs for SUA tertiles in relation to the examined endpoint (i.e. 10-year fatal/non-fatal CVD incidence) were assessed through multivariable Cox-regression analysis. Proportional hazards' assumption was graphically tested. ROC analysis was also performed, and the AUC was calculated to identify the discriminative effect of SUA on CVD incidence, as well as to detect the SUA cut-off point with the best discriminative ability for evaluating CVD events, separately for men and women.

4.6.1.3 Findings

Results from unadjusted analysis regarding the association between SUA and CVD incidence rate within the 10-year follow-up, separately for men and women, are presented in **Table 4.50**. In particular, women in the highest SUA tertile had almost twice as high risk to develop a fatal/non-fatal CVD event within the decade compared with their counterparts in the lowest tertiles (157 vs. 79 CVD events / 1,000 participants, respectively; *p=0.008*). A similar trend was observed for men (237 vs. 169 CVD events / 1,000 participants, respectively; *p=0.04*). Ranking from the lowest to the highest SUA tertile, the man-to-woman CVD event rate ratio was 2.13, 1.69 and 1.50, respectively.

Results from nested Cox regression models evaluating the association between SUA tertiles and CVD incidence in the total sample are presented in **Table 4.51**. In the unadjusted models, participants in the 2nd and 3rd SUA tertile had about 29% (HR 1.29, 95%CI: 1.19-1.40) and 73% (HR 1.73, 95%CI: 1.23-2.42) higher risk to develop CVD within the decade compared with their 1st tertile counterparts, respectively. In the age- and sex- adjusted model, the aforementioned associations were attenuated but retained the level of significance (Model 2). After adjusting for anthropometric, lifestyle, clinical and biochemical factors, the association between SUA and CVD incidence remained only for participants in the 3rd SUA tertile (Model 5). However, after adjusting for metabolic health status, the level of significance was lost (Model 6).

Table 4.50 Unadjusted 10-year cardiovascular disease incidence rate in men and women from the ATTICA study according to sex-specific serum uric acid tertiles.

	Overall sample	**Sex-specific SUA tertiles**			
		1st tertile	**2nd tertile**	**3rd tertile**	***p-value***
Men, n/cases	825/157	248/42	286/46	291/69	*0.04*
CVD incidence rate per 100 participants	*19.0*	*16.9*	*16.1*	*23.7*	
Women, n/cases	862/96	252/20	317/30	293/46	*0.008*
CVD incidence rate per 100 participants	*11.1*	*7.9*	*9.5*	*15.7*	
Overall, n/cases	1,687/253	500/62	603/76	584/115	*<0.001*
CVD incidence rate per 100 participants	*15.0*	*12.4*	*12.6*	*19.7*	
Man-to-woman CVD incidence rate ratio	**1.72**	**2.13**	**1.69**	**1.50**	

P-values were obtained using chi-squared test. **Abbreviations**: CVD: cardiovascular disease; SUA: serum uric acid

Table 4.51 Nested Cox-regression analysis models to evaluate the association of serum uric acid with 10-year cardiovascular disease incidence (n=1,687).

	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**

	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
SUA tertiles						
1st	Ref	Ref	Ref	Ref	Ref	Ref
2nd	**1.29 (1.19, 1.40)**	**1.16 (1.07, 1.26)**	**1.12 (1.03, 1.21)**	**1.09 (1.00, 1.18)**	1.05 (0.96, 1.15)	1.02 (0.95, 1.07)
3rd	**1.73 (1.23, 2.42)**	**1.55 (1.11, 2.18)**	**1.51 (1.09, 2.11)**	**1.47 (1.05, 2.08)**	**1.42 (1.01, 1.99)**	1.39 (0.98, 1.95)
Age, per 1 year	-	1.08 (1.07, 1.09)	1.08 (1.06, 1.09)	1.07 (1.05, 1.09)	1.07 (1.05, 1.09)	1.07 (1.05, 1.09)
Male gender	-	1.86 (1.41, 2.46)	1.82 (1.36, 2.45)	1.81 (1.17, 2.76)	1.66 (1.07, 2.61)	1.66 (1.07, 2.61)
Years of school, per 1 year	-	-	0.96 (0.92, 0.99)	0.97 (0.92, 1.02)	0.95 (0.90, 1.01)	0.95 (0.90, 1.01)
MedDietScore (range 0-55), per 1/55	-	-	0.98 (0.96, 0.99)	0.98 (0.94, 0.99)	0.97 (0.94, 1.01)	0.97 (0.94, 1.01)
Alcohol consumption, yes vs. no	-	-	0.90 (0.75, 1.10)	0.92 (0.76, 1.11)	0.92 (0.76, 1.11)	0.92 (0.76, 1.11)
Physical activity, yes vs. no	-	-	0.94 (0.70, 1.25)	1.32 (0.88, 1.98)	1.43 (0.94, 2.17)	1.43 (0.94, 2.17)
Current smoking, yes vs. no	-	-	1.27 (0.94, 1.71)	1.50 (1.00, 2.28)	1.45 (0.94, 2.23)	1.45 (0.94, 2.23)
LDL-C, per 1 mg/dL	-	-	-	1.01 (1.00, 1.03)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)
Family history of	-	-	-	1.37 (0.90, 2.08)	1.39 (0.89, 2.17)	1.39 (0.89, 2.17)

CVD, yes vs. no						
ALT, per 1 U/L	-	-	-	1.01 (0.98, 1.04)	1.00 (0.97, 1.04)	1.00 (0.97, 1.04)
AST, per 1 U/L	-	-	-	0.99 (0.95, 1.02)	0.98 (0.94, 1.01)	0.98 (0.94, 1.01)
Waist circumference, per 1 cm	-	-	-	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)
HOMA-IR, per 1 unit	-	-	-	1.06 (0.98, 1.16)	1.06 (0.98, 1.16)	1.06 (0.98, 1.16)
CRP, per 1 mg/L	-	-	-	1.06 (0.98, 1.15)	1.06 (0.98, 1.15)	1.06 (0.98, 1.15)
eGFR, per mL/min/1.73m^2	-	-	-	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)
Obesity, yes vs. no	-	-	-	-	1.65 (1.00, 2.92)	1.61 (0.89, 2.52)
Metabolic health status, healthy vs. unhealthy	-	-	-	-	-	0.43 (0.17, 0.99)

HRs and their corresponding 95%CIs were obtained from Cox regression analysis. **Bold** indicates statistically significant outcomes i.e. *p<0.05*. ***Abbreviations:*** SUA: serum uric acid; ALT: alanine transaminase; AST: aspartate transaminase; CVD: cardiovascular disease; CI: confidence interval; CRP: C-Reactive Protein; eGFR: estimated glomerular filtration rate; HR: Hazard ratio; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; LDL-C: low density lipoprotein cholesterol

Significant interactions were observed with sex (*p for interaction=0.003*). In particular, in **Table 4.52**, multi-adjusted sex-based sensitivity analysis revealed that the direct association between SUA and CVD incidence was independent only in women; women in the 3rd SUA tertile had about 79%

higher risk to develop CVD compared with their 1st tertile counterparts (HR=1.79, 95%CI 1.04, 3.17). However, the level of significance was lost when metabolic health status was taken into account (Model 4).

Table 4.52 Sex-based sensitivity analyses to evaluate the association of serum uric acid with 10-year cardiovascular disease incidence (n=1,687).

		Men	**Women**	
N, cases		825/157	862/96	
		HR (95%CI)	HR (95%CI)	**Models adjusted for**
Model with SUA as continuous variable				
	per 1 mg/dL	1.11 (0.97, 1.27)	1.34 (1.13, 1.58)	
Model with SUA tertiles				Crude model
	1st	*Ref*	*Ref*	
	2nd	0.94 (0.59, 1.48)	1.21 (0.67, 2.19)	
	3rd	**1.52 (1.10, 2.33)**	**2.16 (1.24, 3.76)**	
Model with SUA as continuous variable				
	per 1 mg/dL	0.99 (0.87, 1.14)	1.20 (1.02, 1.77)	Model 1: Age, years of school, MedDietScore, alcohol consumption physical activity, current smoking
Model with SUA tertiles				
	1st	*Ref*	*Ref*	
	2nd	0.85 (0.52, 1.31)	1.09 (0.60, 1.97)	
	3rd	1.35 (0.98, 2.09)	**1.94 (1.11, 3.38)**	
Model with SUA as continuous variable				
	per 1 mg/dL	0.95 (0.48, 1.24)	1.01 (0.55, 1.85)	Model 2: Model 1 plus LDL-C, family history of CVD, ALT, AST, waist circumference, HOMA-IR, CRP, eGFR, menopause status (*only in women*)
Model with SUA tertiles				
	1st	*Ref*	*Ref*	
	2nd	0.82 (0.51, 1.30)	1.04 (0.57, 1.94)	
	3rd	1.27 (0.97, 2.05)	**1.85 (1.05, 3.29)**	
Model with SUA as continuous variable				
	per 1 mg/dL	0.93 (0.47, 1.21)	0.98 (0.53, 1.81)	
Model with SUA tertiles				Model 3: Model 2 plus obesity
	1st	*Ref*	*Ref*	
	2nd	0.78 (0.48, 1.24)	1.01 (0.55, 1.85)	
	3rd	1.21 (0.93, 1.96)	**1.79 (1.04, 3.17)**	

Model with SUA as continuous variable			
	per 1 mg/dL	0.89 (0.45, 1.16)	0.94 (0.50, 1.73)
Model with SUA tertiles			Model 4: Model 3 plus metabolic health status
	1st	*Ref*	*Ref*
	2nd	0.76 (0.47, 1.21)	0.98 (0.53, 1.81)
	3rd	1.18 (0.91, 1.92)	1.75 (0.97, 3.01)

HRs and their corresponding 95%CIs were obtained from Cox regression analysis. **Bold** indicates statistically significant outcomes i.e. *p<0.05*. ***Abbreviations:*** SUA: serum uric acid; ALT: alanine transaminase; AST: aspartate transaminase; CVD: cardiovascular disease; CI: confidence interval; CRP: C-Reactive Protein; eGFR: estimated glomerular filtration rate; HR: Hazard ratio; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; LDL-C: low density lipoprotein cholesterol

The ROC analysis is presented in **Figure 4.13**. Based on the generated AUC, SUA seemed to better detect 10-year CVD events in women, but this was not significant. Further analysis showed that the cut-off points of SUA levels with the highest predictive capacity for CVD events were 5.05 mg/dL (0.29 mmol/L) for men and 4.15 mg/dL (0.24 mmol/L) for women.

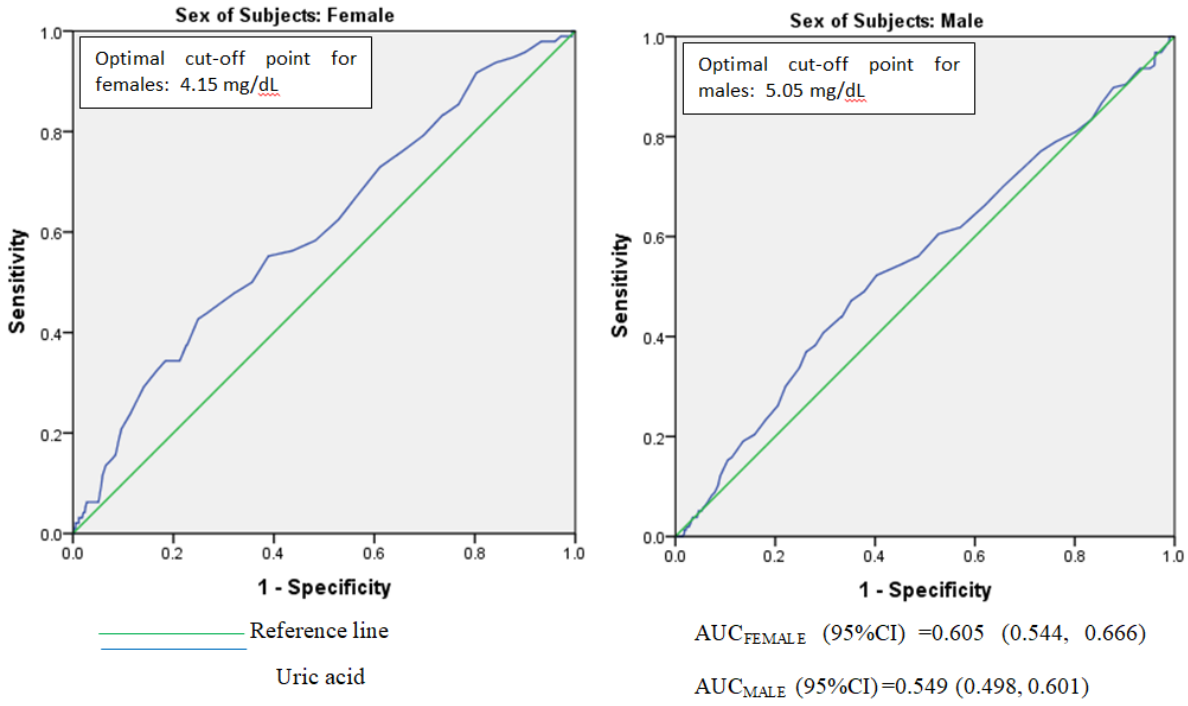

Figure 4.13 Receiver operating characteristic curve to evaluate the predictive capacity of serum uric acid on 10-year cardiovascular disease incidence through the area under the curve (AUC) and the corresponding 95% confidence intervals (95%CI) in male and female participants of the ATTICA study (n=1,687).

4.7 Nutrition-related microsimulation analysis: sex-specific results for primary and secondary CVD prevention

4.7.1 A Mediterranean diet-related microsimulation modelling approach in relation to ten-year first and recurrent CVD incidence

4.7.1.1 Scope and research hypothesis

The scope here was to quantify the potential changes in long-term CVD onset or recurrence within apparently healthy individuals and patients with established ACS, respectively, transitioning from low to higher level of adherence to Mediterranean diet.

4.7.1.2 Methods and microsimulation analysis

An individual-based microsimulation was created, to estimate the impact of improving adherence to Mediterranean diet on CVD outcomes, among adults, being either healthy or ACS patients diagnosed with ACS. Microsimulation (**Figure 4.14**) was held in three stages: (i) A random proportion of the participants in the two aforementioned epidemiological studies was drawn, (ii) in each random sample a scenario regarding the improvement of MedDietScore was applied and (iii) the effect of MedDietScore on CVD onset, or recurrence, or mortality was estimated. Four different distributions were chosen by which the increment of adherence to Mediterranean diet was drawn, applied to twenty different random proportions of total sample and samples of men and women, leading to 80 unique scenarios. However, emphasis was given to the following scenarios:

Scenario 1: The increment in the level of adherence to the Mediterranean diet was drawn by the Uniform distribution with parameters a=1 and b= 10.

Scenario 2: The increment in the level of adherence to the Mediterranean diet was drawn by the Multinomial distribution with support s={1, 2, …, 10} and event probabilities={20%, 20%, 20%, 20%, 5%, 5%, 2.5%, 2.5%, 2.5%, 2.5%}.

In both of which Mediterranean diet adherence was improved in 10%, 25%, 50% and 75% of the sample, respectively.

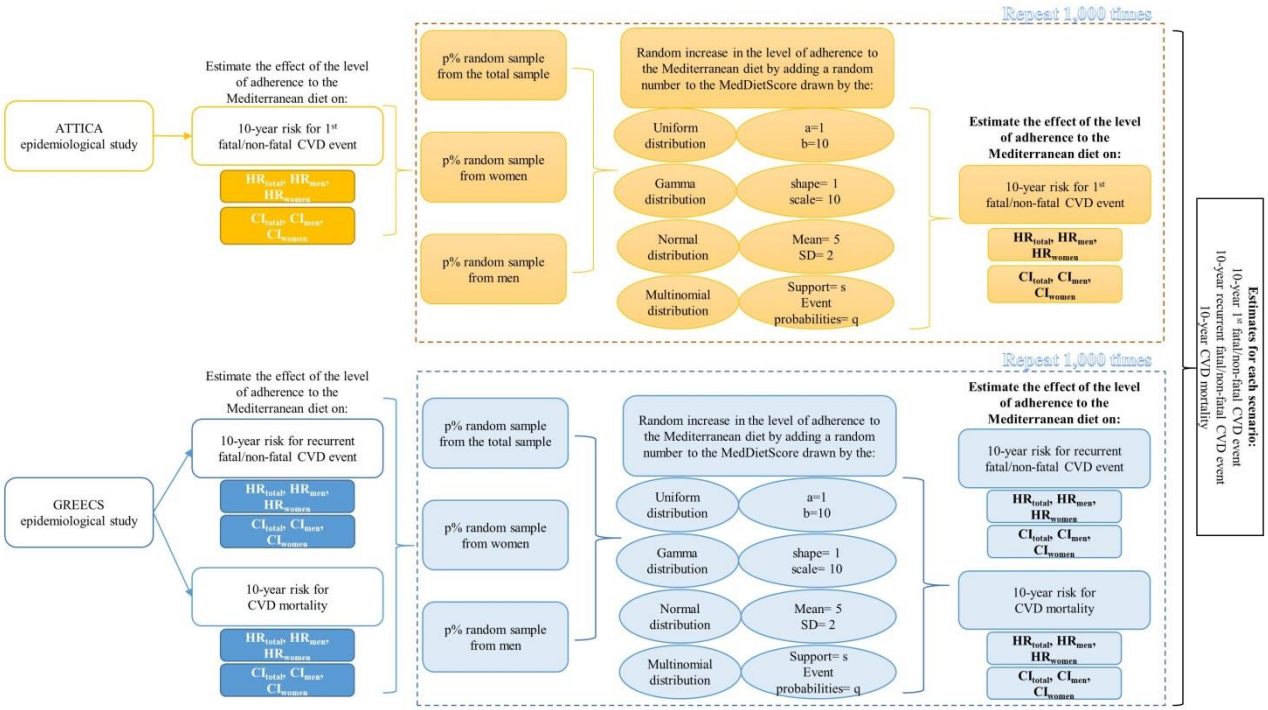

Figure 4.14 Methodological framework of the microsimulation study

p= {5%, 10%, …, 100%}; The support of the Multinomial distribution was the s= {1,2,…,10} and the event probabilities were the q= {20%, 20%, 20%, 20%, 5%, 5%, 2.5%, 2.5%, 2.5%, 2.5%}; The effect of the level of adherence to the Mediterranean diet on the risk of first/ recurrent fatal/ non- fatal CVD event, as well as, on the risk of CVD- related death, was based on the multivariable Cox proportional hazards model and it is adjusted for the individuals': Age (Continuous; in years), Sex (Male/ Female), Physical activity level (Sedentary/ Active), Current smoking status (Yes/ No), Body Mass Index (Continuous; in kg/m2), History of hypertension (Yes/ No), History of hypercholesterolemia (Yes/ No), History of diabetes mellitus (Yes/ No), Educational level (Continuous; in years) and Family CVD history (Yes/ No); Abbreviations: CVD= Cardiovascular disease, HR= Hazard ratio, CI= Confidence Interval, SD= Standard Deviation

4.7.1.3 Findings

Ten-year first CVD incidence in ATTICA study (reference sample) was 15.7% (19.7% in men and 11.7% in women). Improving adherence to Mediterranean diet, even in 10% of the population (scenario 1), a significant relative percentage reduction in the CVD incidence was observed compared to the reference sample (reduction by 6.9%; *p=0.038*), with this reduction being 2 times higher in women compared to men [reduction by 11.7% in women (*p=0.037*) and by 5.8% in men (*p=0.025*)]. In a more optimistic scenario, where the simulated policy scenario effectively improved the level of adherence to the Mediterranean diet in 25% of the population (scenario 2), a significant reduction in CVD incidence was pointed out both for the total sample and for the sample of men and women [reduction by 13.2% in the total sample (*p=0.021*), by 19.0% in women (*p=0.017*) and by 10.8% in men (*p=0.028*)] compared to the reference sample (**Figure 4.15**).

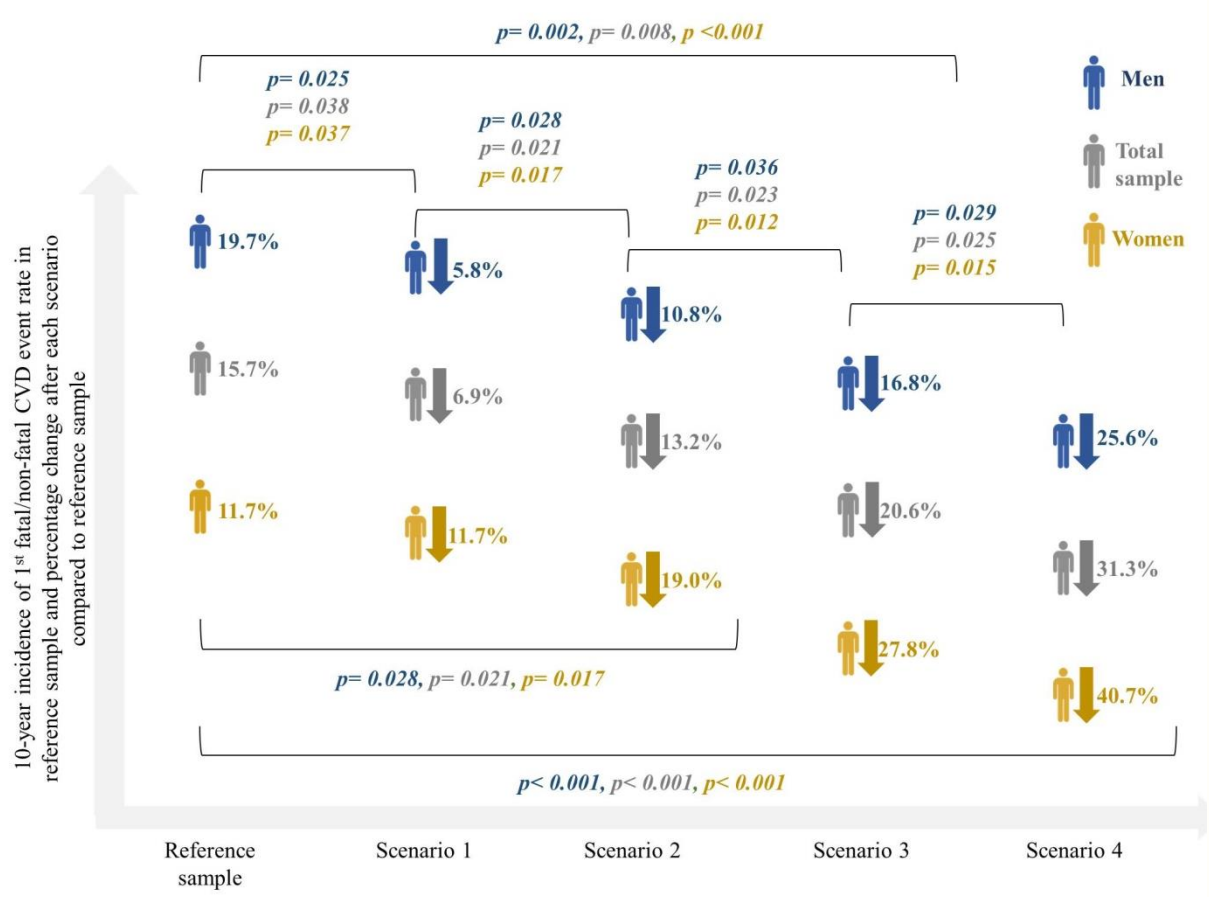

Figure 4.15 Ten-year incidence of first fatal/non-fatal CVD event among apparently healthy men and women from the ATTICA epidemiological study and its relative percentage change after each simulation scenario

Reference sample: 2020 apparently healthy individuals (1006 men and 1014 women) having participated in the 10- year follow-up of the ATTICA epidemiological study; The increment in the level of adherence to the Mediterranean diet was drawn by the Uniform distribution with parameters a=1 and b= 10; Scenario 1: The level of adherence to the Mediterranean diet was improved in 10% of the sample; Scenario 2: The level of adherence to the Mediterranean diet was improved in 25% of the sample; Scenario 3: The level of adherence to the Mediterranean diet was improved in 50% of the sample; Scenario 4: The level of adherence to the Mediterranean diet was improved in 75% of the sample; The effect of the level of adherence to the Mediterranean diet on the risk of first fatal/ non- fatal CVD event, was based on the multivariable Cox proportional hazards model and it is adjusted for the individuals': Age (Continuous; in years), Sex (Male/ Female), Physical activity level (Sedentary/ Active), Current smoking status (Yes/ No), Body Mass Index (Continuous; in kg/m2), History of hypertension (Yes/ No), History of hypercholesterolemia (Yes/ No), History of diabetes mellitus (Yes/ No), Educational level (Continuous; in years) and Family CVD history (Yes/ No); Abbreviation: CVD= Cardiovascular disease

Additionally, as depicted in **Table 4.53** with a policy scenario which could improve the level of adherence to the Mediterranean diet even in 10% of the population, at least 851 CVD events (95%CI=838-864) or 1081 CVD events (95%CI=1067-1097) per 100,000 of population could be prevented, with the preventable CVD events in women being almost 1.5 times higher compared to men.

Table 4.53 Estimated number of preventable first and recurrent CVD events, as well as CVD-related deaths, under different simulation scenarios regarding the improvement of the level of adherence to Mediterranean diet, in a population of 100,000 individuals, both for the total population and separately according to sex

	Number of preventable first CVD events per 100,000 of population		
	Total population	**Men**	**Women**
Simulated scenario			
Uniform distribution			
Scenario 1	1081 (1067,1097)	573 (554,617)	684 (663,717)
Scenario 2	2076 (2049,2106)	1063 (1024,1153)	1109 (1072,1192)
Scenario 3	3233 (3190,3279)	1628 (1615,1685)	1655 (1644,1716)
Scenario 4	4916 (4850,4986)	2382 (2312,2504)	2517 (2355,2651)
Multinomial distribution			
Scenario 1	851 (838,864)	471 (370,527)	538 (482,547)
Scenario 2	1534 (1509,1555)	777 (661,849)	887 (826,902)
Scenario 3	2535 (2495,2571)	1226 (1132,1277)	1400 (1332,1422)
Scenario 4	3217 (3166,3263)	1531 (1444,1577)	1749 (1677,1777)
	Number of preventable recurrent CVD events per 100,000 of population		
	Total population	**Men**	**Women**
Simulated scenario			
Uniform distribution			
Scenario 1	482 (475,530)	255 (247, 266)	304 (293, 327)
Scenario 2	913 (879, 926)	412 (398, 443)	563 (542, 612)
Scenario 3	1422 (1411, 1443)	605 (600, 627)	877 (871, 910)
Scenario 4	2162 (2141, 2194)	886 (859, 932)	1334 (1248, 1406)
Multinomial distribution			
Scenario 1	374 (318, 368)	175 (138, 196)	285 (255, 290)
Scenario 2	674 (626, 663)	289 (246, 315)	470 (438, 478)
Scenario 3	1096 (1073, 1115)	456 (421, 475)	742 (706, 754)
Scenario 4	1391 (1347, 1415)	569 (537, 586)	927 (888, 943)
	Number of preventable CVD- related deaths in ACS patients per 100,000 of population		
	Total population	**Men**	**Women**
Simulated scenario			
Uniform distribution			
Scenario 1	318 (314, 374)	158 (151, 180)	176 (168, 200)

Scenario 2	771 (739, 782)	371 (355, 408)	414 (396, 456)
Scenario 3	1303 (1296, 1321)	627 (624, 652)	699 (695, 727)
Scenario 4	2077 (2060, 2105)	999 (931, 1056)	1114 (1079, 1178)
Multinomial distribution			
Scenario 1	205 (146, 208)	62 (3, 96)	144 (121, 147)
Scenario 2	514 (468, 522)	231 (163, 273)	295 (270, 300)
Scenario 3	967 (934, 982)	478 (422, 509)	517 (489, 525)
Scenario 4	1276 (1221, 1296)	646 (595, 674)	668 (638, 678)

2020 apparently healthy individuals (1006 men and 1014 women) participated in the 10-year follow up of the ATTICA epidemiological study and 2172 patients with diagnosed acute coronary syndrome (1649 men and 523 women) participated in the 10-year follow up of the GREECS epidemiological study; The results are presented in the form: Number of events/ deaths (95% Confidence Interval); ***Scenario 1***: The level of adherence to the Mediterranean diet was improved in 10% of the sample; ***Scenario 2***: The level of adherence to the Mediterranean diet was improved in 25% of the sample; ***Scenario 3***: The level of adherence to the Mediterranean diet was improved in 50% of the sample; ***Scenario 4***: The level of adherence to the Mediterranean diet was improved in 75% of the sample; The Uniform distribution with parameters a=1, b=10 and the Multinomial distribution with support {1, 2, …, 10} and event probabilities {20%, 20%, 20%, 20%, 5%, 5%, 2.5%, 2.5%, 2.5%, 2.5%} were used, for the simulation of the increment in the level of adherence to the Mediterranean diet (as measured by the MedDietScore scale); Deaths and events (first or recurrent) averted, were calculated for a population of 100,000 individuals, with 50% of them being women; The effect of the level of adherence to the Mediterranean diet on the risk of first/ recurrent CVD event, as well as, on the risk of CVD- related death was based on the multivariable Cox proportional hazards model and it is adjusted for the individuals': Age (Continuous; in years), Sex (Male/ Female), Physical activity level (Sedentary/ Active), Current smoking status (Yes/ No), Body Mass Index (Continuous; in kg/m^2), History of hypertension (Yes/ No), History of hypercholesterolemia (Yes/ No), History of diabetes mellitus (Yes/ No), Educational level (Continuous; in years) and Family CVD history (Yes/ No); Abbreviation: ACS= acute coronary syndrome; CVD= Cardiovascular disease.

Ten-year incidence of recurrent CVD incidence in GREECS study was 37.3% (38.8% in men and 32.9% in women) and CVD mortality rate was 17.7% (17.3% in men and 18.7% in women). A relative percentage change of 2.9% [2.9% in men (*p=0.049*) and 4.0% in women (*p=0.026*)] and 5.6% [5.5% in men (*p=0.025*) and 6.7% in women (*p=0.017*)] was presented in CVD recurrence when adherence to Mediterranean diet was improved in 10% and 25% of the population, respectively (**Figure 4.16 (A)**), while a relative percentage change of 1.8% [1.8% in men (*p=0.048*) and 1.9% in women (*p=0.043*)] and 4.4% [4.3% in men (*p=0.013*) and 4.4% in women (*p=0.025*)] was presented in the 10-year CVD mortality rate, under the same scenarios (**Figure 4.16 (B)**).

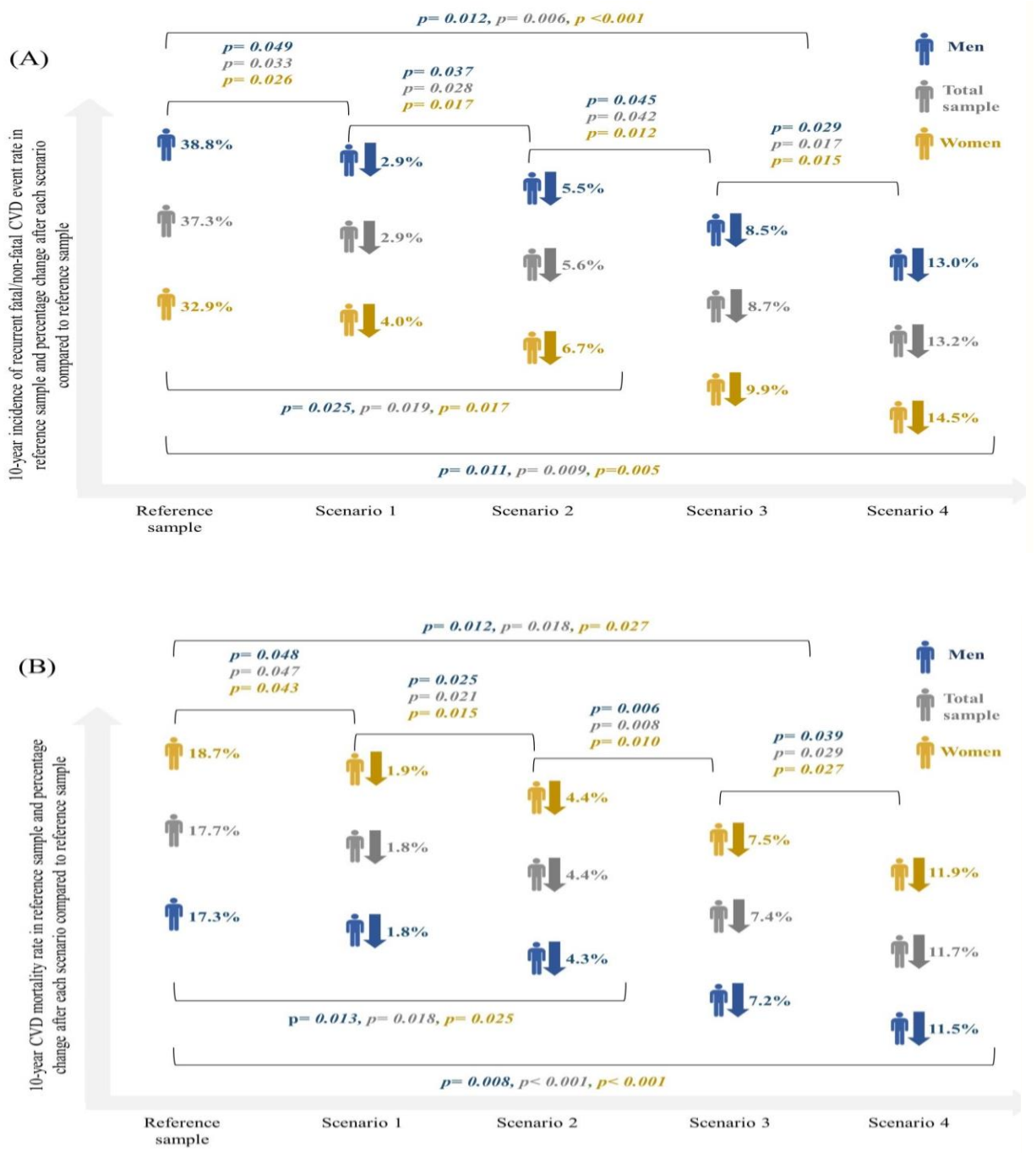

Figure 4.16 (A) Ten-year incidence of recurrent fatal/non-fatal CVD event and (B) Ten-year CVD mortality rate among men and women with established acute coronary syndrome from the GREECS epidemiological study and its relative percentage change after each simulation scenario

Reference sample: 2172 patients with diagnosed acute coronary syndrome (1649 men and 523 women) having participated in the 10- year follow up of the GREECS epidemiological study; The increment in the level of adherence to the Mediterranean diet was drawn by the Uniform distribution with parameters a=1 and b=10; ***Scenario 1***: The level of adherence to the Mediterranean diet was improved in 10% of the sample; ***Scenario 2***: The level of adherence to the Mediterranean diet was improved in 25% of the sample; ***Scenario 3***: The level of adherence to the Mediterranean diet was improved in 50% of the sample; ***Scenario 4***: The level of adherence to the Mediterranean diet was improved in 75% of the sample; The effect of the level of adherence to the Mediterranean diet on the risk of recurrent fatal/ non- fatal CVD event, as well as, on the risk of CVD- related death, was based on the multivariable Cox proportional hazards model and it is adjusted for the individuals': Age (Continuous; in years), Sex (Male/ Female), Physical activity level (Sedentary/ Active), Current smoking status (Yes/ No), Body Mass Index (Continuous; in kg/m^2), History of hypertension (Yes/ No), History of hypercholesterolemia (Yes/ No), History of diabetes mellitus (Yes/ No), Educational level (Continuous; in years) and Family CVD history (Yes/ No); ***Abbreviation***: CVD= Cardiovascular disease

5 Discussion

5.1 Sex-specific associations of risk factors with long-term CVD, primary and secondary prevention spectrum

The present work, based on two large-scale prospective epidemiological studies from a Mediterranean region, suggested the existence of sex-specific associations of conventional and non-conventional/emerging risk factors with long-term CVD, highlighting in some cases differentiations between primary and secondary prevention spectrum.

It was revealed that the magnitude of the association of common lifestyle factors on 10-year first and recurrent CVD events is different and probably sex-oriented. In particular, the lifestyle pattern that determined men's CVD onset namely included physical activity as well as smoking habits yet in case of CVD recurrence, physical activity and adherence to Mediterranean diet came at the top of the ranking. On the other hand, in apparently healthy women adherence to Mediterranean diet was the dominant CVD risk factor while risk for recurrent cardiac episodes seemed to be highly determined by the total lifestyle pattern. Additionally, inadequate sleep duration was an important contributor to the first CVD event risk, only for women. Focusing on the dietary part, various sex-specific associations were revealed regarding specific food groups, nutrients and other dietary features such as the consumption of dairy products, the dietary vitamin D intake, meat consumption and the anti-inflammatory potential of diet in relation to major events (i.e. fatal/non-fatal CVD events) as well as to other CVD risk factors (e.g., depressive symptomatology), intermediate cardiometabolic conditions (e.g., diabetes, hypertension, dyslipidaemia, metabolic status, NAFLD) and various biomarkers related with inflammation, insulin resistance or redox stress. Among the most important nutrition-related findings of the present work was the fact that two individuals with similarly moderate to high level of adherence to Mediterranean diet were not equal against CVD; with the high consumption of fruits, vegetables, whole wheat products and legumes being more important than the low consumption of meat and full fat dairy products. Most importantly, this plant-based orientation was more frequent in case of women. This challenges the dietary interventions giving higher weights to the plant-based part.

The psychological part was an indispensable part of the present work. Drawing on the most updated evidence, depression is a major driver of overall quality of life. The women's two-fold higher depression prevalence in general and namely in cardiac population sets the hypothesis for a more amplified comorbidity in this target group. In a combined re-analysis of two prospective epidemiological studies, higher magnitude of association between depressive symptomatology and 10-year first or recurrent CVD event was revealed for women. Most

importantly, 35% of excess first CVD event risk and nearly half of excess risk for recurrent CVD events due to depressive symptomatology in women subgroup was mediated by conventional factors while different patterns of ranking were observed regarding the separate mediating effect of adjustment factors oriented by CVD prevention stage.

Considering that body composition stands among the most important differences between men and women, the sex-specific role of lean and fat mass on 10-year CVD onset and recurrence was also examined here, suggesting that the association of predicted lean and fat mass, generated from population-based equations, on long-term CVD incidence may vary according to sex as well as according to CVD prevention stage. In particular, it was revealed that lean mass may have an independent cardioprotective role not only in patients with established CVD and advanced age, but also in apparently healthy middle-aged individuals. LMI seemed to independently protect against CVD even in the context of increased weight status or adiposity in male free-of-CVD subjects. In case of female ACS patients, it is noteworthy that the same association followed a U-shape trend. On the other side, FMI seemed to significantly aggravate cardiac health in free-of-CVD women as well as in men with established disease.

MHO status is another condition highly observed in women. Hence, this was another issue examined in the present work, revealing interesting outcomes. In brief, MHO status was an independent CVD risk factor only in women while the stability of this condition throughout a 10-year follow-up period was challenged principally in women (i.e. the men-to-women MHO-to-MUO transition rate was 0.81 i.e. in favour of women). An intermediate path suggested by the results presented here may be related with the increased likelihood of MHO women to have NAFLD.

Various biomarkers were examined in relation to CVD following a sex-specific orientation. In particular, this work suggested different predictive ability among various lipid markers and their apolipoproteins specifying the outcomes to men and women. It was revealed that non-HDL-C, non-HDL-C/HDL-C and TC/HDL-C ratio had a generally higher discriminative ability against 10-year CVD onset, principally in women, compared to conventional lipid biomarkers. For men, LDL-C seemed to ameliorate the predictive ability of conventional risk prediction models even if the association was not significant. As for apolipoproteins, the ApoB100/ApoA1 ratio was a better CVD predictor in case of men. Lp(a) is another biomarker highly discussed for its potential sex-specific association with CVD. The present work suggests that the role of Lp(a) on cardiac health may be more important for men, probably presenting higher predictive ability, yet more complex in case of women. Sensitivity analyses revealed that the independent aggravating role of Lp(a) was more evident in the context of total cholesterol, HDL-C, ApoA1 and triglycerides above the normal range as well as in participants with low

adherence to Mediterranean diet; further interaction analysis suggested that some of these observations, mainly from the standpoint of lipidemic profile, could be sex-mediated. Finally, SUA was as well investigated for its potential to predict 10-year CVD onset separately for men and women revealing stronger associations for women. In addition to this, the present work suggested the optimal sex-specific SUA thresholds to detect 10-year CVD incidence rate.

5.2 Lifestyle and psychological factors, sex and long-term CVD onset and recurrence

5.2.1 Mediterranean diet, physical activity, smoking and sleep duration

Very few studies have investigated to what extent each lifestyle factor contributes to the CVD burden separately for men and women, mainly on the primary prevention setting. Focusing on the dietary habits, in CARDIO2000 case-control study, the effect of Mediterranean diet on ACS onset likelihood was higher in women compared with men, which seems to be completely aligned with the outcomes presented here (Chrysohoou et al 2003). In the INTERHEART study, the effect of a "high risk" diet was as well slightly higher in women; yet in that case, "high risk" diet was defined, by the contributors of the study, only according to fruits and vegetables consumption instead of a holistic dietary pattern, as performed in the present work (Anand et al 2008). Another interesting observation of this study was that physical activity was protective for both sexes against first CVD event, yet the effect was more evident in women (Anand et al 2008). Much as the beneficial effects of exercise in primary CVD prevention spectrum are well documented, the hypothesis that an equitable exposure on a physically active or sedentary lifestyle between men and women may have a greater impact for the latter, has been stressed in the literature (Alves et al 2016, Shiroma et al 2010). In this context, in the present work, the magnitude of physical activity-first CVD association was suggested to be higher in men compared with women; considering that physical activity status in terms of absence of a sedentary lifestyle, was almost at the same level between sexes, other factors such as duration or intensity of exercises could be moderators or mediators in this work as well as other similar studies (Alves et al 2016, Shiroma et al 2010). The implications of cigarette smoking on cardiac health were highlighted in the present analysis, yet the outcomes were more evident for men, in line with the results of the INTERHEART study (Anand et al 2008). Nevertheless, recent meta-analyses about the sex-specific effect of smoking on first CVD event suggested a higher effect size in women with CHD as main outcome. Subsequently, the positive effect of smoking cessation was similar in both sexes (Huxley et al 2011). Another issue investigated here was the extent to which sleep duration could modify the cumulative effect of the aforementioned lifestyle factors on CVD risk. Inadequate sleep duration as regards the development of a first CVD event

was more evident in the case of women. It is widely adopted that sleep attitude plays a key role on health and general quality of life (Sofi et al 2014). Poor sleep quality has been associated with inflammation and redox stress, as well as unhealthy dietary habits, all contributing to the progression of atherosclerosis and resulting in increased CVD risk (Dominguez-Rodriguez et al 2014). Different trends oriented by sex are currently suggested with the women being more susceptible to the aggravating effect of inadequate sleep (Georgousopoulou et al 2017, Mallampalli et al 2014).

Due to the fact that sex incorporates both psychosocial and cultural aspects, it is obvious that behavioural and lifestyle patterns are expected to be discriminated between men and women (Kouvari et al 2018). As mentioned above, younger women are more likely to follow healthy dietary practices (GBD 2015) mostly for healthy weight maintenance and better body image reasons (Hattori et al 2017). On the other side, women are less physically active probably due to their role in workplace and household tasks (Yahia et al 2016). In contrast, it is documented that men are more likely to have poor health behaviours such as active smoking, unhealthy dietary habits and alcohol overconsumption; however, they spend more time in exercises and physical activities (Hattori et al 2017, Spence et al 2015).

Even if scientific research has been slightly focused on the interaction between sex and lifestyle on the CVD onset, the sex interacting effect on lifestyle-CVD progression association remains vague. Indeed, in secondary CVD prevention, focus is always being oriented towards medical treatment and invasive therapeutics (Kouvari et al 2018). An additional outcome of interest in the present work was the impact of disease onset on the lifestyle-cardiac health association separately for men and women. In women, the whole lifestyle pattern seemed to independently impact their risk for recurrent cardiac episodes. In comparison with the first CVD event risk, healthy dietary habits in terms of adherence to Mediterranean diet retained their significant protective role yet a reduced effect size was highlighted. At the same time, unhealthy habits such as sedentary lifestyle and smoking came on the short list. Several hypotheses could be performed to interpret the differences between the two prevention stages. CVD manifestation is accompanied by a large psychological burden which strongly affects women (Hare et al 2014). Considering that a serious depression symptomatology status is related to unhealthy lifestyle habits, women cardiac patients seem rather susceptible to high risk behaviours (Hare et al 2014). At the same time, lifestyle counselling is usually underrepresented in cardiac rehabilitation programs, even more in women patients who are generally susceptible to inadequate medical advice (Panagiotakos et al 2007b). The life-long consolidation of severe cardiac complications is expected to gradually limit patients' intention – to – adapt lifestyle modifications and this has been suggested to be even more evident in women (Kouvari et al 2017a, Kadda et al 2016,

Panagiotakos et al 2016). In case of smoking paradigm an additional hypothesis may exist. Women tend to smoke later in life presenting a similar smoking pattern with their men-smokers' counterparts while they have greater difficulty in quitting this habit (Appelman et al 2015). This may be the reason for which the disproportionally larger CVD burden in women smokers, suggested in the literature (Huxley et al 2011), was observed in the present study, in post-ACS period (e.g. at more advanced age). As for the men, a higher positive effect of Mediterranean diet was highlighted in terms of recurrent CVD events risk while smoking lost its significance and physical activity status remained an important prognostic factor. Hence, it could be speculated that men ACS patients tend to modify their lifestyle towards a healthier approach. One of the very few studies in the secondary prevention spectrum investigating such issues is the GENESIS-PRAXY prospective study, yet with adults with premature ACS; the tendency of men to ameliorate their lifestyle and the difficulty of women in adapting to healthier behaviours in the post-ACS period was an important highlight of the study (Leung Yinko et al 2015). In accordance with this, in the present work, besides the 2-fold higher cumulative risk of unhealthy lifestyle pattern in apparently healthy men compared with their women counterparts, this was not the case when CVD was established. In particular, the respective men-to-women cumulative risk ratio was towards 1-to-1; probably suggesting a hypothesis that men in their post-cardiac period are more motivated to ameliorate their lifestyle while women patients are less willing to adapt.

5.2.2 Different clusters of Mediterranean diet

An additional finding here is related with the clustering of Mediterranean diet components within a population of men and women. Although sodium, sugars, fat and red meat have been the main focus of diet policy debate within the past two decades (WHO 2009, WHO 2013b) the assessment performed in the present work shows that increasing the consumption of plant-based products should be the leading target to achieve better cardiometabolic health and preventing CVD deaths. Scoring higher in the consumption of fruits, vegetables, legumes and whole-wheat cereals was more important than achieving a better score in low consumption of animal-based products such as meat or full-fat dairy products. The beneficial role of plant-based diets in cardiovascular health has been increasingly recognized, with a vast and accumulating evidence-base documenting their health effects (Satija et al 2018). On the other side, complementary non-significant findings from studies of individual animal foods or nutrients including saturated fatty acids, red meat, full-fat dairy products regarding their association with CVD (Fontecha et al 2019, de Souza et al 2015) lend further support to the probably higher cardiometabolic effects of plant-based products. Giving higher weight to the plant-based part of Mediterranean diet seems an approach quite similar with the five dietary metrics suggested by the Nutrition Committee of the American Heart Association to define the ideal cardiometabolic health having the Dietary

Approaches to Stop Hypertension (DASH)-type eating plan as a basis; among these metrics fruits, vegetables and whole-wheat products were on the top of the rank with processed meats and saturated fatty acids set as secondary metrics (Lloyd-Jones et al 2010). What should be underscored here is that the woman – to – man ration for the pattern oriented by the high intake of plant-based products was close to 3:1 while within the pattern oriented by the low intake of animal-based products was close to 1:4. This may be another reason to which the protective effect of Mediterranean diet against CVD onset and recurrence was stronger in case of women.

5.2.3 Specific food groups, nutrients and other dietary features

5.2.3.1 Dairy consumption

Among the most debated issue regarding dairy products is their fat content. In line with the outcomes presented here, recent meta-analyses suggest from an inverse (Mishali et al 2019, Schwingshackl et al 2017, Lee et al 2018) to a non-significant (Gholami et al 2017, Zhang et al 2020, Alexander et al 2016) association between total dairy products and CVD or metabolic syndrome irrespective to their fat content. Similarly, meta-analyses that provide separate results for low-fat and full-fat dairy products reveal mostly non-significant outcomes (Hu et al 2014, de Goede et al 2016). Another important finding here was the fact that not all kind of dairy products are the same. In particular, milk presented a non-significant association in relation to CVD incidence. In contrast, fermented products independently protected against 10-year CVD onset. This comes in line with recent longitudinal cohort studies (Farvid et al 2017, Dehghan et al 2018). Additionally, the latest meta-analysis on the role of fermented dairy products on primary CVD prevention revealed similar inverse associations (Zhang et al 2019). As for the cheese, hitherto evidence is mixed, yet interestingly tend to inverse correlations with coronary heart disease or stroke (Farvid et al 2017, Chen et al 2017, Guo et al 2017). In the present work, cheese was significantly associated with lower CVD risk, yet the effect size was quite small probably due to the reported low consumption level on the sample; the matter of quantity above which cheese may exert its cardioprotective properties has been previously reported [36]. The stronger protective effect of yogurt – and not cheese – could also suggest that the principle mechanism through which fermented dairy products exert their role on cardiac health is related with probiotics (Chen et al 2017). Yogurt contains strain-specific probiotics that affect gut microbiota (Fernandez et al 2017). On the other side, in case of butter intake no aggravating– as normally expected – effects were observed. This finding comes in line with the results from the multinational PURE study (Dehghan et al 2018) as well as a recent meta-analysis (Pimpin et al 2016). High concentration of saturated fatty acids in butter was usually related with impaired health outcomes. Nevertheless, recent works suggest that dairy fat is not that dangerous for humans' health (Vissers et al 2019).

A paucity of research exists regarding the sex-specific effect of dairy products on CVD onset (Goldbohm et al 2011, Kondo et al 2013). Here, it was revealed that the aforementioned associations were principally retained in women. This comes in line with a very recent meta-analysis where high consumption of dairy products – any kind – was associated with about 20% lower risk to develop CVD (Mishali et al 2019). In the same meta-analysis, a similar association was observed regarding diabetes development (Mishali et al 2019). In a cohort study with Korean participants, women reported a high consumption of dairy products on a daily basis, were protected against metabolic abnormalities and obesity while in men no significant associations were observed (Lee et al 2018). Additionally, in a recent clinical trial, different effects of full-fat or low-fat cheese on LDL particles size were observed between men and women (Raziani et al 2018). Hence, the protective effect of dairy products on such intermediate cardiometabolic conditions may subsequently reduce women's CVD risk. Moreover, a diet rich in milk-derived proteins has been suggested to affect fat distribution in postmenopausal women causing more subcutaneous fat relative to visceral fat to accumulate (Chen et al 2019). Adipose tissue in women tends to be subcutaneous, whereas in men, there is a tendency towards visceral accumulation which may explain any sex-specific associations between dairy intake and CVD. In line with the previously reported hypotheses, in the present work adjusting for obesity (expressed through BMI) as well as conditions related with impaired glycaemic, lipidemic and blood pressure profile alleviated the examined associations in women – even if the level of significance was retained. Moreover, in case of further adjustment for visceral adiposity (expressed through waist circumference) and biomarkers related with inflammation, liver fat accumulation and insulin resistance – all paths of central obesity – the level of significance was lost; suggesting that regular dairy intake (mainly yogurt intake) may be related with better cardiometabolic health in women with impaired weight status. This comes in line with recent evidence (Buziau et al 2019).

5.2.3.2 Dietary vitamin D intake

Considering that emerging data suggest that vitamin D status is highly dependent on genetic regulation and sex, the role of this nutrient and its metabolites on CVDs should be specified to men's and women's special features (Mithal et al 2009). Here, the significant inverse association of dietary vitamin D with CVD event was retained only in men. This finding partially contradicts previous reports suggesting a more pronounced protective effect of vitamin D in women especially those in the postmenopausal life stage (Al Mheid et al 2013). Several mechanistic as well as methodological hypotheses could be performed. Firstly, vitamin D and its metabolites interact with sex hormones. An in vitro experiment revealed the interaction between vitamin D and oestrogenic hormone pathways, with vitamin D restoring endothelial function in cells isolated from ovariectomized rats, normalizing the expression of cyclo-oxygenase-2,

antagonizing thromboxane-prostanoid receptor and inducing nitric oxide production (Dong et al 2013). In accordance with this, in Multi Ethnic study of Atherosclerosis vitamin D deficiency was cross-sectionally related with lower estradiol levels in women yet no interaction between vitamin D and testosterone levels in men was observed (Zhao et al 2017); this comes in line with the present analyses since the modest association between dietary vitamin D and CVD onset in the crude model of women was lost after adjusting for age and menopause status. Secondly, the generated outcomes may be a matter of chronicity since women are exposed to major cardiac events about one decade later than men (Mosca et al 2011a). In the present work, despite the lack of association between dietary vitamin D and hard endpoints in women, significant inverse associations in intermediate cardiometabolic conditions (i.e. transition to unhealthy metabolic status) were observed which subsequently increases CVD risk.

5.2.3.3 Dietary anti-inflammatory factors and cardio-metabolic disease

The association between D-AII and MetS is still a relatively new area of research with only nine studies investigating this association, seven of which were of a cross-sectional design. The findings of these studies indicated inconsistent results ranking from neutral (Sokol et al 2016, Wirth et al 2014, Pimenta et al 2015, Carvalho et al 2019, Ren et al 2018) to positive associations (Neufcourt et al 2015, Mazidi et al 2018, Phillips et al 2018, Kim et al 2018). Moreover, the results of the cross-sectional analysis in this study indicated neutral or borderline significant association between MetS and the inflammatory load of participants' dietary pattern irrespective to the defining criteria used. Heterogeneity of findings could be attributed to several factors, such as the different set of criteria or the different study settings and participants' characteristics (age, gender, geographic origin and ethnicity). To minimize the effects of these issues, three alternate official criteria to define MetS were used to be associated with the D-AII. Although most of this sensitivity analysis revealed generally neutral associations, it was of interest that in case of the revised NCEP ATP III definition the examined relation was borderline significant. Considering that the principle difference among the three definitions is related to the waist circumference cut-off points, this could also be suggested as a potential mediator or moderator. The highest cut-off points in waist circumference can imply that the protective effect of D-AII may be principally exerted in the context of severe visceral adiposity with central obesity-related inflammation being an underlying mechanism (Mtintsilana et al 2019). Additionally, official analysis of interaction was performed accompanied by sampling stratifications when needed and beyond the principle analysis with MetS, sensitivity analysis was performed to examine the separate association of MetS components with D-AII. Focusing on the latter, it was revealed that a diet with low pro-inflammatory load was inversely associated with abnormal waist circumference in men while in women more predominant inverse associations were observed with insulin resistance and low

HDL-C levels similarly to the findings reported in previous studies (Rubio-Ruiz et al 2015, Neufcourt et al 2015). Based on these observations, it can be proposed that MetS traits 'per se' may differentially react to diet-related parameters such as the duration of exposure to pro-or anti-inflammatory dietary patterns in men and women.

5.2.3.4 Depressive symptomatology

Secondary prevention in women

Psychological health interventions are currently a "Grade A" guideline for established CVD. Women cardiac patients have a two-fold higher likelihood to suffer from depressive symptoms (Piepoli et al 2016). Although this prevalence points to a greater degree of adverse events in women patients with psychological stressors, hitherto literature is not well-defined (Shanmugasegaram et al 2012). In the present work, sex-based analysis revealed –in line with similar works (Parashar et al 2019, Orth-Gomér et al 2009)– that depressive symptomatology was an independent prognostic risk factor for recurrent CVD event only in ACS women. In the latest meta-analysis of prospective studies, post-AMI depression presented a two-fold higher 2-year risk of impaired outcomes (van Melle et al 2004). What should be outlined here is that no sex-stratified analysis was performed while the women-patient representativeness did not exceed the 26% of total meta-analysis sample (van Melle et al 2004). Interestingly, sex differences in depressive symptomatology and post-AMI prognosis were recently investigated in an individual patient data meta-analysis (MINDMAPS study); the conclusion was that besides the more frequent symptoms in women, the aggravating effect was stronger for men (Doyle et al 2015). Nonetheless, this "men- privilege" was attenuated by disease status.

In ACS women patients of the present work, the effect of examined mediators was higher, interpreting almost the half of depressive-symptomatology aggravating effect. Among the tested mediators, low adherence to medication came on the top of the rank. This is in accordance with recent highlights from GENESIS-PRAXY, related to a very low women's intention–to–adapt recommendations in their post-ACS period of life, due to depressed mood oriented by cardiac-complication consolidation (Leung Yinko et al 2015). Another important observation here was that disease severity indicators were not as important mediators as normally expected. This finding comes in line with recent suggestions that depressive symptoms in women are irrelevant with disease burden (Pelletier et al 2016, Smolderen et al 2015).

Primary prevention in women

Even if it has been four years since when AHA stated depression as "risk factor" for subsequent cardiac episodes, no convincing evidence exists for CVD onset, even more when it comes to sex interactions (Lichtman et al 2014). Some meta-analyses (*please see* 1.4.6.2 section) have served interesting findings being in line with the outcomes discussed here. Several putative mechanisms

have been also suggested (Hare et al 2014, Kollia et al 2017). One hypothesis investigated here was the extent to which the depressive symptoms-CVD association in women was mediated by conventional risk factors. Sex role-related stressors i.e. role overload, interpersonal orientation and decreased self-esteem, are the most discussed mediators driven by low socioeconomic status (Low et al 2010). Here, modest mediating effects of socioeconomic factors were revealed, overlapped by clinical/biochemical factors i.e. excess body weight, abnormal glycaemic status and inflammation; similar underlying mechanisms have been previously suggested as more evident in women (Webb et al 2017). Another finding here was that suboptimal adherence to Mediterranean diet and inadequate sleep were the most important behavioural mediators. A bidirectional association between unhealthy nutrition and depressive symptoms has been profoundly proposed in the literature (Yannakoulia et al 2008) while this is currently suggested for sleep quality, as well (Georgousopoulou et al 2014, Bao et al 2017). Lipid markers are also presented as mediators (Hare at al 2014). In the present work, high density lipoprotein and triglycerides were the lipid markers with the biggest mediating effect; women-specific reports for lipid profile may explain this ranking (Kouvari et al 2018).

The important observation here was that all the aforementioned factors accounted for only 35% of the examined association; suggesting a biological relation between the examined disease states that remains unexplained. Increasing evidence suggests that some psychological factors are influenced by genes and that some of these genes may have cardiac implications (Mulle et al 2013). Twin-study analyses have shown that various genetic factors contribute to depression-CVD comorbidity, yet interestingly only in women; hence, higher heritability and stronger genetic basis are inferred (Mulle et al 2013). Another hypothesis that could be performed here is the depressive-symptom chronicity in women. Women predominance regarding psychological stressors begins in adolescence and persists into middle and early old age (Low et al 2010). Since there is evidence that depressive symptoms are more persistent and severe for women, this could be considered as an unmeasured confounder. Moreover, women-specific conditions have been suggested to exacerbate the effect of depressive symptoms on vascular system. Serotonin, involved in depression-disrupted behaviours (mood, appetite, sleep), presents reciprocity with female sex hormones while an immunological estradiol-mediated path has been currently suggested (Steiner et al 2011).

Depressive symptomatology and CVD in men

As for men sample of the present work, the aggravating effect of depressive symptoms on CVD event rate was observed, yet it did not reach the level of significance. The more prevalent behavioural and clinical risk factors in men along with the lower likelihood to suffer from depressive symptoms might contribute to that outcome. Men are more likely to suffer from

depressive symptoms later in life and after a chronic disease establishment while, in contrast with women (Smolderen et al 2015, Samad et al 2014, Roumia et al 2017), the increased subsequent CVD event risk is to be attributed to frailty– and disability– related confounders in advanced age (Doyle et al 2015). Finally, different depression features and response to psychological/mental stress have been suggested for men pointing to a non-well defined and probably underdiagnosed male depression syndrome hypothesis (Samad et al 2014, Azorin et al 2014).

5.2.3.5 Depressive symptomatology and meat consumption

In the present work, a non-linear trend between meat consumption and depressive symptomatology was observed affecting the risk for CVD onset or recurrence; suggesting that a moderate consumption of meat i.e. about 2-3 servings per week may have more benefits for psychological and in turn cardiac health compared with a lower consumption level. This non-linear trend could be explained by the fact that the mechanisms through which meat consumption exerts its effect on psychological health could support either a positive or an inverse association. An additional hypothesis investigated here was the extent to which meat consumption is equally related with depressive symptomatology and cardiac health between men and women. Interestingly, the present work revealed that the suggested non-linear U-shape trend in total as well as red meat intake seemed to be statistically significant only in women. These findings are somehow consistent with previous works. For instance, in a sample of Australian women, lower red meat intake was associated with nearly twice as high risk of major depressive and anxiety disorders. A trend towards a U-shaped curve was as well reported, with lower than the habitual consumption of red meat having aggravating effect on psychological health (Jacka et al 2012) What is more, in another work where three dietary patterns were examined against various psychological disorders it was revealed that much as in men significant inverse and positive associations were observed in relation to plant-based and meat-based food patterns, respectively, in case of women these generally expected trends did not reach the level of significance (Noguchi et al 2013). The mechanisms through which the suggested beneficial effect of meat and especially red meat on women's psychological health is exerted are unknown. Several hypotheses related with the mediating effect of micronutrients highly met in red meat, such as iron, have been suggested in women (Noguchi et al 2013). In specific, disturbances in iron metabolism have been associated with impaired psychological health; considering that women are more susceptible to iron deficiency and that meat –principally– red meat is the main food source of iron may imply that the observations raised here for women are attributed to this critical micronutrient (Wojciak et al 2014).

5.3 Body composition, sex and long-term CVD onset and recurrence

5.3.1 Body composition in secondary prevention

Lean mass as a novel prognostic factor is attracting considerable attention in patients with established CVD. The present work sets implications that high lean mass accompanied by obese and/or excess-body-fat status may not be that protective which comes in line with previous studies (Srikanthan et al 2016, Spahillari et al 2016). Moreover, sensitivity analysis, revealed that women in 2nd LMI tertile presented the best prognosis compared with 1st and 3rd LMI tertile while this trend was not confirmed in men. This comes in line with a very recent work from a Greek sample (Kouvari et al 2019). There are indications that women in advanced age (Yang et al 2019) and/or with an established catabolic disease (Anderson et al 2017) may be more vulnerable in lean mass loss compared with men of similar age and disease profile probably due to lifestyle or biological factors (Vest et al 2015, Kouvari et al 2019). Additionally, what is highly suggested for women patients, is a more pronounced BMI-related paradoxical association, also confirmed here (Atkins et al 2014). This has been attributed greater myocardial fatty acid uptake and lower myocardial utilization in women (Yoowannakul et al 2018). Additionally, the present work suggested that women's "overweight paradox" may be attributed not only to their resistance to adiposity but also to sex-specific responses of lean mass. In particular, ACS women patients with moderate LMI presented the best prognosis; this patient's category was mostly overweight and/or with low to medium total fat mass. On the other side, further raise in FMI, probably accompanied by visceral adiposity considering the life stage of women participants, attenuated the potential protective effect of lean mass which has been observed elsewhere (Dereziński et al 2018).

5.3.2 Body composition in primary prevention

Body composition in apparently healthy younger individuals has been scarcely studied. In the present work, BMI and FMI presented an independently positive association with CVD incidence only under the context of highest adiposity rates. On the other side, LMI had a protective effect against CVD. Examining the combined role of FMI and LMI on CVD onset, FMI remained an independent risk factor even in high LMI values yet with lower effect size compared with lower LMI. This independent association of FMI comes in line with a very recent big data analysis (UK Biobank) (Iliodromiti et al 2018); yet the added value of this work was the consideration of lean mass on this association which has mostly been applied in patient or at-advance-age populations. The sex-stratified analysis revealed that the aforementioned associations were retained only in women; while in men LMI had a stronger protective effect against CVD even in the context of high adiposity estimations. These indications are partially aligned with previous works. Sex-specific analysis in UK Biobank revealed that adiposity metrics had a stronger aggravating for women compared with men (Iliodromiti et al 2018). In the PREVEND cohort study, adiposity was independently associated with CVD onset even after adjusting for a muscle mass indicator

with marginally stronger association in women, yet only on the basis of total fat mass (Byambasukh et al 2019). Different fat distribution between sexes may partially explain these observations. To this issue, there are indications that total body fat may have a higher predictive ability in women whereas in men focus should be oriented towards visceral adiposity metrics (Onat et al 2010). Additionally, sex is to influence adipocyte size in specific anatomic regions. Given the same weight gain, intraperitoneal visceral adipocyte size in men marginally increases compared with the rise in women; large adipocytes are associated with more proinflammatory hormone-mediated adipokine secretion which may explain the susceptibility of women in excess body fat (Hung et al 2017). Moreover, the observed association may be gene-mediated; in Women's Genome Health study it was revealed that a fat-mass related gene had an independent aggravating effect on women's CVD risk especially in those with low physical activity (Ahmad at al 2010). Finally, several hypotheses could be performed regarding the protective effect of lean mass against excess adiposity only in men. Firstly, physiologically middle-aged men have higher lean mass compared with women. Secondly, within younger ages men present a higher level of physical activity compared with women which may affect their lean mass on the basis of quantity, metabolic activity and strength (Shiroma et al 2010). Thirdly, in the ATTICA study sample, men with high LMI had significantly lower waist circumference compared with their counterparts in lower LMI tertiles which could have driven the final outcome. Lastly, this observation may be mediated by testosterone levels. Lean mass is highly correlated with testosterone levels. Hence, behind the lower lean mass metrics, testosterone deficiency may be hindered; considering the mounting evidence suggesting that normal testosterone levels are beneficial to men's vascular system and that testosterone deficiency is associated with an unfavourable metabolic profile, including increased adiposity and insulin resistance, the findings of the present work may be attributed to this intermediate path (Elagizi et al 2018).

5.4 Metabolic status, NAFLD, sex and long-term CVD onset

5.4.1 MHO status and CVD

Most recently, it is conferred that MHO status may be transient in nature. Prospective population-based studies have revealed that a considerable proportion, ranging between 33% to 52%, of MHO individuals lose such status over time (Mongraw-Chaffin et al 2018). However, these rates have been mainly documented in MHO subjects with ≤2 metabolic abnormalities. It is upheld, that these estimates may underestimate true rates as individuals with two established unhealthy conditions are more likely to be diagnosed with MetS the following years. On the other hand, the transition of obese individuals without metabolic abnormalities has scarcely been investigated. In the present work, MHO participants without metabolic abnormalities at baseline subsequently progressively increased likelihood to present at least one metabolic abnormality

within the 10-year follow-up period. This is in line with the hitherto evidence yet comes to highlight that even the "healthiest" obese confer a high risk to develop conventional risk factors and become MUO later in their life. On the other hand, little evidence exists regarding CVD risk of non-persistent MHO persons. In the present work, this MHO subgroup was independently associated with increased CVD risk within the decade, strengthening the hypothesis that the initially observed non-significant outcomes might be hindered by a lag in risk till the transition to unhealthy metabolic status. An additional gap in knowledge is potential differences in MHO between sexes. Interestingly, in the present work significant sex-related interactions were observed with stratified analysis revealing that MHO status was an independent CVD risk factor only in women. This finding contradicts a previous report suggesting a more pronounced aggravating effect in case of men (Hansen et al 2017). However, as the only group that directly addressed the question of transition of MHO to MUO status separately in women, *Echel and colleagues* found that a large proportion of metabolically healthy women converted to an unhealthy phenotype over time which then was associated with increased CVD risk (Eckel et al 2018). In line with this, here, the men-to-women MHO-to-MUO transition rate was 0.81 i.e. in favour of women.

5.4.1.1 The mediating effect of NAFLD

Another core finding of the present work was that temporally MHO participants had an independently higher likelihood for NAFLD compared with MHN counterparts strengthening the hypothesis that the initially observed non-significant outcomes might be hindered by a lag in risk till the transition to unhealthy metabolic status. In addition to this, the association between NAFLD and transition to unhealthy metabolic status was prospectively examined within a 10-year follow-up revealing an independent positive association. Such observation comes in line with previous findings suggesting NAFLD as an independent risk factor for diabetes mellitus and MetS (Lonardo et al 2015; Mantovani et al 2018) while sets implications for potential inclusion of NAFLD in the definition of MHO status to catch the stability or temporality of metabolically health status. In addition to this finding, significant sex-related interactions were observed with stratified analysis revealing that MHO status was independently associated with increased NAFLD likelihood only in women. This finding comes in line with the sex-specific analysis performed by *Chang and colleagues* (Chang et al 2016). The reasons for a stronger association of MHO status with incident NAFLD in women are unclear. Excess adiposity and lower lean mass in women as well as the different fat distribution between men and women (i.e. more subcutaneous fat in women vs. more visceral and ectopic fat in men) – for the same fat content – may explain this variation (Stefanska et al 2015).

5.4.2 NAFLD and Mediterranean diet

The present work examined the sex-specific role of Mediterranean diet and separate macronutrients on liver steatosis presence. In particular, it was revealed that Mediterranean diet was significantly associated with liver steatosis only in men; while in case of women the protective role of this pattern was alleviated firstly when adjusting for age and secondly when weight status and body fat distribution were taken into account. In the context of liver pathologies, dissimilarities of sex hormones are listed among the main reasons for the differences in the prevalence of liver diseases, even if in case of NAFLD less consistency exist (Cvitanović et al 2018). In the meanwhile, development and progression of liver steatosis in men is independent to their age while in women NAFLD occurrence is accelerated in postmenopausal life stage (Ballestri et al 2017). This may partially be attributed to oestrogens and other female-specific factors (Ballestri et al 2017). Considering that the effect of genetic and hormonal status may be stronger than environmental factors such as nutrition, this may explain the moderating effect of age on the association between Mediterranean diet and liver steatosis in the subsample of women, observed here. Partition of fatty acids to ketone body production, very low density lipoprotein cholesterol synthesis and fatty acids oxidation, together with deposition of triglycerides as lipid droplets are considered as parts of NAFLD pathology, principally in the context of adiposity (CvitanovićTomaš et al 2018). A high-fat diet has been suggested to alter these conditions only in women (CvitanovićTomaš et al 2018). In Mediterranean diet, dietary fat is 35-40% of total energy intake implying another explanation of the present outcomes. On the other side, another finding arisen here was related with the carbohydrate content of participants' diet; revealing that in women only Mediterranean diet with carbohydrate content <35% of total energy intake was inversely associated with liver steatosis. Women are more likely to consume refined grains, sweets and other products with high added sugar and fructose content of high energy density even in the context of a generally healthy dietary pattern which may explain this observation (Kouvari et al 2019). Considering that this observation was generated after adjusting for weight status and total energy intake, this could justify the present finding. Several inconclusive gene-diet interactions have been lastly discussed in relation to liver health accounting for gender, yet further research is demanded (Coltell et al 2019). On the other side, in the present work an alternate mechanism related with adiponectin was to mediate the association between liver steatosis and Mediterranean diet. The sex-specific response of adiponectin in the context of Mediterranean diet has been scarcely discussed yet implications for differences between men and women may explain the conclusions revealed here (Bédard et al 2014, Kyrou et al 2017).

5.5 Various biomarkers, sex, and long-term CVD onset

5.5.1 Lipoproteins, apolipoproteins and CVD

The present work examined the role of conventional and non-conventional lipid biomarkers against hard CVD-related endpoints, specifying the outcomes for men and women. Focusing on the traditional lipid markers, LDL-C was an important CVD predictor only in men yet in case of women HDL-C seemed to contribute more to the overall CVD risk. Men have higher LDL-C levels compared with the age-matched women, till the menopause stage; since then a steep LDL-C raise occurs, predisposing women to a CVD-risk escalation (Mora et al 2011). On the other side, HDL-C and triglycerides have been suggested as stronger lipid indicators in case of women which comes in line with findings arisen here. More specifically, HDL-C addition in SCORE risk stratification led to a modest improvement in its predictive ability for women (Cooney et al 2009). In Women's Health Study, the inverse association between HDL-C and primary CVD incidence was retained across all LDL-C levels, with the exception of women with low total atherogenic particle burden (Mora et al 2011). What is more, the TC/HDL-C ratio has been suggested as a strong independent predictor for acute myocardial infarction in men with only a paucity of studies evaluating this ratio in women (von Mühlen et al 2003, Calling et al 2019, Albrektsen et al 2017). In line with previous works, the findings raised here suggested that TC/HDL-C ratio is independently associated with increased CVD risk in both sexes, yet with the association being stronger in case of women. As for triglycerides, in a relevant meta-analysis, a stronger association of fasting triglycerides with CVD mortality in women was revealed (Liu et al 2013).

The sex-specific effect of alternative lipid-related markers against hard CVD endpoints was as well evaluated. The added value of non-HDL-C and relative derivatives such as the non-HDL-C/HDL-C ratio is increasingly suggested within the last decade yet with inconclusive outcomes. Here, the lowest hazard for CVD was found in women and men with the lowest non-HDL-C concentrations. This is in accordance with outcomes revealed from the Multinational Cardiovascular Risk Consortium where a continuous and linear increase for higher non-HDL-C concentrations was observed (Brunner et al 2019). Additionally, several works suggest that the non-HDL-C/HDL-C ratio is superior to traditional lipid markers in estimating arterial stiffness and atherosclerosis with this association being more evident in case of women (Paul et al 2012, Qin et al 2015). This comes in line with the outcomes revealed here. The exact reason for this result must be determined in future studies. Changes in the levels of both HDL-C and triglycerides found to exert stronger effects among women may partially explain this observation (Shaw et al 2006). In the INTERHEART study, dyslipidaemia, defined as (ApoB100)/(ApoA1) ratio possessed the highest population-attributed risk in both sexes (Anand et al 2008). Here, it was revealed that ApoB100 was a stronger CVD predictor for men yet ApoA1 for women.

However, apolipoproteins seemed to have the lowest discriminative ability against CVD in both men and women which comes in line a meta-analysis of cohort studies implemented by the Emerging Risk Factors Collaboration (ERFC 2012).

In addition to the aforementioned lipid markers highly discussed in the literature, there remains controversy regarding the shape of Lp(a) risk curve and the extent to which Lp(a) confers CVD risk, independently of other lipid markers used in current risk equations. What is more, the role of sex on the association between Lp(a) and risk for incident CVD remains a matter of inconsistency. In particular, the Emerging Risk Factors Collaboration published a meta-analysis in 2009 combining 24 studies and 72,683 individuals to show that a 1 standard deviation (3.5-fold) higher Lp(a) was associated with a hazard ratio of 1.14 for CVD mortality, without any significant differences between sexes (ERFCS 2009). This meta-analysis further confirms the hypothesis that Lp(a) is a promising emerging risk factor in CVD epidemiology (ERFC 2009). Nevertheless, it is only within the last 5 years that the sex-hypothesis has been considered more seriously with cohort studies providing sex-based analyses regarding the role of Lp(a) on CVD onset albeit with contradicting remarks. In this context, considering the recent literature, the magnitude of interaction between Lp(a) and sex on CVD risk seems to be moderate. Very recent highlights by *Cook et al.* with data from Women's Health Initiative and Women's Health study suggested that Lp(a) was independently associated with long-term CVD incidence, yet only in case of women with hypercholesterolemia (Cook et al 2018). This finding was replicated in the female sample of the JUPITER clinical trial while in case of men, Lp(a) retained its significant aggravating effect even at lower total-cholesterol levels (Cook et al 2018). Apart from these highlights corresponding to the American population, a very recent combined analysis of two large-scale cohort studies with a Danish population suggested that Lp(a) is independently associated with long-term CVD mortality in both sexes, with a marginally higher effect size in men (Langsted et al 2019). Additional analyses with different endpoints i.e. non-CVD mortality and all-cause mortality, revealed a retention of the aforementioned association only in men (Langsted et al 2019). The findings of the present work are in line with the conclusions generated by the aforementioned prospective studies, revealing that conventional lipid markers may have a strong moderating effect on the Lp(a)-CVD association in women. In addition to this, the present work suggests that Lp(a) measurement in daily clinical practice may have an added value in the prediction of CVD onset and/or as a therapeutic target only in case of men, while for women other lipid markers may be more important. This claim contradicts previous hypotheses where Lp(a) was put under the umbrella of emerging biomarkers for women's cardiac health (Manson et al 2015).

5.5.2 Serum uric acid and CVD

The present analysis showed that higher SUA levels were significantly related to a greater 10-year CVD incidence, especially in women. Such a sex-specific association has also been reported in a few previous studies (Kivity et al 2013, Chilunga et al 2020, Høieggen et al 2004, Rodrigues et al 2012). Moreover, the present work suggested the existence of sex-specific SUA thresholds for CVD risk assessment, being lower in women [i.e. 5.05 mg/dL (0.29 mmol/L) in men and 4.15 mg/dL (0.24 mmol/L) in women]. A few trials investigated such SUA cut-off points. In brief, the URRAH study found that SUA cut-off values that predicted fatal myocardial infarction were 5.49 mg/dL (0.33 mmol/L) in men and 5.26 mg/dL (0.31 mmol/L) in women (Maloberti et al 2020). In the RODAM study, SUA thresholds for the detection of 10-year CVD risk (defined by the ACC/AHA risk score) were 6.77 mg/dL (0.40 mmol/L) in men and 5.15 mg/dL (0.31 mmol/L) in women (Chilunga et al 2019). Finally, the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) study, involving 1,720 untreated hypertensive patients followed-up a mean of 12 years, reported that the lowest risk for CVD events was observed at SUA levels 4.5-5.2 mg/dL (0.27-0.31 mmol/L) in men and 3.2-3.9 mg/dL (0.19-0.23 mmol/L) in women (Verdecchia et al 2000); the highest CVD incidence was observed at SUA ≥6.2 mg/dL (0.37 mmol/L) in men and ≥4.6 mg/dL (0.27 mmol/L) in women (Verdecchia et al 2000). These findings highlight the need for further research to identify SUA cut-off values predicting CVD incidence in the general population, as well as in certain patient populations.

5.6 Future steps on public health: health policies, practices and initiatives to achieve gender equity in primary and secondary CVD prevention spectrum

5.6.1 Health policy

According to the CDC, health policy is a law, regulation, procedure, administrative action, incentive, or voluntary practice of governments and other institutions (https://www.cdc.gov/policy/analysis/process/definition.html). According to the IOM, policy development is an essential public health function included in three of the 10 Essential Public Health Services (**Figure 5.1**) (IOM 1988, IOM 2002, CDC 2020). Public health professionals play an important role in policy development by conducting policy-relevant research, communicating findings in a manner that facilitates action, developing partnerships, and encouraging the efficient use of resources through the promotion of policies based on science—such as the promotion of evidence-based health interventions.

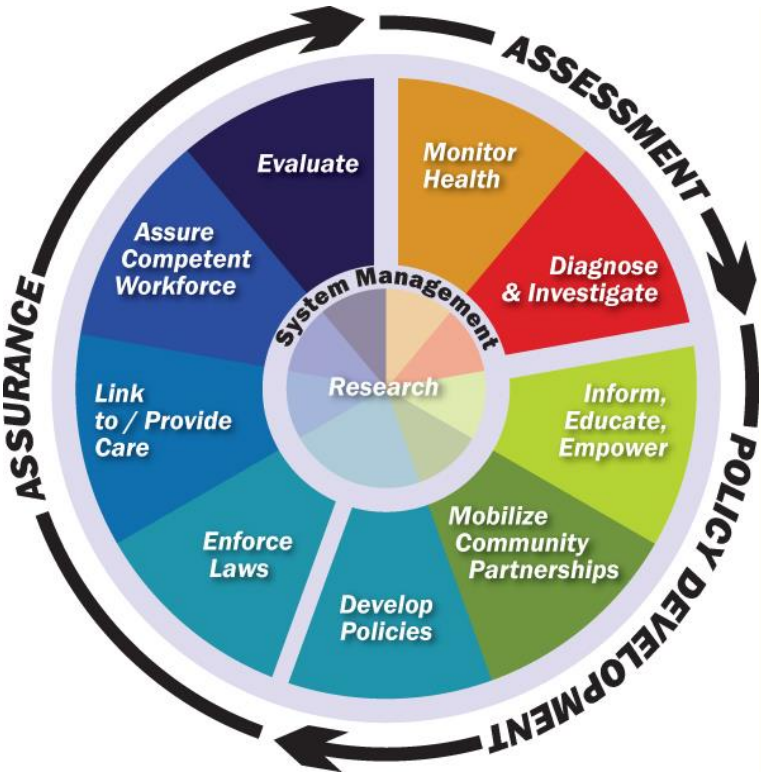

Figure 5.1 The 10 Essential Public Health Services. **Source:** CDC. Available at: https://www.cdc.gov/publichealthgateway/publichealthservices/essentialhealthservices.html

5.6.2 Gender health equity in EU

In June 2006, the Council of the EU adopted a statement on common values and principles in EU healthcare systems, listing the overarching values of universality, access to good-quality care, equity and solidarity (European Council 2006). 'Equity' in healthcare is defined as follows: "Equity relates to equal access according to need, regardless of ethnicity, gender, age, social status or ability to pay."

In 2006, in Council Conclusions on women's health, the Council invited the European Commission to:

- integrate gender aspects in health research
- support the exchange of information and experience on good practice in gender-sensitive health promotion and prevention
- assist Member States in developing effective strategies to reduce health inequalities with a gender dimension
- promote and strengthen the comparability and compatibility of gender-specific information on health across Member States and at EU level through the development of appropriate data
- present a second report on the state of women's health in the EU.

Women's life expectancy has been increasing in the EU-28 and exceeds that of men. In 2017, the average life expectancy at birth in the EU-28 was 83.5 years of age for women and 78.3 years for men (Eurostat, 2017). In all EU Member States – including Greece – life expectancy at birth is higher for women than for men, although the size of the gap varies noticeably (**Figure 5.2**) (Eurostat, 2017).

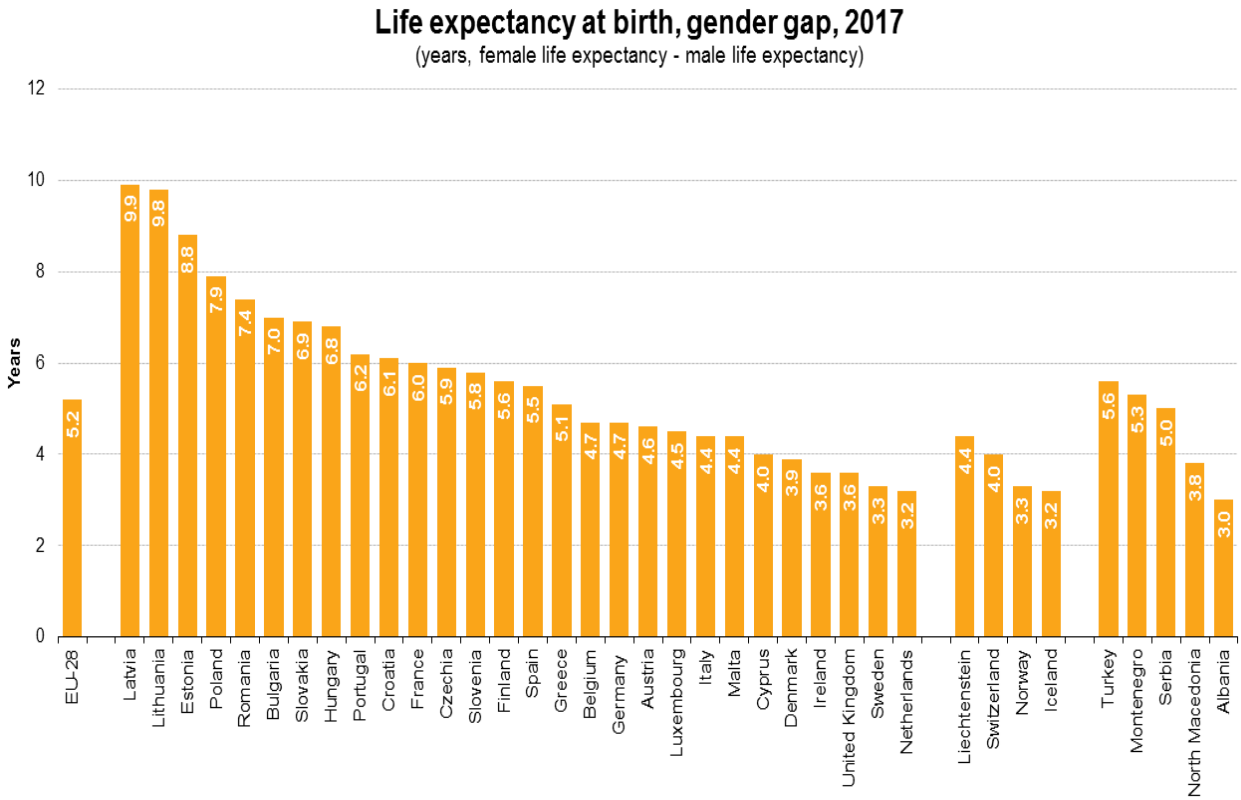

Figure 5.2 Life expectancy at birth, gender gap, 2017 (years, female life expectancy – male life expectancy). **Source:** Eurostat, 2017. Available at: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20190725-1#:~:text=Life%20expectancy%20at%20birth%20in%20the%20European%20Union%20(EU)%20was,of%20the%20gap%20varies%20noticeably.

Neveretheless, besides the longer life expectancy, women present increased disability and morbidity. For instance, in 2018 the number of healthy life years at birth was estimated at 64.2 years for women and 63.7 years for men in the EU, this represented approximately 76.7 % and 81.4 % of the total life expectancy for women and men (**Figure 5.3**) (Eurostat 2018). This correspond to 79% and 74% of total life expectancy for women and men respectively. What should be outlined here is that the gender gap was considerably smaller in terms of healthy life years than it was for overall life expectancy; thereby, women suffer from health problems at a later age yet for a longer time.

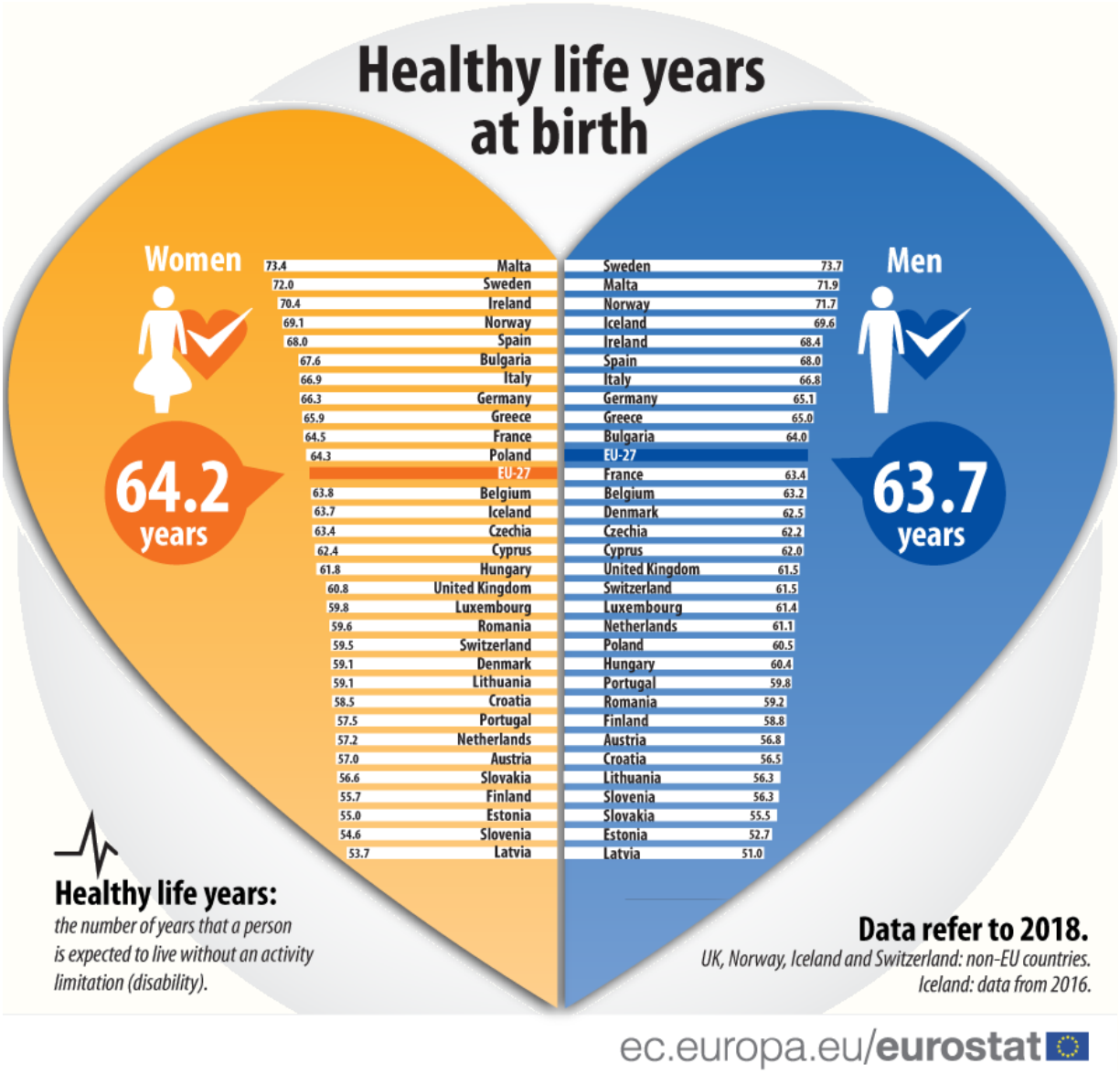

Figure 5.3 Healthy life years at birth, gender gap, 2018. **Source:** Eurostat, 2018. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php/Healthy_life_years_statistics#:~:text=years%20at%20birth-,In%202018%2C%20the%20number%20of%20healthy%20life%20years%20at%20birth,men%20in%20the%20EU%2D27.

5.6.3 Implications for future policies and practices in CVD spectrum to achieve gender equity

After the introduction of the United Nations Millennium Development Goals in 2000, there is an imperative focus of policy makers around the globe to improve women's sexual and reproductive health (WHO 2015a). International and especially national health plans lack in gender sensitivity (Briones-Vozmediano et al 2012). This phenomenon is even more apparent in case of CVDs. The lack of sex- and gender- sensitive studies in CVD spectrum possesses indicative defaults in the NCDs research field (Garcia et al 2016). Underrepresentation of women in studies needs being addressed while important efforts are demanded to effectively include sex/gender in health research and funding (Sharman et al 2012).

Too few women are aware of CVD. This had been firstly identified by the AHA in 1997 where it was found that only 1 out of 3 women correctly identified heart disease as their leading

cause of death (Mosca et al 2000). The aforementioned campaigns and other initiatives to educate the public and increase support for women's heart disease contributed to a significant amelioration in the level of women's awareness regarding their cardiac health which has doubled since 1997. Nevertheless, this remains substandard and has not significantly improved significantly since 2006, particularly in younger and ethnic minority women as well as those being at low socioeconomic status (Marcuccio et al 2003, Mosca et al 2013). Younger women and racial and ethnic minorities have lower rates of awareness and higher rates of CVD mortality and more risk factors (Mosca et al 2013). In particular, they are less aware of their risks, have delayed diagnosis, face inconsistent responses from the healthcare system, have underestimation of their disease severity, receive suboptimal treatment, and ultimately have worse outcomes. This is more evident in women aged 35 to 54 years, in those with lower levels of education, and among racial and ethnic minorities. Focusing on younger patients, prospective cohort studies such as GENESIS-PRAXY and Variation in Recovery: Role of Gender on Outcomes of Young AMI Patients (VIRGO), have revealed some sex differences in demographic, cardiovascular risk factors, symptoms, and treatment (Khan et al 2013, Lichtman et al 2014). Additionally, the social determinants of CVD in women have been highly discussed including health literacy, lower education achievements, low-wage jobs, higher rates of poverty, and more familial responsibilities, coupled with societal discriminatory norms and practices (Shaw et al 2017).

At the same time, physicians also appear to have gaps regarding women's cardiac health. In particular, physicians are more likely to assign a lower CVD risk category to female patients, as well as underestimate CVD risk in women (Mosca et al 2005). They are also less likely to refer women and ethnic minorities for diagnostic cardiac catheterization (Schulman et al 1999). In a 2012 online survey, only 1 out of 5 women reported that their physicians had ever discussed their risk for heart disease (Mosca et al 2013). Women often receive suboptimal CVD preventative care. A couple of years later, the Women's Heart Alliance survey selected 200 primary care providers and 100 cardiologists to determine their self-reported readiness to address CVD risk in women patients. Primary care providers reported CVD as a principle health concern in women yet less important than breast- and weight- related health. Moreover, most physicians, including 1 out of 2 cardiologists, reported suboptimal training in assessing CVD risk in women.

To optimize women's cardiac health, policy makers should recognize, promote and allocate resources to address sex- and gender- specific issues in the prevention, risk assessment, diagnosis, treatment and rehabilitation in CVDs. Policies should be accordingly designed after a cautious needs assessment process to evaluate the actual demands in nation or country level (Souliotis et al 2018, Souliotis 2015). Based on the discussed public health initiatives, it is suggested that priority setting for CVDs prevention and control include a successful combination

between policy makers/stakeholders, researchers and health care systems. Thereby, a multidimensional approach – as suggested in **Figure 5.4** – is demanded to support the generation of evidence-based, cost-effective and tailor-made decisions in policy making. Through this multidimensional approach specific goals should be set including, among others, the incorporation of sex- and gender- based guidelines in CVD prevention, management and rehabilitation, the provision of comprehensive patient-centered care customized to address cultural, ethnic, spiritual, and social determinants of the patient as well as the implementation of multidisciplinary healthcare teams for women incorporating clinicians who care for women to improve the quality and equitable healthcare gaps in women including family physicians, primary care physicians, obstetricians and gynaecologists, nurse practitioner, emergency department physicians, and nurses. What is more, women's education in terms of health literacy amelioration regarding CVD is equally important. Finally, community partnership and commitment to research with the focus oriented towards the most vulnerable subgroups (e.g., younger women, women at low socioeconomic status, ethnic minorities etc) should be an indispensable part of such approaches.

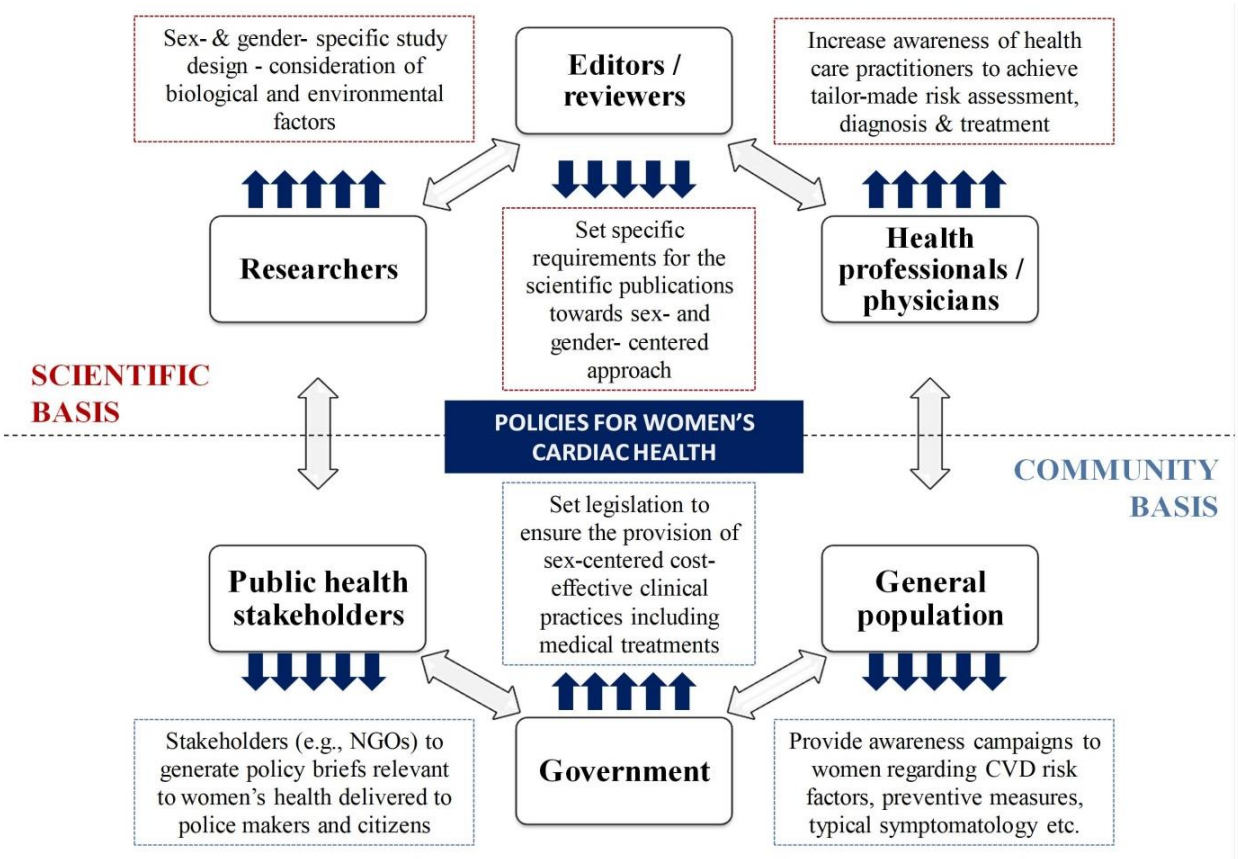

Figure 5.4 Policy making to address gender equity in cardiovascular disease (CVD) spectrum; the target audiences – **Abbreviations:** Non-Governmental Organizations (NGOs)

5.7 A nutrition-related, sex-oriented microsimulation scenario to reduce CVD burden

Current observations forecast a new epidemic within the young segment of the population as they age for both men and women (Timmis et al 2020). The third Sustainable Development Goal recognized the importance of CVD by targeting a one-third reduction in premature mortality due to NCDs (UN 2015a). To meet this target, countries have to contend with various barriers limiting their ability to improve health care. Estimations show that 80% of CVD is preventable earlier in life (Timmis et al 2020). This calls for cost-effective preventive strategies in first place. There is a longstanding recognition that diet plays a major role in the aetiology of many chronic diseases, thereby contributing to significant geographic variations in morbidity/mortality rates worldwide. It is estimated that among all behaviours, nutrition makes the largest contribution to CVD morbidity across Europe (Wilkins et al 2017). The relationship between nutrition and CVD has been extensively investigated on the basis of food groups such as fruits and vegetables as well as on the basis of dietary patterns. Mediterranean diet stands among the most discussed dietary patterns in CVD prevention mostly primary and recently secondary (Rees et al 2019). These findings have been widely used to inform guidelines. However, to inform and prioritize national public health policies, specific questions should be addressed, such as: "How much reduction – if any – in CVD cases would be expected from an increase in the level of adherence to a cardiac-friendly dietary pattern?" or "Which should be the dynamic of a dietary intervention to be recognised as an effective strategic plan for a country?". Microsimulation models are served as sophisticated tools well-suited to address such questions and guide evidence-based public health policy (Arnold et al 2019).

As mentioned above, the microsimulation modelling performed using the data from ATTICA and GREECS observational studies, confirmed the added value of Mediterranean diet in primary as well as secondary CVD prevention yet this time on a population basis. The present work suggested that increasing adherence to Mediterranean diet even in 10% of a population can significantly protect against CVD onset as well as from CVD recurrence and mortality; preventing from more than 851/100,000 new cardiac episodes in apparently healthy subjects, 482/100,000 recurrent cardiac episodes as well as 205/100,000 deaths in ACS patients. Subsequently, it was revealed that achieving a small percentage of the population to comply with this pattern was of added value for both men and women with women coming first in this rank. This observation is important from a public health perspective since it confirms the fact that women are more likely to adapt to healthier dietary habits compared to men who are more exposed to unhealthy lifestyle behaviours. What is more, this gender gap may be explained by another highlight of the present work related with the existence of different clusters – probably

sex-oriented – within one dietary pattern; a woman vs. a man with increased level of adherence to Mediterranean diet consumed more plant-based products without major reductions in the consumption of animal-based products. Thereby, these findings challenge also the orientation of dietary interventions giving higher weights to the plant-based part.

5.8 Limitations and Strengths

The main strength here that compensates the following limitations is that this in one of the very few works that evaluated the association of conventional and non-conventional/novel CVD risk factors providing sex- as well as CVD-prevention-stage specific remarks. Nevertheless, some limitations should be reported for better interpretation of the outcomes. Firstly, present findings should be considered with caution due to studies' observational nature. Secondly, it should be noted that only baseline measurements were taken into account for the research hypotheses raised here; hence misclassifications of transitions regarding anthropometric, clinical or lifestyle measurements cannot be precluded. Lastly, statistical power reduction due to sex-related stratified analysis should be considered during the interpretation of outcomes.

5.9 Conclusions

CVD still remains the largest single contributor to global disability and mortality with up to date evidence indicating abatement in the rate of the promising mortality decline observed the last four decades. Overall, the annual cost of CVD in Europe reaches the $210 while in United States the total direct medical costs between 2012 and 2030 are projected to increase from $396 to $918 billion. Much as the past two decades substantial efforts have been performed to ameliorate women's health on the whole, there is still much to be done. Giving a broader definition to women's health, primary and secondary prevention of CVDs has started to be prioritized and important initiatives have been launched towards this approach. To achieve this, global and most importantly national sectors should perform appropriate policy making; to support a sex- and gender- sensitive collection, usage and interpretation of health data and to enhance the level of awareness in citizens and health professionals. Considering the imperative need to maximize the cost-effectiveness of prevention and management strategies, health disparities have to be addressed by health practitioners and the case of women in CVD care remains an ongoing public health concern. In this context, "female" and "male" CVD pattern accompanied by the underlying metabolic and genetic mechanisms needs to be better clarified and presented in a coherent manner; so as to reduce the gender biases in cardiovascular care and to give the potential for more tailored made national health policies. Building upon this need the present work suggested sex-based specifications in relation to lifestyle and psychological patterns, revealed differences in the magnitude of the associations of conventional and non-conventional cardiometabolic risk

factors with long-term CVD onset or recurrence while suggested cardiometabolic paths specified to men and women to achieve earlier or more valid CVD risk assessment and prevention.

On the other side, the findings of the present work were based on two extended databases from Greece. Greece is a European country with limited initiatives on the gender equity in primary and secondary CVD prevention. To this issue, the National Action Plan on CVDs in Greece makes a short reference on "CVDs in women" highlighting only a couple of risk factors being more prevalent in women. Nevertheless, the incorporation of gender equity in the Strategic Plan for effective CVD prevention and management is missing (Hellenic Ministry of Health 2008). Thereby, the present work builds also upon the insufficient documentation of sex-specific conclusions in both primary and secondary prevention spectrum as well as the fact that men and women are equally treated against CVD – this time on a national basis – not only through highlighting sex-specific remarks regarding CVD risk factors but also through developing a nutrition-related, sex-oriented microsimulation scenario for primary and secondary prevention spectrum which could be taken into consideration in the development of effective strategic plans to decrease CVD burden in both men and women.

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Annex I

Scientific publications in peer-reviewed journals from the present dissertation

1. Kouvari M, Boutari C, Chrysohoou C, Fragkopoulou E, Antonopoulou S, Tousoulis D, Pitsavos C, Panagiotakos DBP, Mantzoros CS. Mediterranean diet is inversely associated with steatosis and fibrosis and decreases ten-year diabetes and cardiovascular risk in NAFLD subjects: results from ATTICA prospective cohort study. Clinical Nutrition. 2020. Proofs phase [indexed in PubMed, Scopus etc; IF=6.360].
2. Kouvari M, Souliotis K, Yannakoulia M, Panagiotakos DB. Cardiovascular diseases in women: policies and practices around the globe to achieve gender equity in cardiac health. Risk Management and Healthcare Policy. 2020. Proofs phase. [indexed in PubMed, Scopus etc; IF=2.429].
3. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou EN, Yannakoulia M, Tousoulis D, Pitsavos C; ATTICA study Investigators. Dairy products, surrogate markers and cardiovascular disease; a sex-specific analysis from the ATTICA prospective study. Nutrition, Metabolism and Cardiovascular Diseases. 2020. Proofs phase. [indexed in PubMed, Scopus etc; IF=3.318].
4. Kouvari M, Panagiotakos DB, Chrysohoou C, Yannakoulia M, Georgousopoulou EN, Tousoulis D, Pitsavos C; ATTICA study Investigators. Meat consumption, depressive symptomatology and cardiovascular disease incidence in apparently healthy men and women: highlights from the ATTICA cohort study (2002-2012). Nutr Neurosci. 2020 Apr 11:1-10. [indexed in Pubmed, Scopus etc; IF=3.950].
5. Kouvari M, Panagiotakos DB, Chrysohoou C, Yannakoulia M, Georgousopoulou EN, Tousoulis D, Pitsavos C; ATTICA study Investigators. Dietary vitamin D intake, cardiovascular disease and cardiometabolic risk factors: a sex-based analysis from the ATTICA cohort study [published online ahead of print, 2020 Apr 7]. J Hum Nutr Diet. 2020;10.1111/jhn.12748. [indexed in Pubmed, Scopus etc; IF=3.088].
6. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou EN, Tousoulis D, Pitsavos AC. Sex-Related Differences of the Effect of Lipoproteins and Apolipoproteins on 10-Year Cardiovascular Disease Risk; Insights from the ATTICA Study (2002-2012). Molecules. 2020;25(7):E1506. [indexed in Pubmed, Scopus etc; IF=3.060].
7. Kouvari M, Panagiotakos DB, Naumovski N, et al. Dietary anti-inflammatory index, metabolic syndrome and transition in metabolic status; a gender-specific analysis of ATTICA prospective study [published online ahead of print, 2020 Jan 28]. Diabetes Res Clin Pract. 2020;161:108031. [indexed in Pubmed, Scopus etc; IF=3.239].
8. Kouvari M, Panagiotakos DB, Chrysohoou C, Notara V, Georgousopoulou E,Tousoulis D, Pitsavos C; ATTICA & GREECS Studies Investigators. Sex-discrete roleof depressive symptomatology on 10-year first and recurrent cardiovasculardisease incidence: results from ATTICA and GREECS prospective studies. Hellenic J Cardiol. 2019;S1109-9666(19)30295-7. [indexed in PubMed, Scopus etc; IF=2.269].
9. Kouvari M, Panagiotakos DB, Chrysohoou C, Notara V, Georgousopoulou EN, Yannakoulia M, Tousoulis D, Pitsavos C; ATTICA and GREECS study Investigators. A sex-specific evaluation of predicted lean and fat mass composition and cardiovascular disease onset and progression: A combined analysis of the ATTICA and GREECS prospective epidemiological studies. Obes Res ClinPract. 2019 Sep - Oct;13(5):469-477. [indexed in PubMed, Scopus etc; IF=2.370].
10. Kouvari M, Panagiotakos DB. Vitamin D status, gender and cardiovascular diseases: a systematic review of prospective epidemiological studies. Expert Rev Cardiovasc Ther. 2019;17(7):545–555. [indexed in PubMed, Scopus etc; IF=2.000].
11. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou EN, Yannakoulia M,Tousoulis D, Pitsavos C. Lipoprotein (a) and 10-year Cardiovascular DiseaseIncidence in Apparently Healthy Individuals: A Sex-based Sensitivity Analysis from ATTICA Cohort Study. Angiology.2019;70(9):819–829. [indexed in PubMed, Scopus etc; IF=3.022].
12. Kouvari M, Panagiotakos DB. The role of lipoprotein (a) in primary and secondary cardiovascular disease prevention: a systematic review of epidemiological studies. Current Opinion in Cardiology. 2019;34:424-434[indexed in PubMed, Scopus etc; IF=2.006].
13. Kouvari M, Panagiotakos DB, Yannakoulia M, Georgousopoulou E, Critselis E, Chrysohoou C, Tousoulis D, Pitsavos C; ATTICA study Investigators. Transition from metabolically benign to metabolically unhealthy obesity and 10-year cardiovascular disease incidence: the ATTICA cohort study. Metabolism. 2019;93:18-24. [indexed in PubMed, Scopus etc; IF=5.963].
14. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou E, Notara V, Tousoulis D, Pitsavos C, The ATTICA GREECS Studies Investigators. Gender-specific, Lifestyle-related Factors and 10-year Cardiovascular Disease Risk; the ATTICA and GREECS Cohort Studies. Curr Vasc Pharmacol. 2019;17(4):401–410. [indexed in Pubmed, Scopus etc; IF=2.391].
15. Kouvari M,Yannakoulia M, Souliotis K, Panagiotakos DB. Challenges in sex- and gender- centered prevention and management of cardiovascular disease; implications of genetic, metabolic and environmental paths. Angiology. 2018; 69:843-853 [indexed in PubMed, Scopus etc; IF=3.085]. 2019 Angiology Top 10 Cited Article.

Scientific publications in peer-reviewed journals (under review)

1. Kouvari M, Tsiampalis T, Chrysohoou C, Georgousopoulou E, Notara V, Souliotis K, Psaltopoulou T, Pitsavos C, Panagiotakos DB. A Mediterranean diet-related microsimulation modelling approach in relation to cardiovascular disease burden: the ATTICA and GREECS epidemiological studies.
2. Kouvari M, Chrysohoou C, Georgousopoulou E, Fragkopoulou E, Pitsavos C, Panagiotakos DB, Mantzoros CS. Non-alcoholic fatty liver disease, metabolically healthy obesity and 10-year cardiovascular disease incidence: evidence from the ATTICA cohort study.
3. Katsiki N, Kouvari M, Panagiotakos DB, Borghic C, Papanikolaou A, Chrysohoou C, Mikhailidis D, Pitsavos C. The association between serum uric acid levels and 10-year cardiovascular disease incidence: results from the ATTICA prospective study.

Annex II

Scientific presentations in international congresses

1. Kouvari M, Panagiotakos DP, Chrysohoou C, Notara V, Yannakoulia M, Georgousopoulou E, Tousoulis D, Pitsavos C. The role of triglycerides-glucose index to predict 10-year first and recurrent cardiovascular disease events: a sex-based analysis from ATTICA and GREECS prospective studies. ESC Congress, on-line 29 August – 4 September 2020.
2. Kouvari M, Panagiotakos DP, Chrysohoou C, Yannakoulia M, Georgousopoulou E, Tousoulis D, Pitsavos C. A U-shape trend between total and red meat consumption and depressive symptomatology in apparently healthy women: highlights from the ATTICA prospective (2002-2012) study. ESC Congress, on-line 29 August – 4 September 2020.
3. Kouvari M, Panagiotakos DP, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. The gender-specific role of prediabetes on 10-year cardiovascular disease incidence: Highlights from the ATTICA prospective (2002-2012) study. ESC Congress, on-line 29 August – 4 September 2020. (*Advances In Science presentation*)
4. Kouvari M, Panagiotakos DP, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Healthful and unhealthful plant-based dietary patterns and their role on 10-year transition to metabolically unhealthy status in obese participants of the ATTICA prospective (2002-2012) study. ESC Congress, on-line 29 August – 4 September 2020. (*Best poster presentation*)
5. Kouvari M, Panagiotakos DP, Chrysohoou C, Boutari C, Georgousopoulou E, Tousoulis D, Pitsavos C, Mantzoros CM. Non-alcoholic fatty liver disease, Mediterranean diet and 10-year cardiovascular disease incidence: the mediating role of adiponectin. Highlights from the ATTICA prospective (2002-2012) study. ESC Congress, on-line 29 August – 4 September 2020.
6. Kouvari M, Panagiotakos DP, Chrysohoou C, Yannakoulia M, Georgousopoulou E, Tousoulis D, Pitsavos C. The role of dietary vitamin D intake on 10-year cardiovascular disease incidence, intermediate cardiometabolic risk factors and surrogate markers: highlights from ATTICA prospective study. ESC Congress, on-line 29 August – 4 September 2020.
7. Kouvari M, Panagiotakos DP, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Fermented dairy products, gender and 10-year cardiovascular disease onset: the mediating effect of insulin resistance and hepatic steatosis. Highlights from the ATTICA prospective (2002-2012) study. ACC Congress Chicago 28-30 March 2020.
8. Kouvari M, Panagiotakos DP, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Visceral adiposity index, non-alcoholic fatty liver disease and 10-year cardiovascular disease incidence: a gender-based analysis from the ATTICA prospective (2002-2012) study. ACC CongressChicago 28-30 March 2020.
9. Panagiotakos DP, Kouvari M, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. The association between healthful and unhealthful plant-based dietary patterns and 10-year cardiovascular disease incidence in apparently healthy men and women; highlights from the ATTICA prospective (2002-2012) study. ACC CongressChicago 28-30 March 2020.
10. Kouvari M,Panagiotakos DP, Yannakoulia M, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Normal weight central obesity and 10-year cardiovascular disease onset in apparently healthy males and females: the interacting effect of sex. ESC Congress, Paris 31 August-04 September 2019.
11. Kouvari M,Panagiotakos DP, Chrysohoou C, Yannakoulia M, Georgousopoulou E, Tousoulis D, Pitsavos C. The effect of lipoprotein (a) on primary prevention of cardiovascular disease and the interaction with conventional lipid markers: a sex-based sensitivity analysis from a 10-year cohort study. ESC Congress, Paris 31 August-04 September 2019.
12. Kouvari M,Panagiotakos DP, Yannakoulia M, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Stable and temporal metabolically benign obesity and cardiovascular disease onset in males and females: the missing link with adiponectin ESC Congress, Paris 31 August-04 September 2019.
13. Kouvari M,Panagiotakos DP, Chrysohoou C, Notara V, Georgousopoulou E, Tousoulis D, Pitsavos C. Depressive symptomatology, sex and 10-year cardiovascular disease; revealing the mediation ranking of lifestyle, sociodemographic and clinical factors in primary and secondary prevention spectrum.ESC Congress 31 August-04 September 2019.
14. Kouvari M,Chrysohoou C, Dilaveris P, Georgiopoulos G, Magkas N, Aggelopoulos P, Panagiotakos DP, Tousoulis D. The sex-based effect of skeletal muscle mass on 10-year cardiovascular disease prognosis of patients with acute coronary syndrome: the mediating effect of systemic inflammation. ESC Congress, Paris 31 August-04 September 2019.
15. Kouvari M,Panagiotakos DP,Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Metabolically healthy obesity and 10-year cardiovascular disease risk in apparently healthy men and women: the interacting effect with gender. Europrevent Lisbon 11-13 April 2019.
16. Kouvari M,Panagiotakos DP, Chrysohoou C, Georgousopoulou E, Notara V, Tousoulis D, Pitsavos C. The gender-related discrete role of depressive symptomatology on 10-year first and recurrent cardiovascular disease incidence. Europrevent Lisbon 11-13 April 2019.
17. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Gender-specific effect of Mediterranean diet on cardiovascular disease risk; the clustering of MedDietScore components in apparently healthy males and females: 10-year follow-up of the ATTICA study. ESC Congress, Munich 25 - 29 August 2018.
18. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Gender-specific risk stratification of lipid markers on the 10-year cardiovascular disease: the ATTICA study. ESC Congress, Munich 25 - 29 August 2018.

19. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou E, Tousoulis D, Pitsavos C. Metabolic syndrome is an independent predictor of 10-year cardiovascular disease risk in apparently healthy males; the ATTICA study. ESC Congress, Munich 25 - 29 August 2018.
20. Kouvari M, Panagiotakos DB, Chrysohoou C, Georgousopoulou E, Notara V, Tousoulis D, Pitsavos C. Gender-specific hierarchical analysis of behavioural factors on the 10-year primary and secondary prevention of cardiovascular disease: a re-analysis of ATTICA & GREECS observational studies. ESC Congress, Munich 25 - 29 August 2018.
21. Kouvari M, D B. Panagiotakos, V. Notara, Y. Kogias, G. Papanagnou, S. Zombolos, Y. Mantas, C. Pitsavos. Sex differences in the role of Mediterranean diet in secondary prevention of acute coronary syndrome, according to the individual cardiovascular disease history: the Greecs prospective study. EuroPrevent 2018; ESC, Slovenia, 19-21 April, 2018.
22. Kouvari M, D B. Panagiotakos, V. Notara, Y. Kogias, P. Stravopodis, G. Papanagnou, S. Zombolos, Y. Mantas, C. Pitsavos.Sex-specific effect of diabetes mellitus in the secondary prevention of acute coronary syndrome: the Greecs prospective study. EuroPrevent 2018; ESC, Slovenia, 19-21 April, 2018.

Scientific presentations in national congresses

1. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Λιπιδαιμικό προφίλ, φύλο και 10ετής καρδιαγγειακός κίνδυνος: δεδομένα από την επιδημιολογική προοπτική μελέτη ΑΤΤΙΚΗ. 41ο Πανελλήνιο Καρδιολογικό Συνέδριο, Αθήνα, 22-24 Οκτωβρίου 2020.
2. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γιαννακούλια Μ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Μεταβολικά υγιής παχυσαρκία και πρωτογενής εμφάνιση καρδιαγγειακής νόσου σε άντρες και γυναίκες: η διαμεσολαβητική δράση της αδιπονεκτίνης. Επιδημιολογική μελέτη ΑΤΤΙΚΗ.8^{ο} Συνέδριο Ομάδων Εργασίας Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα, 29-30 Νοεμβρίου 2019 (*η ανακοίνωση έλαβε το 3^{ο} Βραβείο*).
3. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γιαννακούλια Μ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ.Λιπώδης και άλιπη μάζα σώματος σε σχέση με την εμφάνιση καρδιαγγειακής νόσου σε υγιείς άντρες και γυναίκες: διαφορές ως προς το φύλο. Επιδημιολογική μελέτη ΑΤΤΙΚΗ. 8^{ο} Συνέδριο Ομάδων Εργασίας Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα, 29-30 Νοεμβρίου 2019.
4. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γιαννακούλια Μ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ· Κεντρικού τύπου παχυσαρκία με φυσιολογικό δείκτη μάζα σώματος και 10ετής εμφάνιση καρδιαγγειακής νόσου: διαστρωματοποιημένη ανάλυση ως προς το φύλο. Επιδημιολογική μελέτη ΑΤΤΙΚΗ. 4^{0ο} Πανελλήνιο Καρδιολογικό Συνέδριο, Ιωάννινα, 17-19 Οκτωβρίου 2019.
5. Κούβαρη Μ, ΠαναγιωτάκοςΔ, Χρυσοχόου Χ, Γιαννακούλια Μ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ.Ο ρόλος της λιποπρωτεΐνης α στην πρωτογενή εμφάνιση καρδιαγγειακής νόσου: η αλληλεπίδραση με το φύλο. Επιδημιολογική μελέτη ΑΤΤΙΚΗ. 40^{ο} Πανελλήνιο Καρδιολογικό Συνέδριο, Ιωάννινα, 17-19 Οκτωβρίου 2019.
6. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Νοταρά Β, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Ιεραρχική ανάλυση παραγόντων του λιπιδαιμικού προφίλ ως προς την πρωτογενή εμφάνιση καρδιαγγειακής νόσου και η διαμεσολαβητική δράση του φύλου. Δεδομένα από την επιδημιολογική μελέτη ΑΤΤΙΚΗ. 8ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα 29 Νοεμβρίου-01 Δεκεμβρίου 2018.
7. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Νοταρά Β, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Ο ρόλος συμπεριφοριστικών παραγόντων στην πρωτογενή και δευτερογενή εμφάνιση καρδιαγγειακής νόσου: διαφορές ανάλογα με το φύλο από τις επιδημιολογικές μελέτες ΑΤΤΙΚΗ και GREECS. 8ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα 29 Νοεμβρίου-01 Δεκεμβρίου 2018.
8. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Μεσογειακό διατροφικό πρότυπο, φύλο και 10ετης καρδιαγγειακός κίνδυνος: συστατική ανάλυση από την επιδημιολογική μελέτη ΑΤΤΙΚΗ. 8ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα 29 Νοεμβρίου-01 Δεκεμβρίου 2018.
9. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Η προβλεπτική ικανότητα του μεταβολικού συνδρόμου ως προς την πρωτογενή εμφάνιση καρδιαγγειακής νόσου: η αλληλεπίδραση με το φύλο: Επιδημιολογική μελέτη ΑΤΤΙΚΗ. 8ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα 29 Νοεμβρίου-01 Δεκεμβρίου 2018.
10. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Νοταρά Β, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Καταθλιπτική συμπτωματολογία και φύλο στην πρωτογενή και δευτερογενή εμφάνιση καρδιαγγειακής νόσου: επιδημιολογικές μελέτες ΑΤΤΙΚΗ και GREECS. 8ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Αθηροσκλήρωσης, Αθήνα 29 Νοεμβρίου-01 Δεκεμβρίου 2018.
11. Κούβαρη Μ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Νοταρά Β, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Ιεραρχική ανάλυση συμπεριφοριστικών παραγόντων κινδύνου ως προς τον 10ετη κίνδυνο πρωτογενούς και δευτερογενούς εμφάνισης καρδιαγγειακής νόσου διακριτά σε άνδρες και γυναίκες: δεδομένα από τις επιδημιολογικές μελέτες ΑΤΤΙΚΗ και GREECS. 39^{ο} Πανελλήνιο Καρδιολογικό Συνέδριο, Αθήνα 18-20 Οκτωβρίου 2018.
12. ΚούβαρηΜ, Παναγιωτάκος Δ, Χρυσοχόου Χ, Γεωργουσοπούλου Ε, Τούσουλης Δ, Πίτσαβος Χ. Ο ρόλος του μεταβολικού συνδρόμου με τη 10-ετήεμφάνιση καρδιαγγειακής νόσου σε υγιείς άντρες και γυναίκες: ανεξάρτητος παράγοντας κινδύνου

και για τα δύο φύλα; Επιδημιολογική μελέτη ΑΤΤΙΚΗ. 39ο Πανελλήνιο Καρδιολογικό Συνέδριο, Αθήνα 18-20 Οκτωβρίου 2018.

13. Κούβαρη Μ, Παναγιωτάκος Δ, Νοταρά Β, Κόγιας Γ, Στραβοπόδης Π, Αντωνούλας Α, Παπανάγνου Γ, Ζόμπολος Σ, Μαντάς Γ, Πίτσαβος Χ. Μεσογειακή διατροφή και πρόγνωση Οξέος Στεφανιαίου Συνδρόμου: η ανεξάρτητη προστατευτική της δράση και η αλληλεπίδραση με κλινικούς/συμπεριφοριστικούς παράγοντες, ανάλογα με το φύλο. Επιδημιολογική μελέτη GREECS. 7ο Συμπόσιο Ομάδων Εργασίας της Ελληνικής Εταιρίας Αθηροσκλήρωσης, Αθήνα 1-2 Δεκεμβρίου 2017. (η εργασία έλαβε το 3ο Βραβείο).
14. Κούβαρη Μ, Παναγιωτάκος Δ, Νοταρά Β, Κόγιας Γ, Στραβοπόδης Π, Αντωνούλας Α, Παπανάγνου Γ, Ζόμπολος Σ, Μαντάς Γ, Πίτσαβος Χ. Ο ρόλος του σακχαρώδους διαβήτη στη δευτερογενή πρόληψη οξέος στεφανιαίου συνδρόμου: διαφορές ανάλογα με το φύλο. Επιδημιολογική μελέτη GREECS. 7ο Συμπόσιο Ομάδων Εργασίας της Ελληνικής Εταιρίας Αθηροσκλήρωσης, Αθήνα 1-2 Δεκεμβρίου 2017.
15. Κούβαρη Μ, Παναγιωτάκος Δ, Νοταρά Β, Κόγιας Γ, Στραβοπόδης Π, Αντωνούλας Α, Παπανάγνου Γ, Ζόμπολος Σ, Μαντάς Γ, Πίτσαβος Χ. Κλινικοί και συμπεριφοριστικοί παράγοντες ως διαμεσολαβητές της σχέσης του δείκτη μάζας σώματος με τη θνησιμότητα από νέο καρδιαγγειακό επεισόδιο στη 10ετία, σε γυναίκες με οξύ στεφανιαίο σύνδρομο: επιδημιολογική μελέτη GREECS. 38ο Πανελλήνιο Επετειακό Συνέδριο της Ελληνικής Καρδιολογικής Εταιρείας, Αθήνα 19-21 Οκτωβρίου 2017.
16. Κούβαρη Μ, Παναγιωτάκος Δ, Νοταρά Β, Κόγιας Γ, Στραβοπόδης Π, Αντωνούλας Α, Παπανάγνου Γ, Ζόμπολος Σ, Μαντάς Γ, Πίτσαβος Χ. Η καταθλιπτική συμπτωματολογία ως παράγοντας κινδύνου στη 10ετή πρόγνωση οξέος στεφανιαίου συνδρόμου: η αλληλεπίδραση με το φύλο. Επιδημιολογική μελέτη GREECS. 38ο Πανελλήνιο Επετειακό Συνέδριο της Ελληνικής Καρδιολογικής Εταιρείας, Αθήνα 19-21 Οκτωβρίου 2017.
17. Κούβαρη Μ, Παναγιωτάκος Δ, Νοταρά Β, Κόγιας Γ, Στραβοπόδης Π, Αντωνούλας Α, Παπανάγνου Γ, Ζόμπολος Σ, Μαντάς Γ, Πίτσαβος Χ. Ιεραρχική ανάλυση παραγόντων κινδύνου στη δευτερογενή πρόληψη οξέος στεφανιαίου συνδρόμου, σε γυναίκες, ανάλογα με την ηλικία: επιδημιολογική μελέτη GREECS 38ο Πανελλήνιο Επετειακό Συνέδριο της Ελληνικής Καρδιολογικής Εταιρείας, Αθήνα 19-21 Οκτωβρίου 2017.