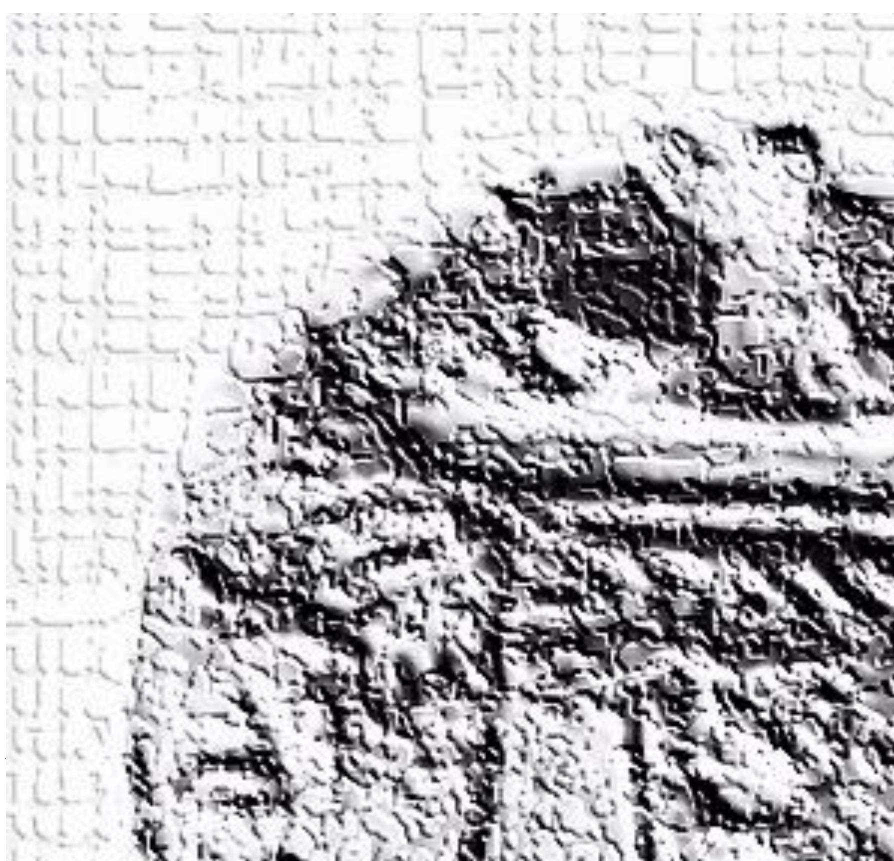
HAROKOPIO UNIVERSITY

ATHENS

ASSESSMENT OF BEHAVIOURAL, ANTHROPOMETRICAL AND BIOCHEMICAL INDICES OF HEALTH STATUS IN PRIMARY SCHOOL CHILDREN FOR THE DEVELOPMENT OF NUTRITION COUNSELING RECOMMENDATIONS.

GEORGE B MOSCHONIS



HAROKOPIO UNIVERSITY
DEPARTMENT OF NUTRITION AND DIETETICS
70 EL. VENIZELOU, AVE. 176 71, ATHENS, GREECE

Assessment of behavioural, anthropometrical and biochemical indices of health status in primary school children for the design and implementation of appropriate nutrition counselling programs.

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ΧΑΡΟΚΟΠΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ
ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΔΙΑΙΤΟΛΟΓΙΑΣ ΚΑΙ ΔΙΑΤΡΟΦΗΣ
ΕΛ. ΒΕΝΙΖΕΛΟΥ 70, 176 71, ΚΑΛΛΙΘΕΑ, ΑΘΗΝΑ

Αξιολόγηση συμπεριφοριστικών, ανθρωπομετρικών και βιοχημικών δεικτών υγείας σε μαθητές πρωτοβάθμιας εκπαίδευσης για τη διαμόρφωση οδηγιών διατροφικής αγωγής

ΓΕΩΡΓΙΟΣ Β ΜΟΣΧΩΝΗΣ

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Abstract

The assessment of anthropometrical, biochemical and clinical indices of health status in children, as well as of associated diet and physical activity behaviours is the first and most important step in the development of appropriate nutrition counselling and health education programs, which can ensure health and normal growth in children.

The objectives of the current PhD Thesis are a) to record a wide range of indices for the assessment of children's health status, b) to record and assess behaviours related to children's health status, c) to identify those factors that determine these behaviours and consequently health status in children, d) to develop easy to use tools that will assist health professionals in designing and implementing effective nutrition counselling and health education programs tailored to the specific needs of individuals or population groups at risk.

To investigate these research hypotheses, we conducted measurements to collect a wide range of data on anthropometrical, biochemical and behavioural indices of health status on a representative sample of 2655 primary schoolchildren, attending schools in four counties of Greece (i.e. Attica, Aitoloakarnania, Thessaloniki and Iraklion-Crete) (the Healthy Growth study).

According to the findings of the present study the prevalence of overweight and obesity was found to be 30.9% and 11.4%, respectively, with higher rates observed for boys compared to girls (13.3% versus 9.6%, $P<0.05$). Moreover, the prevalence of insulin resistance (IR) (HOMA-IR<3.16) was found to be 28.4%, that of dyslipidaemias was found to range from 3.9% (HDL-cholesterol <35mg/dL) to 13.3% (Total cholesterol > 200 mg/dL), while that of iron deficiency (ID) (Transferrin Saturation (TS) < 16%) and ID anaemia (TS<16% and Hgb<12 g/dL) was found to be 15.3% and 2.6%, respectively.

One interesting finding of the present was the strong association of obesity, not only with several cardiometabolic risk indices, such as dyslipidaemias, IR and hypertension, but also with indices of nutritional insufficiencies, such as ID, indicating obesity's central role in children's "poor" health status. More specifically, approximately one third of obese boys and girls (28.6% and 28.9%, respectively) were found to be iron deficient, with these prevalence rates being statistically significantly higher compared to the prevalence of ID in their normal-weight counterparts (15.5% and 14.3%, respectively). The low-grade chronic inflammation induced by excessive adiposity stimulates production of several iron-binding molecules, such hepcidin, which can ultimately lead to low serum iron levels in obese children.

Using Principal Component Analysis we identified certain behavioural indices that were combined into certain lifestyle patterns and were found to be significantly associated with obesity and dyslipidaemias in children. More specifically, a lifestyle pattern comprising higher daily intake of dairy products with consumption of an adequate breakfast; a second pattern characterized by increased consumption of high fibre foods; and a third pattern combining higher physical activity levels with frequent meals during the day were all found to be significantly

associated with a lower likelihood of obesity and increased fat mass levels in children. The latter lifestyle pattern and precisely engagement in more than 45 minutes of moderate-to-vigorous physical activity daily combined with at least five meals per day was also found to significantly decrease the odds of hypercholesterolemia in children.

The association between these lifestyle patterns and health status indices in children is very much determined by several social environmental factors. In this context the present Thesis showed that lower annual family income and grandmother as the child's primary caregiver were the most important socio-economic indices that were found to be related to an increased likelihood of obesity and IR in children. Furthermore, parental underestimation of overweight and obese children's weight status was also found to be another social environmental factor that was found to be related to an increased likelihood of childhood obesity.

Last but not least, via the examination of a wide range of perinatal factors the current PhD Thesis showed that small size of infants at birth was the only perinatal factor that was found to significantly increase the risk of IR in late childhood. Furthermore, increased maternal pre-pregnancy body weight, maternal smoking during pregnancy, rapid growth velocity during infancy and late introduction of solid food at weaning were all found to significantly increase the likelihood of obesity in children.

The findings of the current PhD Thesis were combined into the development of an index that can be used to holistically assess children's diet and lifestyle (Healthy Lifestyle-Diet Index). Higher scores of this index (indicative of a healthier diet and lifestyle) were found to be associated with lower odds of obesity and IR in children.

In conclusion, the current PhD thesis confirmed the high prevalence of overweight and obesity consistently reported for children in Greece, as well as of obesity-related metabolic complications (IR, dyslipidaemias and hypertension) that also include nutritional insufficiencies, such as ID. Furthermore, we identified certain lifestyle patterns as well as the possible determinants of the behaviours comprising these patterns, such as parental misperception of their children's weight status. All these findings were combined into the development of an index, which can be used to holistically assess children's health, diet and lifestyle patterns. This Healthy Lifestyle-Diet index as well as other similar tools can facilitate the development of effective public health recommendations, which can be personalised at an individual level (i.e. according to an individual's behavioural, anthropometric, biochemical, clinical status profile) or tailored to the specific needs of a population group at risk for obesity and related metabolic complications.

Περίληψη

Η καταγραφή και αξιολόγηση ανθρωπομετρικών, βιοχημικών και κλινικών δεικτών υγείας στα παιδιά καθώς και των σχετιζόμενων συμπεριφορών αποτελεί το πρωταρχικό και σημαντικότερο βήμα στη διαμόρφωση αποτελεσματικών συστάσεων διατροφικής αγωγής και προγραμμάτων αγωγής υγείας γενικότερα, που μπορούν να διασφαλίσουν την υγεία και τη φυσιολογική ανάπτυξη. Σκοποί της παρούσας διδακτορικής διατριβής αποτελούν α) η καταγραφή και η αξιολόγηση ενός μεγάλου αριθμού δεικτών υγείας σε μαθητές δημοτικού στην Ελλάδα, β) η καταγραφή και αξιολόγηση των συμπεριφορών που σχετίζονται με τους δείκτες υγείας, γ) η αναγνώριση των παραγόντων που καθορίζουν τις συμπεριφορές και κατ' επέκταση τους δείκτες υγείας και δ) η δημιουργία εύχρηστων εργαλείων για επιστήμονες υγείας που θα τους βοηθήσουν στην ανάπτυξη και εφαρμογή προγραμμάτων διατροφικής αγωγής και αγωγής υγείας προσαρμοσμένων τόσο σε επίπεδο ατόμου όσο και πληθυσμών.

Για τη διερεύνηση των παραπάνω ερευνητικών υποθέσεων πραγματοποιήθηκαν μετρήσεις για τη συλλογή ενός μεγάλου αριθμού δεδομένων σε ανθρωπομετρικούς, βιοχημικούς και συμπεριφοριστικούς δείκτες υγείας από ένα αντιπροσωπευτικό δείγμα 2655 παιδιών ηλικίας 9-13 ετών, που φοιτούσαν σε σχολεία τεσσάρων νομών της ελληνικής επικράτειας (Αττική, Αιτωλοακαρνανία, Θεσσαλονίκη και Ηράκλειο-Κρήτης) (Μελέτη Healthy Growth).

Σύμφωνα με τα ευρήματα της παρούσας διδακτορικής διατριβής ο συνολικός επιπολασμός του υπέρβαρου και της παχυσαρκίας βρέθηκε να είναι 30,9% και 11,4%, αντίστοιχα, με υψηλότερα ποσοστά παχυσαρκίας στα αγόρια σε σύγκριση με τα κορίτσια (13,3% έναντι 9,6%, $P<0.05$). Επιπρόσθετα, ο συνολικός επιπολασμός της ινσουλινοαντίστασης (HOMA-IR<3,16) βρέθηκε να είναι 28,4%, των δυσλιπιδαιμιών να κυμαίνεται από 3,9% (HDL-χοληστερόλη<35mg/dL) έως 13.3% (Ολική Χοληστερόλη > 200 mg/dL), ενώ της ανεπάρκειας σιδήρου (Κορεσμός Τρανσφερίνης (TS) < 16%) και της σιδηροπενικής αναιμίας (TS<16% και Hgb<12 g/dL) να είναι 15,3% και 2,6%, αντίστοιχα. Ένα αρκετά ενδιαφέρον εύρημα της παρούσας μελέτης ήταν το ότι η παχυσαρκία βρέθηκε να σχετίζεται ισχυρά όχι μόνο με δείκτες καρδιομεταβολικού κινδύνου, όπως οι δυσλιπιδαιμίες, η ινσουλινοαντίσταση και η υπέρταση, αλλά και με δείκτες διατροφικών ανεπαρκειών, όπως η ανεπάρκεια σιδήρου, υποδεικνύοντας έτσι τον κεντρικό ρόλο της παχυσαρκίας στη διαμόρφωση μιας γενικότερα «φτωχής» κατάστασης υγείας.

Με τη χρήση της ανάλυσης σε κύριες συνιστώσες αναγνωρίστηκαν συγκεκριμένοι συμπεριφοριστικοί δείκτες που συνδυάστηκαν για πρώτη φορά σε πρότυπα τρόπου ζωής, τα οποία βρέθηκαν να σχετίζονται σημαντικά τόσο με την παχυσαρκία όσο και με τις δυσλιπιδαιμίες στα παιδιά. Συγκεκριμένα, το πρότυπο τρόπου ζωής που συνδύαζε την αυξημένη κατανάλωση γαλακτοκομικών στη διάρκεια της ημέρας με την κατανάλωση ενός πλήρους πρωινού γεύματος; το πρότυπο που χαρακτηριζόταν από την αυξημένη κατανάλωση τροφίμων πλούσιων σε φυτικές ίνες, όπως φρούτα, λαχανικά και δημητριακά ολικής άλεσης; και το πρότυπο που συνδύαζε αυξημένα επίπεδα φυσικής δραστηριότητας με συχνότερα γεύματα στη

διάρκεια της ημέρας βρέθηκαν να σχετίζονται με μειωμένη πιθανότητα εμφάνισης παχυσαρκίας και αυξημένων επιπέδων λιπώδους μάζας σώματος στα παιδιά. Το τελευταίο πρότυπο μάλιστα και συγκεκριμένα η μέτριας έως υψηλής έντασης φυσική δραστηριότητα για διάστημα μεγαλύτερο από 45 λεπτά σε συνδυασμό με πέντε γεύματα ημερησίως βρέθηκαν να σχετίζονται σημαντικά με μειωμένη πιθανότητα εμφάνισης δυσλιπιδαιμιών στα παιδιά.

Η επίδραση των παραπάνω συμπεριφορών και των προτύπων του τρόπου ζωής σε δείκτες υγείας στα παιδιά βρέθηκε να καθορίζεται σε σημαντικό βαθμό από παράγοντες του κοινωνικού τους περιβάλλοντος. Στο πλαίσιο αυτό, το χαμηλότερο οικογενειακό εισόδημα και η γιαγιά ως η κύρια υπεύθυνη για την ανατροφή των παιδιών βρέθηκαν να είναι οι σημαντικότεροι κοινωνικο-οικονομικοί παράγοντες που σχετίζονταν με αυξημένη πιθανότητα εμφάνισης παχυσαρκίας και ινσουλινοαντίστασης στα παιδιά. Επιπλέον, η υποεκτίμηση του βάρους των υπέρβαρων και παχύσαρκων παιδιών από τους γονείς τους βρέθηκε να είναι σημαντικά υψηλότερη για τα υπέρβαρα και παχύσαρκα παιδιά.

Τα παραπάνω ευρήματα συνδυάστηκαν στη δημιουργία ενός δείκτη αξιολόγησης της υγείας, της διατροφής και του τρόπου ζωής των παιδιών γενικότερα (Healthy Lifestyle-Diet Index), η υψηλότερη βαθμολογία του οποίου (ενδεικτική ενός πιο υγιεινού τρόπου ζωής) βρέθηκε να σχετίζεται με μικρότερη πιθανότητα εμφάνισης παχυσαρκίας αλλά και ινσουλινοαντίστασης στα παιδιά.

Συμπερασματικά, η παρούσα διδακτορική διατριβή επιβεβαίωσε τα πολύ υψηλά ποσοστά παχυσαρκίας σε παιδιά ηλικίας 9-13 ετών στην Ελλάδα, καθώς και των συνοδών μεταβολικών της διαταραχών (ινσουλινοαντίσταση, δυσλιπιδαιμίες, υπέρταση), συμπεριλαμβανομένων διατροφικών ανεπαρκειών, όπως του σιδήρου. Επιπλέον, αναγνώρισε συγκεκριμένα πρότυπα τρόπου ζωής και παραγόντων που καθορίζουν τις συμπεριφορές που τα συνιστούν, όπως οι λανθασμένες αντιλήψεις των γονέων για το βάρος του παιδιού τους. Τα παραπάνω συνδυάστηκαν στο σχεδιασμό ενός δείκτη αξιολόγησης της υγείας, της διατροφής και του τρόπου ζωής των παιδιών. Ο συγκεκριμένος δείκτης καθώς και άλλα παρόμοια εργαλεία μπορούν να αποτελέσουν σημαντικό εφόδιο στο σχεδιασμό αποτελεσματικών προγραμμάτων πρόληψης της παιδικής παχυσαρκίας, καθώς και των συνοδών μεταβολικών της διαταραχών, μέσω της διαμόρφωσης συστάσεων διατροφικής αγωγής ειδικά εστιασμένων στις ανάγκες και στα χαρακτηριστικά των ατόμων ή των πληθυσμών.

If we knew what it was we were doing, it would not be called research, would it?

Albert Einstein

Supervisor:

Dr. Yannis Manios (Harokopio University)

Supervisory board:

Dr. Yannis Manios (Harokopio University)

Prof. Dr. George P. Chrousos (School of Medicine, Athens, University of Athens)

Prof. Dr. Christos Lionis (School of Medicine, Heraklion, University of Crete)

Prof. Dr. Antonis Zampelas (Department of Food Science and Technology, Agricultural University of Athens)

Dr. Demosthenes B. Panagiotakos (Harokopio University)

Dr. Meropi D. Kontogianni (Harokopio University)

Dr. Andriana C. Kaliora (Harokopio University)

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List of Papers

The current PhD thesis is supported by the following papers, which are referred to in the text by their Roman numerals

I. **Moschonis G**, Tanagra S, Vandorou A, Kyriakou AE, Dede V, Siatitsa PE, Koumpitski A, Androutsos O, Grammatikaki E, Kantilafti M, Naoumi A, Farmaki AE, Siopi A, Papadopoulou EZ, Voutsadaki E, Chlouveraki F, Maragkopoulou K, Argyri E, Giannopoulou A, Manios Y. Social, economic and demographic correlates of overweight and obesity in primary-school children: preliminary data from the Healthy Growth Study. Public Health Nutrition. 2010;13(10A):1693-700.

II. **Moschonis G**, Mougios V, Papandreou C, Malandraki E, Lionis C, Chrousos GP, Manios Y. Better Cardiometabolic Risk Profile in "Leaner and Less Fit" than "Heavier and More Fit" Children: The Healthy Growth Study. Nutrition Metabolism and Cardiovascular Disease. 2013 *(In press).*

III. **Moschonis G**, Chrousos GP, Lionis C, Mougios V, Manios Y. Association of total body and visceral fat mass with iron deficiency in preadolescents: the Healthy Growth Study. Br J Nutr. 2012; 108(4):710-9.

IV. Manios Y, **Moschonis G**, Chrousos GP, Lionis C, Mougios V, Kantilafti M, Tzotzola V, Skenderi KP, Petridou A, Tsalis G, Sakellaropoulou A, Skouli G, Katsarou C. The double burden of obesity and iron deficiency on children and adolescents in Greece: the Healthy Growth Study. Journal of Human Nutrition and Dietetics: the official journal of the British Dietetic Association. 2012. Epub 2013/01/03.

V. **Moschonis G**, Mavrogianni C, Karatzi K, Iatridi V, Chrousos GP, Lionis C, Manios Y. Increased physical activity combined with more eating occasions is beneficial against dyslipidemias in children. The Healthy Growth Study. Eur J Nutr. 2012.

VI. **Moschonis G**, Kaliora AC, Costarelli V, Papandreou C, Koutoukidis D, Lionis C, Chrousos GP and Manios Y on behalf of the "Healthy Growth Study" group. Identification of Lifestyle Patterns Associated with Obesity and Fat Mass in Children. The Healthy Growth Study. Public Health Nutrition. 2013: p. 1-11. (Ahead of print).

VII. **Moschonis G**, Iatridi V, Mavrogianni C, Siatitsa PE, Kyriakou AE, Dede V, Skouli G, Sakellaropoulou A, Manios Y. Accuracy and correlates of visual and verbal instruments assessing maternal perceptions of children's weight status: the Healthy Growth Study. Public Halth Nutrition. 2011;14(11):1979-87. Epub 2011/07/07.

VIII. Manios Y, **Moschonis G**, Papandreou G, Siatitsa PE, Iatridi V, Lidoriki I, Lionis C and Chrousos GP on behalf of the "Healthy Growth Study" group. Being female, small size at birth and from low income family increases the likelihood of insulin resistance in late childhood: The Healthy Growth study. Pediatric Diabetes 2013 (*In press).*

IX. Manios Y, **Moschonis G**, Papandreou G, Politidou E, Naoumi A, Kourlaba G. Revision of the Healthy Lifestyle-Diet Index and association of the revised index with obesity and iron deficiency in schoolchildren: The Healthy Growth study. 2013 *Submitted manuscript.*

Additional publications:

- Manios Y, Kourlaba G, Grammatikaki E, Koubitski A, Siatitsa PE, Vandorou A, Kyriakou K, Dede V, **Moschonis G**. Development of a lifestyle-diet quality index for primary schoolchildren and its relation to insulin resistance: the Healthy Lifestyle-Diet Index. European journal of clinical nutrition. 2010;64(12):1399-406. Epub 2010/09/03.
- Birbilis M, **Moschonis G**, Mougios V, Manios Y. Obesity in adolescence is associated with perinatal risk factors, parental BMI and sociodemographic characteristics. European journal of clinical nutrition. 2013;67(1):115-21. Epub 2012/12/13.

Contents

1. Introduction

1.1. Nutritional assessment: the ABCD approach.

Nutritional assessment represents a multi-dimensional process that aims to record and evaluate growth, development and the general health status of individuals and population groups. The integral parts of nutritional assessment comprise the assessment of i) Anthropometrical, ii) Biochemical, iii) Clinical and iv) Dietary intake indices, closely followed by information on medical history and socio-economic status. Each of these parts requires the execution of standardised measurements for collecting valid and reliable data and to a further extent the assessment and interpretation of these data so as to have a full picture of an individual's or a population's health status.

One simple way to schematically present the main components of nutritional assessment and their interrelationships is the ABCD approach presented in the figure (*Figure 1*) below.

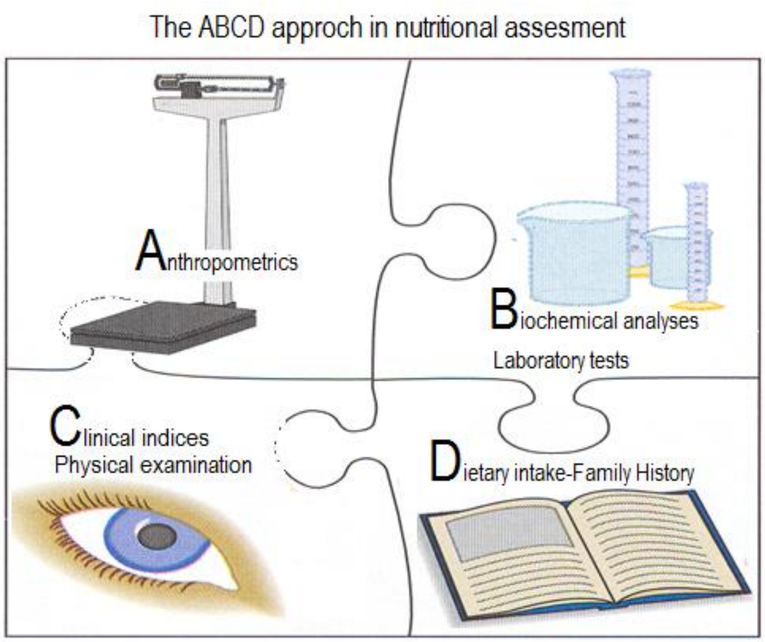

Figure 1. The ABCD approach in nutritional assessment science

Taken as a whole and when it comes to population groups the information collected during nutritional assessment provides answers to the following questions:

- What is the health problem?
- Who has the health problem?
- Why those who have the health problem, have it?

To answer these questions one should bear in mind that increased differentiation of populations on cultural, socio-economic and environmental grounds increases disease vulnerability in certain target groups. For example, groups with lower socio-economic status, individuals living in disadvantaged neighbourhoods, socially-excluded groups, some immigrant communities and ethnic minorities frequently have higher obesity rates and a lower health status. Furthermore, people may also be vulnerable during certain critical periods throughout life, for example during pregnancy, lactation, infancy, childhood, adolescence and in old age.

Consequently, the challenge deriving from all information that nutritional assessment can provide is to combine them into appropriate diet and lifestyle counselling initiatives, tailored to the characteristics of individuals or population groups most in need for such initiatives.

In this context there is evidence that improved lifestyles can reduce the risk of type II diabetes by 58% over four years. Furthermore, population studies have shown that up to 80% of cases of coronary heart disease and up to 90% of cases of type 2 diabetes could potentially be avoided through changing lifestyle factors (1). Physical activity and sedentary behaviour are seen as equally important key modifiable factors as diet contributing to the risk of obesity and associated diseases.

In summary, successful strategies for reducing the rates of nutrition-related health problems, with a special focus on obesity, must target the most vulnerable in all age groups (2). Prevention should start in very early life stages as there is increasing evidence that prenatal and infant nutrition can condition for health problems later in life (3-5). Still, advances in these areas need a better understanding of the underlying mechanisms and effective translation into the public health domain, both of which can stem from nutritional assessment.

1.2. Prevalence of childhood obesity

Childhood overweight and obesity has increased in alarming rates reaching pandemic proportions throughout the world over the last decades, although great regional differences have been detected (6-11). Recent data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) in the US showed that 31.8% of children and adolescents were overweight and obese, with the prevalence of obesity reaching 16.9% (12) (*Figure 2*). Despite these alarming prevalence rates, Olds et al. (13) has recently reported that the prevalence of childhood obesity has been stabilizing thus reaching a plateau. Sill this stabilization does not guarantee that the prevalence will not increase again in the future as similar trends have been observed in the past as well.

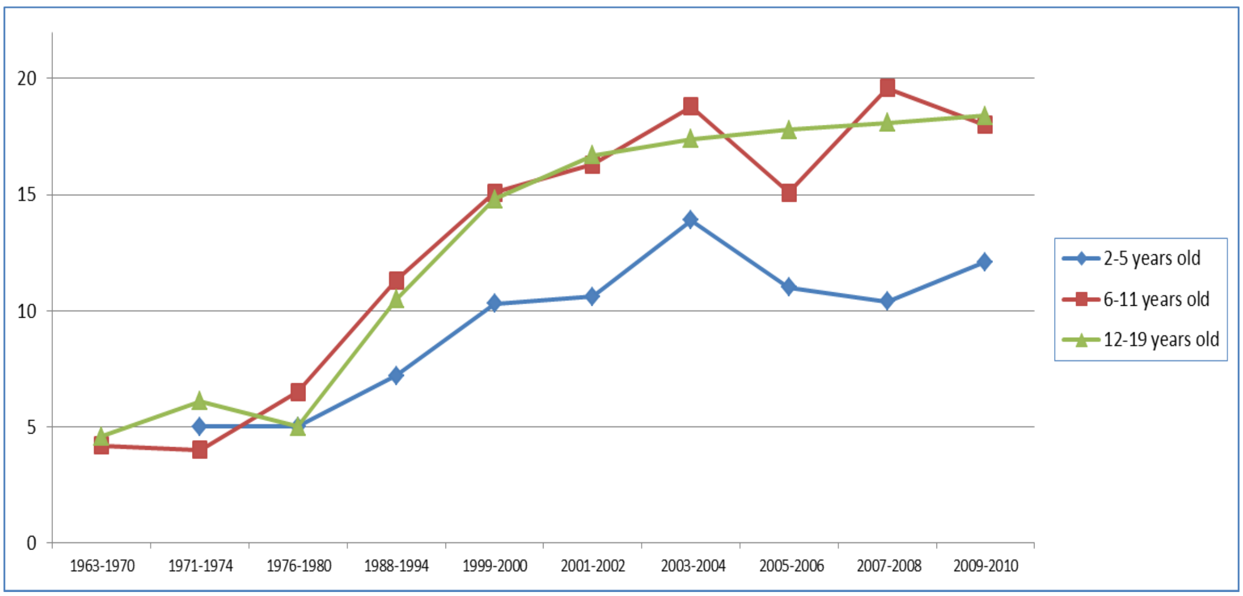

Figure 2. Prevalence of obesity (BMI-for-age > 95^{th} percentile) in children and adolescents in the US (Data from the NHANES study).

Using nationally representative data from eight European countries, the cross-sectional part of the "European Energy balance Research to prevent excessive weight Gain among Youth" (ENERGY)-project (14), has very recently reported a geographic distribution in the prevalence of childhood overweight and obesity in Europe, with southern (i.e. 42.0% in Greece and 23.5% in Spain) and eastern (i.e. 25.8% in Slovenia and 24.3% in Spain) countries having much higher overweight and obesity rates compared to central (i.e. 14.9% in Belgium and 13.8% in

Switzerland) and northern (i.e. 13.9% in Norway and 11.1% in the Netherlands) countries (*Figure 3*). These prevalence rates are higher than those reported in 2005-2006 for 11-year old children from nationally representative samples in the same European countries (11) indicating an increasing trend in Europe even though in a few countries the speed of this increase seems to have levelled off (15-18).

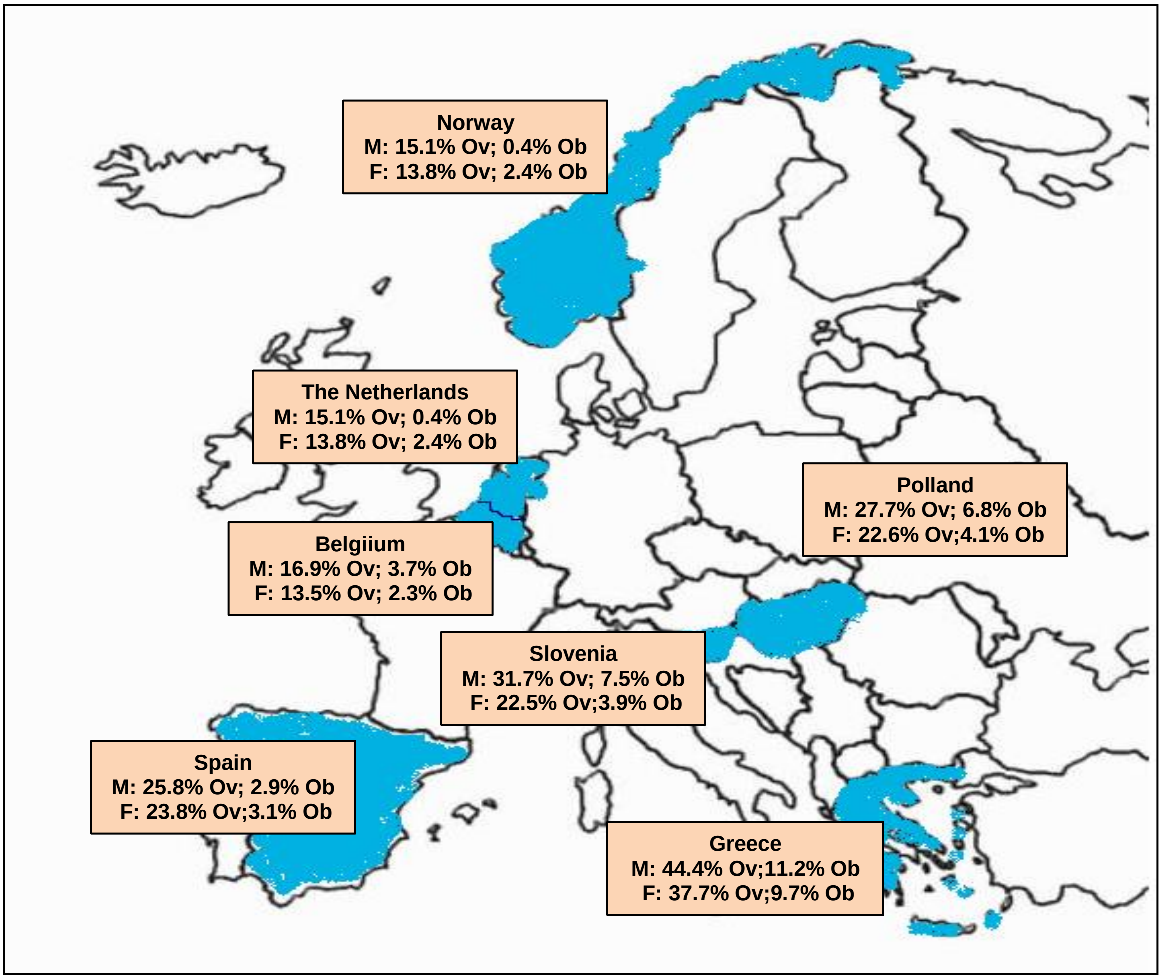

Figure 3. Prevalence of overweight (Ov) and Obesity (Ob) among 10-12 year-old boys (M) and girls (F) in Europe (Data from the ENERGY project).

Following the aforementioned trends in the US and the rest of Europe, the prevalence of overweight and obesity among Greek children and adolescents has also dramatically increased over the last decades (9). This increase places Greece to the top of the list of European countries with the highest prevalence rates of overweight and obesity. *Table 1* presents the prevalence rates of overweight and obesity in different parts of Greece.

Table 1. Prevalence of overweight and obesity in children and adolescents from different parts of Greece.

Region (date of measurement)	**Sample size**	**Age range**	**Definition of Overweight / Obesity**		**Prevalence Overweight / Obesity**	
Crete (1992)[19]	B: 466	6 years old	CDC, 1979	≥95^{o} Percentile	B: 10,9%;	G: 9,2%
	G: 424			≥85^{o} Percentile	B: 23,2%;	G: 28,8%
Crete (1995)[19]	B: 243	9 years old		≥95^{o} Percentile	B: 4,9%; G: 4,5%	
	G: 226			≥85^{o} Percentile	B: 18,9%;	G: 18,1%
Crete (1998)[19]	B: 366	12 years old		≥95^{o} Percentile	B: 8,2%; G: 5,0%	
	G: 359			≥85^{o} Percentile	B: 24,0%;	G: 19,2%
Thessalonica (2000-01)[20]		6-10 years old	Cole et al,	Obese	B: 5,1%; G: 3,2%	
	B: 1226		2000 (IOTF)	Overweight	B: 26,6%;	G: 25,0%
	G: 1232	11-17 years old		Obese	B: 3,7%; G: 1,5%	
				Overweight	B: 25,3%;	G: 13,0%
Volos (2003)[21]	B: 90	11-12 years old	Cole et al,	Obese	B: 6,7%; G: 6,7%	
	G: 105		2000 (IOTF)	Overweight	B: 35,6%;	G: 25,7%
North-Eastern Attica (2003-04)[22]	B: 2054	6-11 years old	Cole et al,	Obese	B: 12,3%;	G: 9,9%
	G: 2077		2000 (IOTF)	Overweight	B: 27,8%;	G: 26,5%
Nation-wide (2003)[23]	B: 6677	12-19 years old	Cole et al,	Obese	B: 6,1%; G: 2,7%	
	G: 7779		2000 (IOTF)	Overweight	B: 23,3%;	G: 14,0%
Nation-wide (2004)[24]	B: 1218	1-5 years old	Cole et al,	Obese	B: 6,2%; G: 8,1%	
	G: 1156		2000 (IOTF)	Overweight	B: 12,9%;	G: 15,5%
Vyronas (2004-05)[25]	B: 1021	12-17 years old	Cole et al,	Obese	B: 4,4%; G: 1,7%	
	G: 987		2000 (IOTF)	Overweight	B: 19,2%;	G: 13,2%
Attica (2005-06)[26]	B: 323	10-12 years old	Cole et al,	Obese	B: 8,6%; G: 9,0%	
	G: 377		2000 (IOTF)	Overweight	B: 33,9%;	G: 22,1%
Ioannina (2004)[27]	B: 153	11 years old	Cole et al,	Obese	B: 11,8%;	G: 7,5%
	G: 159		2000 (IOTF)	Overweight	B: 29,4%;	G: 39,0%
Thessalonica (2007)[28]	B: 275	6-15 years old	Cole et al,	Obese	B: 8,4%; G: 7,3%	
	G: 249		2000 (IOTF)	Overweight	B: 21,1%;	G: 17,6%
Rural Thessalonici - Nea Madytos (2007)[29]	B: 287	6,7,8 and	Cole et al,	Obese	B: 13,6%;	G: 14,4%
	G: 285	10 years old	2000 (IOTF)	Overweight	B: 21,3%;	G: 23,2%

B: Boys ; G: Girls

1.3. Obesity-related metabolic complications in children

Obesity is followed by several metabolic complications. In adults obesity is associated with an increased risk of chronic diseases, such as cardiovascular diseases, mainly heart disease and stroke, several types of cancer (e.g. breast and colon cancers), respiratory complications, such as sleep apnoea, as well as decreased reproductive function in women (30-32). Some of these conditions often cause premature death and their treatment is usually very expensive for the health care system. Obesity is also associated with an increased risk for several metabolic complications, such as insulin resistance, glucose intolerance and type-2-diabetes (33).

1.3.1. Childhood obesity and cardiometabolic risk.

Following the alarming increase of overweight and obesity in childhood and adolescence worldwide (34) and as adipose tissue accumulates in excessive amounts in the body several cardiometabolic alterations begin to occur (35). Research has shown that obese children are at a higher risk for the development of unfavourable lipidaemic and glycaemic profiles compared to lean children (36). This, in turn, is related to an increase in fatty streaks within the arterial endothelium of children, which may lead to atheromatic plaques in adulthood (37).

The term cardiometabolic risk (CMR) has been proposed by the American Diabetes Association and the American Heart Association (38) and reflects an umbrella under which a list of clinical abnormalities that predict cardiovascular disease (CVD) and/or type 2 diabetes (T2D) in adult life are included. Overweight and obesity are on the top of this list closely followed by metabolic and vascular abnormalities, such as insulin resistance, dyslipidaemias and hypertension (39).

The first line of defence against CMR is the modification of related risk behaviours. Several studies have indicated that the roots of chronic disease, both the clinical manifestation of the disease and certain behaviours or lifestyle patterns, may be set early in childhood and track into adulthood (40). The adoption of balanced dietary and physical activity (PA) behaviours in childhood and adolescence may support optimal physical growth, as well as cognitive and emotional development (41). In the long-run, they can also form the basis for a healthy transition to adulthood with lower predisposition to chronic diseases (42).

1.3.2. The double burden of childhood obesity and nutritional insufficiencies.

Further to cardiometabolic risk complications and although contradictory at first sight, obesity has also been associated with certain nutritional insufficiencies (43, 44). Specifically, overweight and obese individuals seem to be at higher risk for iron deficiency (ID) than those having normal body weight (45-47). These observations have been consistent in both children (48-51) and adults (51-53).

Wenzel and colleagues were the first to report lower serum iron levels in obese compared to non-obese adolescents (45). This association has received little attention since then. However, some recent studies have shown that overweight children and adolescents are at higher risk for iron deficiency (ID) compared to their normal-weight counterparts (48, 50, 54). Suggested contributing factors include poor dietary iron intake, probably due to repeated short-term restrictive diets, by overweight individuals and genetics (48, 50). Still, the exact aetiology of obesity-related hypoferremia in children still remains uncertain (52).

Considering that obesity represents a low-grade chronic inflammatory state, the hypoferremia associated with obesity could be attributed to certain metabolic and molecular adaptations induced by inflammation. This hypothesis is supported by the significant inverse correlations observed in overweight and obese individuals between serum iron concentration and the concentrations of a variety of adipocytokines and pro-inflammatory cytokines after controlling for iron intake, iron needs and iron losses (55, 56). Furthermore, certain acute-phase peptides, such as hepcidin, that are usually elevated in obesity have also been found to regulate iron haemostasis, mainly via their iron binding properties (52, 54).

Impaired iron status in children and adolescents could have several important clinical implications for future physical and cognitive development and maturation. Thus identifying overweight/obese children as a high risk group for ID could be very important from a public health perspective for designing and implementing tailored-made dietary intervention approaches to correct both excess body weight and poor iron status, even in developed countries.

1.4. The genetic aetiology of obesity

The current obesity epidemic is to a large extent a result of a complex interplay between behavioural, other environmental and genetic risk factors (57). There are currently numerous theories and models explaining the genetic basis of complex diseases and increased susceptibility but with no real consensus. In this context, 40 years ago James Neel proposed the “thrifty genes hypothesis” in an attempt to explain the increase in the prevalence of type-2 diabetes (58). Based on this hypothesis, obesity originates from natural selection and some genes have evolved in order to protect human populations from starvation by facilitating storage of excess energy as body fat during times of food abundance. During periods of food deprivation those individuals with the capability to store energy combined with its efficient utilization were probably more capable of surviving. Genes or genetic variants of enhancing these features were conserved and prevailed in the human genome during evolution of the human species. However, since not all of us become overweight or obese and thus people are not equally affected by our obesogenic environment the thrifty genes hypothesis has been questioned.

In 2007 the first common genetic variants that undoubtedly affect our susceptibility to obesity were identified. These variants were located in the fat mass and obesity associated gene (FTO) and were identified by Frayling et al (59). In 2010 another study identified the mannosyl (α-1, 3)-glycoprotein β-1,2-N-acetylglucosaminyltransferase, MGAT1, to be significantly associated with body weight among women (60).

1.5. Risk behaviours of childhood obesity and related metabolic complications. What is the state of the art?

Low consumption of fruits and vegetables, breakfast skipping and increased consumption of fruit juices, soft/fizzy drinks, confectionary and fast food have been reported as the most profound unfavourable dietary behaviours that increase obesity and related cardiometabolic and other complications (e.g. nutritional insufficiencies) in children (61, 62). In addition, reduced levels of physical activity in children either in the form of active transportation, of leisure time PA (e.g. walking, cycling) or organized sports, is the other important aspect of the positive energy balance equation that consequently leads to obesity (61, 63). Furthermore, sedentary behaviour (e.g. increased daily time in from of screens, i.e. TV, DVD/video, video games) is beginning to be seen as a separate important risk factor; it appears that being sedentary for large periods of the day may carry a separate risk that is not prevented by short periods of physical activity (64).

Although childhood obesity and related metabolic complications have strongly been associated with several energy balance related behaviours, the actual aetiology of increased adiposity in children requires further investigation due to its complex and multifactorial nature. In this context, it is very important to examine the possible synergistic effect of several lifestyle factors on adiposity in children (65). A common approach to examine the possible synergy of such factors is the development of specific dietary and lifestyle patterns. Principal component analysis (PCA) is frequently used for this purpose, since it enables the grouping of several strongly associated behaviours into patterns.

Recent epidemiological studies have highlighted certain such patterns that when combined can significantly increase the risk of childhood obesity (66, 67). More specifically, dietary patterns such as infrequent meals, breakfast skipping, lower adherence to the Mediterranean diet, higher consumption of sugar sweetened beverages, fruit juice, salty snacks and french fries, as well as lower consumption of dairy products, fruits and vegetables, have consistently been reported as the most important risk factors for childhood obesity (68). Additionally, increased time spent in front of screens (i.e. TV, games consoles and computers), less time spent on physical activity and shorter sleep duration represent other important risk behavioural patterns that have also been implicated in the aetiology of childhood obesity (69, 70). From a public health perspective, these associations are very important, since identification of lifestyle patterns that are strongly related to an increased risk of obesity, could potentially facilitate the formation of practical public health advice, specifically targeted at the prevention and management of childhood obesity. Several previous studies have investigated the association between dietary patterns and childhood obesity; however, to the best of our knowledge, to date no study has attempted to concurrently examine the possible role of specific lifestyle and dietary behavioural patterns in the development of obesity in childhood.

1.5.1. Determinants of obesity and cardiometabolic risk behaviours.

Obesity and cardiometabolic risk behaviours are very much determined by children's intrinsic, personal characteristics (71) as well as their social and physical environment (72). This underlines the need to expand our knowledge and understanding of these determinants of childhood obesity as well as their interactions and their possible role in the development of childhood eating and PA patterns. One of the first theoretical approaches to holistically examine the effect of the duo "personal characteristics-environment" on behaviour was the Theory of Planned Behaviour (TPB). TPB was developed by Icek Azen in 1985 and since then it has been extensively and successfully used mainly in the design of behavioural change intervention programs. Furthermore, TPB has been the basis for the development of other similar theoretical frameworks, such as the Social Ecological Theory and the Ecological Systems Theory, which represent more generalized models for grouping environmental determinants of behaviours (73). To a further extent the Analysis Grid for Environments Linked to Obesity(ANGELO) framework (74), the Environmental Research framework for weight Gain prevention (EnRG) framework (75) and the contextual model of childhood overweight (76), are more recent attempts to specifically describe environmental influences of childhood overweight and obesity.

Based on TBP and the other similar theoretical approaches, children's dietary and PA behaviours seem to be influenced by personal characteristics, such as attitudes, subjective norms, knowledge, self-efficacy and emotions of children themselves but also of their parents (particularly in the case of younger children). Furthermore, social environment, which primarily consists of family (i.e. parents and siblings), peers and teachers, determines children's health behaviour mainly via modelling, encouragement, support, rule setting and rewarding (77). Media (i.e. TV, internet) and food advertisement as well as health professionals are also part of the social environment affecting children's dietary and PA behaviours. Finally, physical environment (i.e. home, neighbourhood and school) can influence obesity risk behaviours mainly through the mediating effect of availability and accessibility (78, 79). The main effects and interactions of all the aforementioned environmental and personal determinants on diet and physical activity behaviours can differentiate, in terms of statistical strength and significance by certain factors which answer the question "For whom these influences are more or less likely?". These factors are called moderators, with the more pronounced ones being age, gender, socioeconomic status, nationality/ethnicity and race (75). For instance, the moderating effect of parental socioeconomic status on the association between parental encouragement and children's obesity risk behaviours, can be reflected on the higher susceptibility of children from less affluent families to skip breakfast more frequently (80) and to engage less in sports activities compared to their peers from more socio-economically advantageous families (81). Another example of the moderating effect of gender is that girls adhere better to guidelines compared with boys (82). However they are less interested in sports or vigorous PA compared to boys of the same age (83) and thus it is possible that they would prefer to engage in moderate PA during their leisure time than increase their vigorous PA levels (84).

1.6. Perinatal risk factors for childhood obesity and related metabolic complications.

Unhealthy eating habits and lack of physical activity are the key environmental contributors leading obesity and related metabolic complications (7). However the aetiology of childhood obesity is far more complex. The rise in the prevalence of childhood obesity over the last decades has led to the development of certain theories that underscore the importance of early life factors on the degree of adiposity in childhood and adulthood (4, 85). Some of these early life factors include maternal smoking during pregnancy, gestational diabetes (86), size at birth (87), breastfeeding or infants' feeding with formula (88) and postnatal growth rate with emphasis on catch-up growth (89). These factors are believed to yield permanent physiological and metabolic adaptations that may become detrimental over the life span. For instance, catch-up growth has been related to insulin resistance in children via the following mechanism: children born small for gestational age when exposed to an energy-rich environment at early infancy are more susceptible to accumulate abdominal visceral fat mass; an adaptation that is caused by energy deprivation and that is originally intended to safeguard foetal survival (6).

1.7. The need to go beyond the state of the art and holistically examine risk factors of childhood obesity and related metabolic complications.

Although childhood obesity has strongly been associated with several energy balance related behaviours, the actual aetiology of increased adiposity and related metabolic complications in children requires further investigation due to its complex and multifactorial nature. Taking into account all aforementioned information regarding behavioural risk factors, their determinants and perinatal risk factors there is a need to holistically record and examine these parameters within a common framework, such as the one proposed and presented in *Figure 4*. This framework could be of great value to public health policy makers and legislators for designing and implementing intervention programs tailored to the needs of susceptible population groups, most in need for such initiatives. Targeting on the determinants of risk behaviours, and not just on the behaviours, has been previously proved as the most effective approach for tackling health issues related to obesity and related metabolic complications in children and adolescents starting from early life stages.

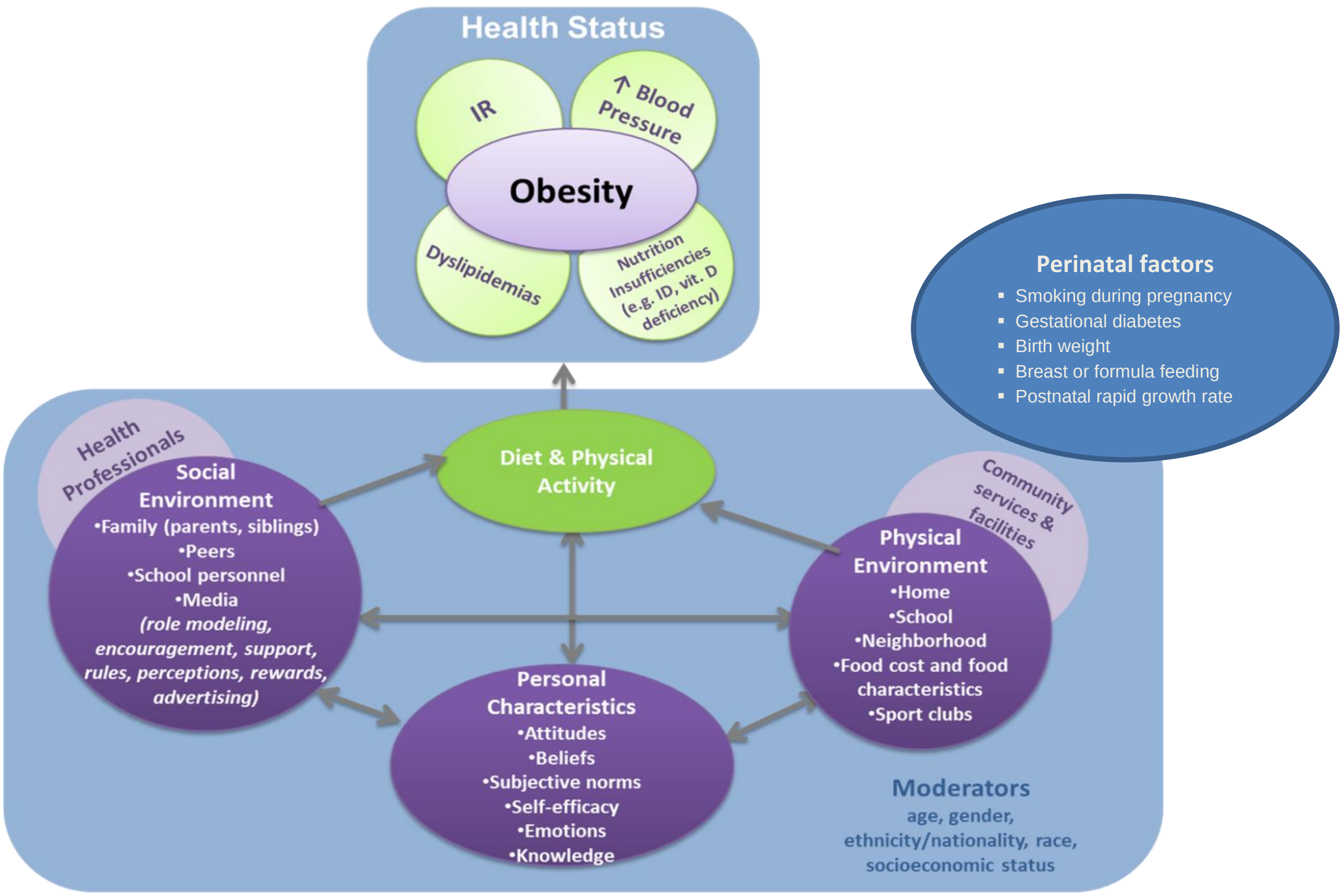

Figure 4. Schematic presentation of a framework that holistically presents risk factors for children's "poor" health status (IR: Insulin Resistance; ID: Iron deficiency).

1.8. Aims and objectives

The objectives of the current PhD Thesis are a) to record a wide range of indices for the assessment of health status in a representative sample of primary schoolchildren in Greece, b) to record and assess behaviours related to children's health status, c) to identify those factors that determine these behaviours and consequently health status in children, d) to develop easy to use tools that will assist health professionals in designing and implementing effective nutrition counselling and health education programs tailored to the specific needs of individuals or population groups at risk..

The specific aims of each scientific paper supporting this PhD thesis are:

Paper I

To explore the prevalence of overweight and obesity among 10 to 12 year-old schoolchildren in relation to several socio-economic and demographic characteristics of children and their families.

Paper II

To record the prevalence of dyslipidaemias, IR and hypertension in children and examine their link with overweight and obesity.

Paper III

To investigate the prevalence of ID and ID anaemia in children and the association of these nutritional deficiencies with central obesity as well as with high percentage body fat and visceral fat mass in children.

Paper IV

To investigate the association between overweight and iron status in children and to identify the main risk factors for iron deficiency.

Paper V

To identify specific lifestyle and dietary patterns associated with obesity and increased levels of total and central adiposity in children.

Paper VI

To examine the associations between dietary and other lifestyle patterns with blood lipid levels in children.

Paper VII

To examine the accuracy of maternal ability to classify their children's weight status correctly using a verbal and a visual classification instrument; and detect possible demographic, socio-economic, maternal and family correlates of maternal underestimation of their children's weight status.

Paper VIII

To examine the association of insulin resistance with perinatal risk factors among 9-13 year-old schoolchildren after controlling for possible confounders with regards to socio-demographic indices and parental characteristics.

Paper IX

To develop a Healthy Diet and Lifestyle-index in order to more accurately assess the degree of children's adherence to existing age-appropriate dietary and lifestyle guidelines and to examine the association of this index with obesity, insulin resistance and iron deficiency.

2. Subjects and methods

2.1. Study design and study population

The "Healthy Growth Study" is a cross-sectional study, the pilot phase of which, was initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethical Committee of Harokopio University of Athens. The population under study comprised of schoolchildren aged 9-13 years attending the 5th and 6th grades from primary schools located in municipalities within the wider region of Athens.

2.2. Randomization and sampling procedures

The sampling of municipalities and schools in the present study was random, multistage and stratified by parental educational level and the total population of 9-13 year-old students, thus yielding a representative sample of primary schoolchildren from the wider urban region of Athens. More specifically, the municipalities in the county of Attica were divided into 3 groups based on the average educational level of their adult population (25 to 65 years old) that was estimated from data provided by the National Statistical Service of Greece (Census 2001). This procedure yielded two parental education cut-off points that allowed categorization of municipalities into 3 categories of different socio-economic level (SEL) i.e. Higher, Medium and Lower SEL. Consequently, based on data from the National Statistical Service of Greece a certain number of municipalities, proportional to the size of their preadolescent population (9-14 years old), was randomly selected from each one of these three SEL groups. Finally, an appropriate number of schools was randomly selected from each one of these municipalities in relation to the population of schoolchildren registered in the 5th and 6th grade in each municipality, based on data obtained from the Greek Ministry of Education.

Weight and height were measured in all pupils attending the 5th and 6th grades in the participating primary schools as part of a school-based health and nutrition education program. Full medical examination (i.e. anthropometrics and body composition measurements, blood collection, clinical examination etc.) and questionnaire data were obtained by a subgroup of pupils whose parents signed an informed consent form. More specifically an extended letter explaining the aims of the current study and a consent form for conducting full measurements was provided to all parents or guardians having a child in these schools. Parents who responded positively had to sign the consent form and provide their contact details. Signed parental consent forms for full measurements were collected for 2655 out of 4145 children (Response rate 64.1%).

2.3. Anthropometrical indices

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. The physical examination included basic anthropometric measurements.

2.3.1 Weight and height measurements

Weight was measured to the nearest 10g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany). Subjects were weighted without shoes in the minimum clothing possible. Height was measured to the nearest 0.1cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Oadby, UK) with subjects not wearing shoes, their shoulders in a relaxed position, their arms hanging freely and their head aligned in Frankfort plane (13). Weight and height were converted to body mass index (BMI) using Quetelet's equation (weight (kg)/height^2 (m^2)).

2.3.2. Circumferences

Waist circumference (WC) was measured to the nearest 0.1 cm with the use of a non-elastic tape (Hoechstmass, Germany) with the student standing, at the end of a gentle expiration. The measuring tape was placed around the trunk, at the level of umbilicus midway, between the lower rib margin and the iliac crest. Hip circumference was measured at the level of greater trochanters and pubic symphisis to the nearest 0.1 cm. Waist to Hip ratio (WHR) was then calculated for each child.

2.3.3. Skinfolds' thickness

The thickness of four skinfolds (triceps, biceps, subscapular and suprailliac) was measured to the nearest 0.1 mm to the right side of the body with a Lange skinfold calliper

(Cambridge, Maryland). Each skinfold was grasped gently, in order to avoid causing any unnecessary discomfort to the child. Triceps and biceps skinfold thickness was measured with the right arm hanging relaxed at the side of the body. The skinfold was picked up about 1 cm bellow the midpoint mark over the triceps and biceps muscle respectively. Measurement of the subscapular skinfold thickness was performed while the child stood with shoulders relaxed and after identifying the inferior angle of the scapula. The skinfold was picked up 1 cm below the subscapular mark. Suprailliac skinfold was measured just above the iliac crest, along the axis of the anterior line. In each case the calliper was applied to the “neck” of the fold just above the finger and thumb, for two repeated measurements.

2.3.4. Definition of overweight/obesity and central obesity

The International Obesity Task Force (IOTF) cut-off points (90, 91) were used to categorize participants as “underweight”, "normal weight", "overweight" or "obese". The age- and sex-specific WC percentiles were used for the classification of central obesity (≥ 90th percentile) (92).

2.4. Body composition indices

Bioelectrical impedance analysis (BIA) was used for the assessment of percentage total body fat (Akkern BIA 101, Akkern Srl., Florence, Italy) and for abdominal-visceral fat mass (Tanita Viscan AB-140, Kowloon, Hong Kong). In abdominal BIA an electric current is passed between the regions near the umbilicus and spinal cord at the umbilicus level and the voltage generated in the lateral abdomen is recorded. Because the equipotential line that passes through visceral fat appears on the lateral abdominal surface, the amount of visceral fat can be estimated by measurement of the voltage generated at this location using a regression equation determined by computed tomography (93). Participants were instructed to abstain from any food or liquid intake and from any intensive exercise for four hours before measurement. They were also instructed not to wear any metal object during measurement. The assessments took place with the pupils lying on a nonconductive surface at ambient room temperature. Percentage body fat was calculated from the resistance and reactance values using valid equations derived from a similar preadolescent population (94), while percent trunk fat mass and visceral fat rating (rating scale from one to 59 units, with 0.5 increment) were read directly from the instrument. As there were only two Tanita Viscan devices available, data on trunk-visceral fat mass were collected for a representative sub-sample of 1500 children.

2.5. Physical examination-Clinical indices

2.5.1. Blood pressure measurements

Blood pressure was recorded from the right arm while in a sitting position and after five minutes of rest. A valid automatic Omron M6 Blood Pressure Monitor (Omron Healthcare Europe BV, Hoofddorp, The Netherlands) (95) was used. The measurement was taken twice with a two-minute interval between readings. A third measurement was taken if there was a difference of over 10 mmHg between the previous measurements. The average value of the measurements was used in analysis. Systolic (SBP) and diastolic blood pressure (DBP) were recorded. Systolic or diastolic hypertension was defined as SBP or DBP above the 95th percentile for gender, age and height (96).

2.5.2. Biological/sexual maturation stage by Tanner.

One well-trained and experienced female paediatrician in each prefecture determined pubertal maturation (Tanner stage) after thorough visual inspection of breast development in girls and genital development in boys [(97)]. Finally, each girl was asked by the paediatrician about her menstruation status and age of menarche.

2.5.3. Cardiorespiratory fitness levels

Cardiorespiratory fitness was estimated indirectly according to children's performance in the 20-m endurance shuttle run test (ERT). The ERT is a field test included in the European battery of physical fitness tests and recommended by the Committee of Experts on Sports Research (98). Based on the test's instructions participants start running at a speed of 8.5 km/h and the speed increases in stages. Children shuttle between two lines placed 20m apart, at a pace dictated by a sound signal on an audiotape, which gets progressively faster (by 0.5 km/h every minute). Each stage of the test is made up of several shuttle runs and the score of the participant is the half-stage completed before the child drops out (thus, scores can be 0, 0.5, 1, 1.5, 2 etc.). The higher the ERT score, the better the cardiorespiratory fitness. Prior to the test all children received clear and comprehensible instructions on rules and procedures, while during the test they were verbally encouraged by the researchers to succeed their maximal number of laps. The ERT is recommended for large groups of children, since it is reliable, valid, non-invasive, and requires limited facilities (99).

2.6. Behavioural indices

2.6.1. Dietary intake

2.6.1.1 Twenty four-hour recalls

Dietary intake data were obtained by trained dieticians and nutritionists via three 24-hour recall morning interviews (i.e. in two consecutive weekdays and one weekend day) conducted with children at school-site. Specifically, all study participants were asked to describe the type and amount of foods and beverages consumed, during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimize the interviewer's effect, reviewed the collected data with the respondent in order to clarify entries, servings and possible forgotten foods. Food intake data were analysed using the Nutritionist V diet analysis software (version 2.1, 1999, First Databank, San Bruno, California, USA), which was extensively amended to include traditional Greek dishes and recipes (100). Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

The food-grouping scheme was designed for all foods or entries (core and recipe) appearing in Nutritionist V. In total, 47 food groups were initially established, based on similar source characteristics and nutrient content. Composite food items, such as recipes, were analysed and were assigned to food groups according to primary ingredients. A similar methodology for the extraction of food groups was previously reported in studies with a smaller sample size, but with only one 24-h recall available (101). Examples of foods included in the food groups were documented previously (102). In total, 18 large food categories (whole grain bread and cereals; refined bread and cereals; dairy products; vegetables; fruits; red meat; processed meat; eggs; potatoes; legumes; fish; other seafood; white meat; fats and oils; fresh fruit juices; sugar-sweetened beverages (i.e. soft drinks and packed fruit juices); salty snacks; and sweets) were created and the relevant values were calculated as consumption in grams per day.

2.6.1.2. Breakfast and Meal Frequency Patterns

Although there is no widely acceptable definition for breakfast, still according to a recent study on children and adolescents an ideal breakfast is the one that would typically include as the first meal of the day (i.e. within two hours after getting up in the morning at weekdays e.g. at home, at school, on the way to school or before entering the school class and before 11.00 a.m. on weekends) choices from the following three food groups: Milk and milk-derived products; cereals (preferably whole grain); and fruits (fresh fruits or natural juices; no sugar) (103). Using data from the 24-h recalls and on the basis of the aforementioned definition of ideal breakfast, a categorical variable was developed to assess the adequacy of children's breakfast consumption. More specifically the following categories were created: 0 = salty, sweet or other savoury snacks, 1 = zero portions of dairy products, cereals and fruits, 2 = one portion of dairy products or cereals or fruits, 3 = one portion of dairy products and one portion of cereals or fruits, 4 = one or more portions of dairy products, cereals and fruits. Data from 24-h dietary recalls were also used to define meal frequency. In turn, meal frequency (i.e. no of meals per day) was assessed by summing the number of meals in which any portion of food was consumed.

2.6.1.3. Assessment of dietary intake underreporting

In order to detect those children that were underreporting their dietary intake the ratio of daily energy intake to estimated basal metabolic rate was calculated. The basal metabolic rate was assessed according to the equations of Schofield (104), taking into account age, sex and weight of children. To identify subjects that underreported their dietary intake during the 24-hour recalls the cut-off points of energy intake / basal metabolic rate ratio proposed by Sichert-Hellert al. (105) were used for boys and girls aged 6-13 years, respectively. Those subjects found to underreport their energy intake were excluded from further analysis with regards to 24-hour recall dietary intake data.

2.6.2. Physical activity levels

2.6.2.1. Subjective and objective methods to assess physical activity levels.

Physical activity during leisure time was assessed using a standardized activity interview, based on a questionnaire completed by the participants in the presence of a member of the research team. This questionnaire is a valid one that has been extensively used to assess physical activity levels in children and further details regarding its reliability and validity are provided elsewhere (106, 107). Respondents reported the time spent on various physical activities on two weekdays and one weekend day, most preferably Sunday. Reported activities were grouped by a member of the research team into moderate-to-vigorous physical activities (MVPA) (intensity higher than 4 metabolic equivalents, METs), including activities such as brisk walking, bicycling, gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, jumping rope and general participation in active outdoors games. Given the age group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity, rather than the strict aerobic definition of 20 continuous minutes appropriate for adults. Physical activity levels that have been proposed to positively affect cardio-respiratory function and to produce desirable health outcomes (i.e. better weight, glycaemic, lipidaemic control etc.) is those higher than 4 METs, which corresponds to the level of moderate, vigorous and very vigorous physical activity (108, 109).

To assess step count as an objective estimate of physical activity, study participants were provided with and instructed to wear waist-mounted pedometers (Yamax SW-200 Digiwalker, Tokyo, Japan) for one week, i.e., from Monday to Sunday. The pedometer was positioned according to the manufacturer’s instructions on the right waistband, vertically aligned with the patella. Children were instructed to wear the pedometer from the time they woke up in the morning until the time they went to bed at night (except when taking a shower, bathing or swimming) and to record their total number of daily steps displayed by the pedometer in diary template before bedtime.

2.6.2.2. Sedentary activities

Children's TV/video viewing and computer games playing time was assessed by children's report with regard to their TV/video viewing time and time playing computer games during a usual weekday and a usual weekend. The mean daily TV/video viewing and computer games playing time was calculated using the following equation: daily TV/video viewing and computer games playing hours =((weekday TV/ video viewing and computer games playing hours x 5) + weekend TV/video viewing and computer games playing hours)/7.

2.7. Haematological and biochemical indices.

2.7.1. Total Blood Count

Blood samples were obtained for biochemical and haematological screening tests between 08.30 and 10.30 after a 12-h overnight fast. Reminders were distributed the previous day to both parents and children in order to ensure compliance with fasting. Professional staff performed venipuncture, using two types of test tubes, one of which contained EDTA, to obtain a maximum of 10 ml blood. EDTA-blood was transferred on the same day of collection to a local laboratory located, where it was analysed in a CELL-DYN haematological auto analyser (Abbott Diagnostics, Abbott Park, IL, USA) for the determination of haematological indices, including red blood cell (RBC) count, haemoglobin and mean corpuscular volume (MCV). The remaining blood was collected in plain test tubes for the preparation of serum, which was divided into aliquots and stored at -80^{o}C. When blood collection was completed in Aitoloakarnania, Thessaloniki and Iraklion, all serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University in Athens, where biochemical analyses and central storage of back-up samples at –80^{o}C took place.

2.7.2. Glycaemic profile

Plasma glucose was determined using commercially available enzymatic colorimetric assays (Roche Diagnostics SA, Vassilia, Swiss). Serum insulin was determined by a Chemiluminescence immunoassay (Kyowa Medex Ltd, Minami-Ishiki, Japan, for Siemens Diagnostics USA). IR was measured through homeostasis model assessment (HOMA-IR) (Matthews et al., 1985). This index was calculated using fasting glucose (FG) and fasting insulin (IF), as follows: HOMA-IR = (IF (μunits/ml) x FG (mmol/l)/22.5. HOMA-IR > 3.16 (110)) was used as a cut-off point to define insulin-resistant schoolchildren. HOMA-IR has been validated as a surrogate measure of insulin resistance in non-diabetic children, with studies showing correlations as high as 0.91 with the hyperinsulinemic-euglycemic clamp, a frequently sampled intravenous glucose tolerance test (111).

2.7.3. Lipidaemic profile

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined in duplicate using commercially available enzymatic colorimetric assays (Roche Diagnostics SA, Vasilia, Switzerland) on an automated analyser (Roche/Hitachi Modular). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation (112). The LDL-C/HDL-C and the TC/HDL-C ratios were also estimated. The National Cholesterol Education Program (NCEP) cut-off points for blood lipids were used to define dyslipidaemias (113).

2.7.4. Iron status

Serum iron and total iron binding capacity (TIBC) levels were determined by colorimetric assays (Roche Diagnostics SA, Basel, Switzerland). Transferrin saturation (TS) was calculated by dividing serum iron by TIBC and multiplying by 100. Finally, serum ferritin was measured by using a chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Tarrytown, New York, USA). Iron deficiency (with or without anaemia) and iron deficiency anaemia (IDA) were defined using the following age- and sex-specific thresholds proposed by UNICEF and the World Health Organization (114): ID was defined as TS < 16%; IDA was defined as TS < 16% and haemoglobin concentration < 12 g/dl, which is the threshold value for anaemia for children aged 9-13. The Mentzer Index [MCV (fl)/ RBC (M/μl)] [115] was also calculated for all pupils participating in the current study to differentiate beta-thalassemia from ID. On the basis of this index, children with thalassemia minor (18 cases) were excluded from further analysis.

2.8. Information obtained by parents (parental questionnaire)

Socio-demographic data, maternal weight and perinatal data were either reported by the parents or recorded from the children's birth certificates and medical records that the parents were instructed to bring along during scheduled interviews conducted at school-site. If parents were unable to attend the meeting (approximately 5% of the total sample) data were collected via telephone interviews. All interviews were conducted with the use of a standardized questionnaire by members of the research team who had been rigorously trained to minimize the interviewer's effect.

2.8.1. Perinatal factors

Mothers were asked to recall and report the following perinatal information: (a) age of maternal menarche; (b) parental weight and height from which body mass index was calculated and used to categorize each parent into normal-weight, overweight and obese on the basis of IOTF's cut-off points (116); (c) maternal weight before pregnancy and weight gained during pregnancy based on the classification recommended by the Institute of Medicine (IOM) (117)); (d) type of conception, i.e., natural conception or *in vitro* fertilization; (e) smoking habits during pregnancy; (f) use of nutritional supplements during pregnancy; (g) medical history of gestational diabetes mellitus and high blood pressure; (h) type of delivery (normal *vs.* caesarean); (i) parity; (j) children's feeding practices from birth to 6 months of age, i.e., exclusive breastfeeding *vs.* use of formula or mixed feeding; age at which formula was introduced; and weaning age; and (k) children's growth rate during the first 6 months of life. Birth weight and gestational age were recorded from each child's birth certificate and medical record and were used for the classification of children into small for gestational age (SGA, <10th percentile), appropriate for gestational age (AGA, 10 to 89th percentile) and large for gestational age (LGA, ≥ 90th percentile).

2.8.2. Family socio-demographics and parental anthropometric characteristics.

Data on the socio-economic and demographic background of the families having at least one child participating in the study were collected from the parents (most preferably from the mother) during the scheduled face-to-face interviews at the school-site All interviews were conducted by a research team of ten members who were rigorously trained to minimize interviewer's effect with the use of a standardised questionnaire. More specifically the data collected by parents included (a) demographic characteristics, such as parent's age, educational level (years of education), origin and nationality (b) economic characteristics, such as family mean annual income over the last three years (€ per year), family residence ownership (c) social characteristics, such as family size, i.e. the number of family members living in the family residence; the primary caregiver(s) of the child, i.e. parents, grandparents, nanny or someone else; family type based on parental marital status, i.e. dual-parent and single-parent families with parents that were either not married, widowed or divorced. Finally, parents reported their weight and height, from which body mass index was calculated and used to categorize each parent into normal-weight, overweight and obese on the basis of IOTF's cut-off points

2.8.3. Primary caregiver's perceptions for their child's weight status

Maternal perceptions for their child's weight status and body image were assessed using a verbal and a visual classification instrument, respectively. Regarding verbal classification of a child's weight status, the child's primary caregivers were asked to provide their answers to the following two questions: "According to your perception, what is the current weight of your child?", and "According to your perception, what is the current height of your child?" Children's weight and height, as perceived by parents, were also converted to BMI by using Quetelet's equation (weight (kg)/height^2 (m^2)) and thereupon their weight status was classified into different weight categories (underweight, normal-weight, overweight and obese) by using Cole's cut-off points (90, 91). Regarding visual classification of a child's body shape this was estimated by using the "Children's Body Image Scale" proposed by Truby et al. (118). In brief, 2 sets of age- and gender-specific photographs were available. Each photograph corresponded to a 10 year-old boy or a girl with a BMI on or around each of the seven conventional 1979 NCHS percentiles (i.e. 3^{rd}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th} and 97^{th}) (119) More specifically, in each set one photograph corresponded to an underweight child (3^{rd} percentile), four photographs corresponded to children within the normal BMI-for-age percentile range (10^{th} – 75^{th} percentile), one photograph corresponded to an overweight child (90^{th} percentile) and the last photograph to an obese child (97^{th} percentile). After presenting this set of photographs in random order, mothers were asked to choose the photograph that most resembled their child's body shape.

2.9. Statistical analyses

Power Analysis

Power calculations for obesity associations and for the regression models tested have been conducted with the Power and Sample Size Calculation software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Power calculations indicated that the total sample size of over than 2000 subjects in all analyses provided enough power.

Variables

Some of the independent variables used in the current analyses were continuous while others were categorical. Normality of the distribution of continuous variables was analysed using the Kolmogorov-Smirnov test. Normally distributed continuous variables were expressed as mean values ± standard deviations (s.d.), skewed variables were reported as medians (25th, 75th interquartile) and categorical variables were reported as frequencies (%).

Associations

Associations between continuous and categorical variables were examined using Student's *t*-test or one-way Analysis Of Variance (ANOVA) for normally distributed variables using the Bonferroni correction to account for increase in type I error owing to *post-hoc* multiple comparisons and the non-parametric Mann-Whitney or Kruskal-Wallis tests for skewed variables even though logarithmic transformations were made. The associations between categorical variables were assessed using the chi square (χ^2) test and the 2-sample Z-tests for proportions for bivariate associations whenever appropriate. In order to test the unadjusted associations between each one of the independent variables examined and the dependent variable, univariate logistic regression analyses were conducted and crude odds ratios (OR) with 95% confidence intervals (CI) were computed. Furthermore, adjusted OR and 95% CI were also estimated using multivariate logistic regression analyses, including all variables which were found to be significantly ($P<0.05$) associated with childhood overweight/obesity at a univariate level.

Principal components analysis (PCA)

Principal components analysis (PCA) was used to identify lifestyle patterns. The Kaiser-Meyer-Olkin (KMO) criterion was applied and it was equal to 0.6. The orthogonal rotation (varimax option) was used to derive optimal non-correlated components (lifestyle patterns). To decide the number of components to retain, Kaiser criterion (Eigen values>1) was used. Factor loadings represent the correlations of each habit with the lifestyle pattern score. Higher absolute values of factor loadings indicate that the habit predictor contributes most to the construction of this particular component. The lifestyle components (patterns) were named according to the factor loadings of those behaviours correlated most with the component (factor loadings > |0.4|). When a behaviour had a factor loading value > |0.4| in more than one component, then this behaviour was used to name the component for which it had the higher factor loading value.

Level of statistical significance and statistical analyses software

All reported *P*-values were based on two-sided tests. The level of statistical significance in all analyses was set at $p<0.05$. The SPSS v. 13.0, 16.0, 17.0 and 21.0 (SPSS Inc., Texas, USA) and STATA (Stata Corp., College Station, TX, USA) statistical softwares were used for all statistical analyses and whenever appropriate.

3. Results and discussion

3.1. Assessment of children's health status (Papers I, III, IV, V and VIII).

Paper I

The prevalence of overweight and obesity among Greek children has increased over the last decades (9). The rates of childhood overweight and obesity in Greece are similar or slightly higher to those reported in other European countries (120, 121). As with adults (122), the adoption of adverse obesogenic behaviours by children seem to have a socio-economic, demographic and cultural pattern (123, 124). Taking the above into consideration, the preliminary Healthy Growth Study data collected from measurements conducted in the county of Attica during the first year of the study were used to record the prevalence of overweight and obesity in urban primary schoolchildren in relation to several family socio-demographic factors. A representative sample of 729 schoolchildren (379 males and 350 females) aged 9-13 years old, stratified by parental educational level, was examined in the wider urban region of Athens. Weight and height were measured using standard procedures and equipment. The International Obesity Task Force thresholds were used for the definition of overweight/obesity (125). Several socio-economic and demographic data and child's "popularity" score were also recorded with specifically designed standardized questionnaires.

The overall prevalence of overweight and obesity was found to be 27.9% and 10.9%, respectively, with the prevalence of obesity being significantly higher in boys compared to girls (15.1% vs. 6.6%, $P<0.001$). Furthermore, non-participation to the full measurements of the study was not found to affect the representativeness of study sample since no statistically significant differences were observed between "full participants" and "weight and height participants" with respect to prevalence rates of overweight and obesity ($P=0.270$), thus reducing the chance of any potential bias deriving from non-participation to all measurements described in the full protocol of the present study. This should be highlighted as one of the novelties and strengths of the present study, since a main source of bias in epidemiological studies is the lack of the representativeness of the study population due to non-participation. *Table 2* presents the prevalence rates of overweight and obesity observed for "full participants" and "weight and height participants" in the first year of measurements in the Healthy Growth Study.

Table 2. Prevalence of overweight and obesity in "full participants" and "weight and height participants". Preliminary data from the Healthy Growth Study.

Weight Groups	Full participants (n=729)			Weight and height participants (n=472)	All Subjects (n=1201)
	Male (n=379)	Female (n=350)	Total (n=729)		
Underweight	7 (1.8)	16 (4.6)	23 (3.2)	19 (4.0)	42 (3.5)
Normal-weight	201 (53.0)	208 (59.4)	409 (56.1)	284 (60.2)	693 (57.7)
Overweight	113 (29.8)	103 (29.4)	216 (29.6)	119 (25.2)	335 (27.9)
Obese	58 (15.1)	23 (6.6)*	81 (11.1)	50 (10.6)	131 (10.9)

All data are presented as n (%); * $P<0.001$ for differences between genders derived from the χ^2-test.

Comparing the findings of the present study regarding the prevalence of overweight and obesity with those reported for other European countries (121) there seems to be a geographic variation. Precisely, the prevalence reported from studies conducted with children living in southern European countries surrounding the Mediterranean, including the current study, ranged between 20 and 41% and was considerably higher compared to the prevalence reported for northern areas in Europe that showed prevalence rates in the range of 10 to 20% (121). Several potential explanations have been proposed for these north-south differences in the prevalence of childhood overweight/obesity in Europe including higher stature and resting energy expenditure for children living in northern latitudes and the genetic predisposition towards weight gain in children living in southern Europe (121). As far as Greece is concerned the prevalence of overweight and obesity reported in the present study was comparable to the prevalence reported by other recent studies conducted with 10 to 12 years old children living in urban and semi-urban areas from southern (29.8% overweight and 14.9% obesity) (9), northern (25.3% overweight and 5.6% obesity) (20), western (34.3% overweight and 9.6% obesity) (27) and eastern (30.3% overweight and 6.7% obesity) (9) parts of Greece. Similarly to the current study the prevalence of obesity was also found to be significantly higher in male than female subjects in almost all of the aforementioned studies. The IOTF cut-off points were used for screening children's overweight/obesity in all of these studies.

Papers V

The results of Paper V on the prevalence rates of dyslipidaemias are summarized in *Table 3*. These data are consistent with those presented by similar studies conducted in Greece (126), and the United States (127). However, gender-specific prevalence of hypertriglyceridemia has been rarely investigated in children and adolescents and has produced inconsistent results (128, 129), precluding comments on our findings for higher prevalence in girls.

Table 3. Prevalence of overweight, obesity and dyslipidaemias[†] in children.

	Boys (n=1018) %	**Girls** (n=1025) %	**Total** (n=2043) %
Weight status			
Normal-weight	56.0	62.1	59.0
Overweight	30.7	28.6	29.7
Obese	13.3*	9.3	11.3
Total cholesterol (mg/dL)			
<170 [a]	55.7	57.9	56.8
170-199 [b]	30.7	29.2	30.0
≥200 [c]	13.6	13.0	13.3
HDL cholesterol (mg/dL)			
≥35 [a]	96.6	95.6	96.1
<35 [d]	3.4	4.4	3.9
LDL cholesterol (mg/dL)			
<110 [a]	71.5*	75.5	73,5
110-129 [b]	18.1	16.0	17,0
≥130 [c]	10.4	8.5	9,4
Triglycerides (mg/dL)			
<75 [a]	80.3*	72.8	76.5
75-99 [b]	13.0*	16.7	14.8
≥100 [c]	6.8*	10.5	8.7

*$P < 0.05$ for differences between sexes based on the chi-square test and the two-sample z-test for proportions.
[†]Adapted from NCEP guidelines for children and adolescents. Letter superscripts indicate grouping based on the proposed thresholds of serum lipids, i.e. [a] Acceptable levels; [b] Borderline-high levels; [c] High levels, [d] Low levels

Papers VIII

Paper VIII revealed a relatively high prevalence of IR (i.e. HOMA-IR < 3.16) in the total sample of children (i.e. 28.4%) (*Table 4*). Previous epidemiological studies conducted in the USA (130) and Italy (131) showed that weight status had a strong impact on the prevalence of

IR in adolescents. More specifically, in the first study, the prevalence of IR was 3.1%, 15% and 52.1% in normal-weight, overweight and obese adolescents, respectively (using HOMA-IR > 4.39 as the threshold), while in the second study IR was present in 3% and 40.8% of normal-weight and obese subjects, respectively (using HOMA-IR > 2.5 as the threshold). All aforementioned findings are indicative of a direct positive association between children's BMI and HOMA-IR levels, already confirmed by earlier studies (132). In this context, the considerably high prevalence of overweight and obesity (29.7% and 11.3%, respectively) recently reported for the current population (133) could provide the basis for the high prevalence of IR observed in this study. However, as there is no widely acceptable definition for IR, comparisons of prevalence data reported in the current and by other studies are not feasible (134)

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Table 4. Prevalence of insulin resistance in children.

	Boys (n=1097)	Girls (n=1098)	Total Sample (n=2195)
	n (%)	n (%)	n (%)
Insulin Resistance (HOMA-IR<3.16)	249 (22.7)	375 (34.2)[†]	624 (28.4)

[†]Significantly different from boys (chi-square test).

Papers II

Greece is one of the leading countries in Europe with respect to the prevalence of childhood obesity (135, 136). As adipose tissue accumulates in excessive amounts in the body several metabolic alterations begin to occur (35). Research has shown that overweight and obese children are at higher risk for the development of adverse lipidaemic and glycaemic profiles compared to lean children (137). While levels of obesity among Greek children are increasing, physical activity and consequently fitness levels are decreasing (138). There is strong evidence for an inverse association of physical activity and fitness levels with cardiometabolic risk (139).

In adults, there is strong evidence supporting that low fitness is a stronger risk factor for CVD than obesity (140, 141). However, in children, there are few studies examining the combined effect of different levels of body weight and physical fitness on cardiometabolic risk factors (142). Taking the above into consideration Paper II aims to confirm the strong link between childhood obesity and cardiometabolic risk and to examine whether the negative effect of fatness is greater than the protective effect of fitness on several cardiometabolic risk indices in children.

The findings of the present study indicate that the lipidaemic and glycaemic profiles were better in the ‘leaner and less fit’ compared to the ‘heavier and more fit’ groups in both sexes (*Table 5*). The interpretation of the diametrically opposed findings reported for children in the current study especially when compared to adults could be based on a developmental basis. More specifically, younger people have healthier tissues to begin with and their homeostatic mechanisms work better (143). A young, lean and unfit person may deal better with the cacostatic load of unfitness than a middle-aged, lean and unfit person. The opposite may be true for a young, fat but fit person, on whom the cacostatic load of obesity may be higher compared to that of a middle-aged, fat and fit person.

Table 5. Cardiometabolic risk profile indices across BMI and fitness groups presented by gender.

	Leaner and less fit	**Intermediate groups**	**Heavier and more fit**	***P*-value***
Boys	**(n=158)**	**(n=957)**	**(n=107)**	
TC (mg/dl)	170.5 ± 30.5	167.3 ± 32.5	168.1 ± 33.4	0.500
HDLC (mg/dl)	63.3 ± 15.3†	58.6 ± 15.2	57.6‡ ± 12.3	0.003
LDLC (mg/dl)	96.5 ± 25.7	96.6 ± 28.1	99.3 ± 30.5	0.196
Triacylglycerols (mg/dl)	53.6 ± 23.6	60.8 ± 29.6	56.4 ± 24.0	0.049
TC/HDLC	2.8± 0.7 †	3.0 ± 0.8	3.02‡ ± 0.7	0.005
HDLC/LDLC	0.7 ± 0.2	0.6 ± 0.3	0.6 ± 0.6	0.846
Glucose (mg/dl)	92.7 ± 10.1	93.3 ± 10.2	93.8 ± 10.0	0.270
Insulin (μU/ml)	8.4 ± 4.6†	11.3 ± 9.2	11.1‡ ± 6.1	0.002
HOMA–IR	1.9± 1.1†	2.6 ± 2.2	2.5‡ ± 1.3	0.005
SBP (mmHg)	114.2 ± 10.6†	121.3 ± 13.4	123.7‡ ± 14.4	<0.001
DBP (mmHg)	65.8 ± 9.6†	69.3 ± 10.0	70.0‡ ± 9.3	<0.001

Girls	(n=231)	(n=849)	(n=108)	
TC (mg/dl)	164.2 ± 32.2	166.0 ± 30.9	162.4 ± 30.6	0.883
HDLC (mg/dl)	60.5 ± 14.2†	57.3 ± 14.5	56.3 ± 14.0‡	0.003
LDLC (mg/dl)	92.0 ± 25.6	92.7 ± 25.1	94.3 ± 25.6	0.682
Triacylglycerols (mg/dl)	58.7 ± 18.6†	67.9 ± 29.7	66.7 ± 25.0‡	0.001
TC/HDLC	2.8 ± 0.5†	3.0 ± 0.8	3.0 ± 0.7‡	0.005
HDLC/LDLC	0.7 ± 0.2†	0.6 ± 0.3	0.6 ± 0.3‡	0.002
Glucose (mg/dl)	91.1 ± 9.2	91.1 ± 9.3	90.8 ± 8.2	0.525
Insulin (μU/ml)	9.7 ± 4.9†	14.0 ± 8.2	15.5 ± 7.7‡	<0.001
HOMA–IR	2.2 ± 1.1†	3.1 ± 1.7	3.4 ± 1.7‡	<0.001
SBP (mmHg)	117.0 ± 12.3†	121.3 ± 13.5	120.6 ± 13.3‡	<0.001
DBP (mmHg)	69.3 ± 9.7	71.3 ± 9.7	71.0 ± 9.8	0.214

TC, Total cholesterol; HDLC, High-density lipoprotein cholesterol; LDLC, Low-density lipoprotein cholesterol; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.
*Leaner and less fit: 1st quartile of BMI and 1st quartile of ERT score; Heavier and more fit: 4th quartile of BMI and 4th quartile of ERT score; Intermediate groups: all other children
*Derived from a four-level random intercept linear regression analysis, after controlling for children's Tanner stage and parental educational level
† *P<0.05* "Leaner and less fit" group vs. other two groups; ‡ *P<0.05* "Heavier and more fit" group vs. other two groups

In summary, Paper II reported a lower cardiometabolic risk for 'leaner and less fit' children compared to their 'heavier and more fit' peers. These differences suggest that fatness may attenuate the benefits of fitness and physical activity on CVD risk factors in children and that, contrary to the findings in adults, leanness may be more important, from a cardiometabolic health benefit perspective, compared to fitness in children. An intriguing question, then, is at what age the situation is reversed, suggesting the need for future research.

Papers III and IV

It is well established that excess adiposity in both adults and children is strongly associated with several cardiometabolic complications (i.e., metabolic syndrome, insulin resistance, and dyslipidaemias) (144, 145). In addition, although contradictory at first sight, obesity has also been associated with nutritional insufficiencies (43, 44). Specifically, overweight and obese individuals seem to be at higher risk for iron deficiency (ID) than those having normal body weight (45-47) and these findings have been consistent in both children (48-51) and adults (51-53). In papers III and IV we set out to investigate the double burden of iron deficiency, with or without anaemia, and excess adiposity assessed either with BMI and WC or with total and visceral fat mass levels in 9-13 year children.

Overall, the prevalence of ID reported by the present study was found to be remarkably high for obese as well for centrally obese children and for children in the highest quartiles of percentage body fat and visceral fat mass quartiles, ranging from 16.0 to 27.0%. *Table 6* presents the prevalence rates of ID and ID anaemia by weight status groups in the population under study.

Table 2. Prevalence of iron deficiency and iron deficiency anaemia by weight group in children.

Iron status categories (% of total)	**Normal-weight**	**Overweight**	**Obese**	*P*-value[‡]
Boys	**(n = 685)**	**(n = 391)**	**(n = 165)**	
ID	15.5[a,b]	20.3[b,c]	28.6[a,c]	< 0.001
IDA	1.4[a]	2.8	5.3[a]	0.029
Girls	**(n = 751)**	**(n = 380)**	**(n = 120)**	
ID	14.3[a]	18.2[b]	28.9[a,b]	0.002
IDA	2.0[a]	3.3[b]	8.2[a,b]	0.003

ID, iron deficiency (with or without anaemia); IDA, iron deficiency anaemia;
[‡]Derived from the Pearson χ^2 test. Values marked with the same superscript letters are significantly different ($P < 0.05$ after two-sample Z-test, as appropriate).

These values are similar or higher than those reported for overweight Swiss and Israeli children and adolescents (i.e., 20% and 12%, respectively) (50, 146). In these two studies, as well as in the current one, the definition of ID was based on reliable biochemical markers of iron status, such as serum transferrin receptor concentration, TS and free erythrocyte protoporphyrin concentration. However, the prevalence of ID reported by the present and the two aforementioned studies was considerably higher compared to similar data from the United States of America (48), since, according to the 3rd National Health and Nutrition Examination Survey (NHANES), the prevalence of ID in obese children and adolescents was 2.4% and 9.1%, respectively. The use of serum ferritin as one of the diagnostic criteria for ID could probably provide an explanation for the lower prevalence of ID observed in the American population. Ferritin is an acute-phase protein and its serum levels are plausibly elevated in states of chronic or acute inflammation (147). Considering that excess total and, especially, visceral fat mass represents a chronic inflammatory state, the increased levels of serum ferritin in overweight and obese children could have contributed to an underestimation of the prevalence of ID in this cohort. This hypothesis is strengthened by a more recent study that also used NHANES data to examine the incidence of ID among overweight female adolescents (46).

The mechanism linking obesity with ID has not yet been elucidated. One proposed such mechanism is low dietary iron intake by overweight individuals. Still, the available literature in this regard remains controversial. Although a study on Greek adolescents reported low iron intakes by overweight pupils (148), other recent studies did not find any significant differences in iron intake between obese and non-obese children and adolescents (46, 52, 146). Similarly, the present study did not show any significant differences in dietary iron intake between weight and waist circumference groups and among quartiles of total and visceral fat mass. Differences in the dietary intakes of specific nutrients that either enhance (vitamin C) or inhibit (calcium, dietary fibre) intestinal non-haeme iron absorption (149) could also provide an explanation for the depleted iron stores observed in children with increased adiposity. However, in line with other recent studies (52, 56, 146), the present study did not detect any such differences, with the exception of dietary fibre intake, which was higher in obese compared to normal-weight girls.

The positive associations found in the present study between total and regional excess adiposity indices and ID in both sexes (*Table 7*) support the hypothesis that the bioavailability of iron may be modulated by factors linked to chronic inflammation induced by excess adiposity. In particular, the increased levels of inflammatory cytokines found in obese individuals (150) have been inversely associated with serum iron levels (56). Low iron bioavailability as a result of increased sequestration by high amounts of ferritin has also been proposed as a factor contributing to the hypoferremia of obesity (151). Furthermore, serum levels of hepcidin, which is the main regulatory hormone of iron absorption and recirculation, were higher in overweight children, adolescents (54, 146) and adults (56) than in normal-weight peers. Adipose tissue-derived cytokines (such as interleukin-6 and leptin), produced in response to obesity related-inflammation, promote hepcidin gene transcription (152). This results in increased serum hepcidin levels that lead to the sequestration of iron within the reticuloendothelial system and to decreased dietary iron absorption from the intestine by controlling the expression of ferroportin 1 at the basolateral membranes of the enterocytes. Consequently, there is hypoferraemia and diminished availability of iron for erythropoiesis (153).

Table 7. Adjusted odds ratios (95% confidence intervals) for Iron Deficiency with and without anaemia among children with different BMI, waist circumference, percentage of body fat and visceral fat mass, controlling for other important covariates.

	Boys (n= 740)			**Girls (n= 753)**		
	n	Model 1	Model 2	n	Model 1	Model 2
Weight Categories						
Normal-weight	421	1.00	1.00	461	1.00	1.00
Overweight	226	2.14 (1.28-3.54)	2.13 (1.27-3.60)	215	1.09 (0.65-1.84)	1.11 (0.64-1.94)
Obese	93	1.64 (0.80-3.34)	1.62 (0.78-3.37)	77	2.43 (1.29-4.58)	2.25 (1.14-4.46)
Waist Circumference groups						
Normal	613	1.00	1.00	614	1.00	1.00
Central Obesity	127	1.62 (0.93-2.84)	1.64 (0.92-2.95)	139	2.39 (1.44-3.96)	2.23 (1.30-3.83)
Body Fat Quartiles						
Lower quartile	183	1.00	1.00	189	1.00	1.00
Medium quartiles†	374	1.24 (0.65-2.35)	1.19 (0.62-2.30)	371	1.31 (0.71-2.43)	1.12 (0.59-2.15)
Higher quartile	183	2.48 (1.28-4.81)	2.48 (1.26-4.88)	193	2.23 (1.16-4.27)	2.12 (1.07-4.20)
Visceral Fat Quartiles						
Lower quartile	217	1.00	1.00	188	1.00	1.00
Medium quartiles†	346	1.81 (0.98-3.35)	1.71 (0.92-3.20)	381	1.27 (0.70-2.32)	1.30 (0.69-2.45)
Higher quartile	177	2.16 (1.11-4.23)	2.12 (1.07-4.19)	184	2.06 (1.08-3.93)	2.17 (1.09-4.34)

Model 1: unadjusted; Model 2: adjusted for dietary iron, calcium, vitamin C and fiber intake, maternal educational level, Tanner stage and menarche (girls only).
†Quartiles 2 and 3 in each case were combined to the "medium" quartiles.

Further to the above and as summarized in *Table 8* overweight and obese children were found to have significantly higher serum levels of cytokines (i.e. IL-6 and CRP) and hepcidin (at borderline significance) as well as in the prevalence of ID compared to their normal-weight peers.

Table 8. Differences in serum cytokines and hepcidin levels (all as mean ± SD) and in the prevalence of iron deficiency in children of different weight status (n=1812)

	Normal-weight	**Overweight**	**Obese**	***P* value**
CRP (ng/ml)	769.9 ± 1465.1[a,c]	1608.7 ± 2222.6[b,c]	2900.1 ± 2902.4[a,b]	< 0.001[§]
IL-6 (pg/ml)	0.98 ± 0.95[a,c]	1.17 ± 1.04[b,c]	1.29 ± 0.80[a,b]	< 0.001[§]
Hepcidin (ng/ml) [†](n=395)	170.0 ± 93.3	201.9 ± 122.2	196.7 ± 120.9	0.050[*]
Iron deficiency (%)	17.4[a]	19.6[b]	28.2[a,b]	0.001[‡]

[§]Derived from the non-parametric Kruskal-Wallis test; [*]Derived from one-way ANOVA, [‡]Derived from Pearson chi-square test.

[a,b,c]: Identical superscript letters denote significant differences between groups ($P < 0.05$ after *post-hoc* multiple comparisons using the Bonferroni rule in the case of normally distributed continuous variables, Mann-Whitney test in the case of non-normally distributed continuous variables and the 2-sample Z-test for proportions in the case of categorical variables).

[†]: Parameter was log-transformed.

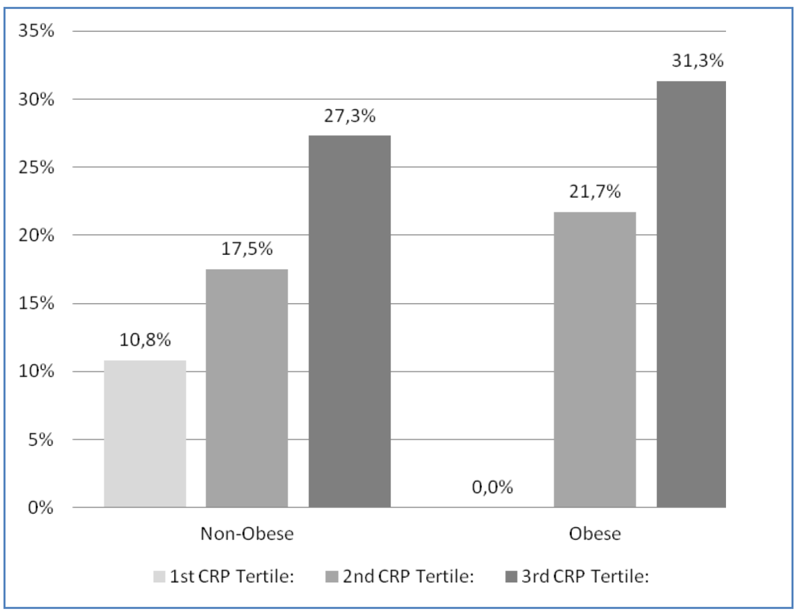

Figure 5. Prevalence of iron deficiency by tertile of CRP levels in non-obese (that is, normal-weight and overweight) and obese children

In an attempt to further elucidate the association between ID and obesity-induced inflammation the present study showed an increasing trend in the prevalence of ID with increasing CRP levels, in obese children ($P< 0.001$).

These associations may have important public health implications, since they point to the need of taking into account children's weight status when providing recommendations for dietary iron intake or when assessing children's iron status.

3.2. Diet and physical activity behaviours related to children's health status (Papers V and VI)

Papers V and VI

Although childhood obesity has strongly been associated with several energy balance related behaviours, the actual aetiology of increased adiposity in children requires further investigation due to its complex and multifactorial nature. In this context, it is very important to examine the possible synergistic effect of several lifestyle factors on adiposity and related metabolic complications, such as dyslipidaemias, in children (65). A common approach to examine the possible synergy of such factors is the development of specific dietary and lifestyle patterns. Principal component analysis (PCA) is frequently used for this purpose, since it enables the grouping of several strongly associated behaviours into patterns. Therefore, the aims of papers V and VI were to examine dietary and other lifestyle patterns associated with adiposity and blood lipid levels in children, offering valuable information in the field and possibly in childhood obesity and adult cardiovascular risk reduction strategies.

The principal component analysis indicated five lifestyle components (*Table 9*) explaining 52.7% of the total variance with regards to the examined variables. These components were characterized as follows: higher dairy consumption and more adequate breakfast consumption (component 1); higher consumption of high fibre foods (component 2); more screen time, shorter sleep duration and higher consumption of sugared-sweetened beverages (component 3); more time spent on MVPA and more eating occasions during the day (component 4); higher red meat and lower fish consumption (component 5).

Table 9. Factor loadings derived from principal component analysis regarding dietary and lifestyle factors of children 9-13 years old.

	Component				
Predictors	**1**	**2**	**3**	**4**	**5**
Dairy consumption [a]	0.79*	0.15	-0.11	0.10	0.002
Fruit consumption	0.05	0.62*	-0.01	0.15	0.000
Vegetable consumption	-0.05	0.74*	0.11	-0.04	0.04
Whole grain products consumption [b]	0.11	0.50*	-0.22	-0.09	-0.11
Red meat consumption	0.01	-0.06	0.07	0.12	0.72*
Fish consumption	-0.01	-0.01	0.07	0.11	-0.75*
Sugared drinks consumption [c]	-0.17	-0.15	0.56*	0.36	0.11
Adequate breakfast consumption	0.76*	-0.09	-0.02	-0.13	0.03
Meal frequency	0.45*	0.35	0.12	0.48*	-0.04
Screen time [d]	0.04	-0.06	0.68*	-0.21	-0.02
Time spent on physical activity [e]	-0.04	-0.004	-0.12	0.82*	-0.001
Sleep time [f]	0.03	-0.07	-0.58*	0.01	0.03
Explained variance (%)	12.1	11.4	10.2	9.7	9.3

[a] Consumption of milk, yogurt and cheese
[b] Consumption of whole grain bread and cereal
[c] Consumption of regular soft drinks and sugared fruit juices
[d] Time spent on watching TV/DVD/video and/or using games consoles/computer for fun
[e] Time spent on moderate to vigorous physical activity
[f] Night sleep time on weekdays
[*] Predictor with the highest factor loading (>|0,4|) within the component

The findings of papers V and VI are supportive of the synergistic effects of distinct lifestyle-related behaviours on childhood obesity and related cardiometabolic risk prevention. More specifically, the following three dietary and lifestyle behavioural patterns were negatively associated with several indices of total and regional adiposity in children: a) higher consumption of dairy foods and adequate breakfast consumption, b) increased consumption of high-fibre foods and c) more time of children's engagement into MVPA combined with an frequent meals consumed during the day. Last but not least and as presented in *Table 10*, the pattern comprising more than approximately 45 min of MVPA and more than 5 eating occasions per day was found to be significantly associated with reduced likelihood of dyslipidaemias in schoolchildren.

Table 10. Mean levels of behaviors comprising lifestyle components 3 and 4 and likelihood of dyslipidemias across quartiles of lifestyle components 3 and 4.

	Quartiles of lifestyle component 3 (more screen time, shorter sleep duration and higher consumption of sugared beverages)				
	1st quartile	**2nd quartile**	**3rd quartile**	**4th quartile**	***P*-value**
Behaviours of component 3	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Screen time (hrs/day)	1.35±0.69	1.85±0.83	2.42±0.97	3.61±1.68	<0.001
Sleep duration (hrs/day)	9.42±0.58	8.98±0.52	8.73±0.55	8.35±0.73	<0.001
Sugared beverages consumption (grams/day)	21.9±57.8	44.2±82.7	82.5±117.1	221.9±222.4	<0.001*
Serum lipids		OR (95% CI)	OR (95% CI)	OR (95% CI)	
HDL cholesterol<35mg/dl†	1.00	0.650 (0.309-1.368)	1.025 (0.530-1.983)	1.480 (0.802-2.731)	
Total: HDL cholesterol >4.5†	1.00	1.056 (0.563-1.979)	1.229 (0.671-2.252)	0.929 (0.490-1.764)	
	Quartiles of lifestyle component 4 (more time spent on physical activity and more eating occasions)				
	1st quartile	**2nd quartile**	**3rd quartile**	**4th quartile**	***P*-value**
Behaviors of component 4	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Time spent on MVPA (min/day)	20.0±22.6	44.8±28.6	72.4±37.7	142.0±67.7	<0.001*
Meal frequency (no. of meals/ day)	4.10±0.84	4.70±0.74	5.05±0.75	5.22±0.72	<0.001
Serum lipids		OR (95% CI)	OR (95% CI)	OR (95% CI)	
Total cholesterol>170 mg/dl†	1.00	0.703 (0.554-0.909)	0.705 (0.544-0.914)	0.619 (0.476-0.805)	
LDL cholesterol>110 mg/dl†	1.00	0.674 (0.507-0.896)	0.695 (0.522-0.925)	0.667 (0.499-0.890)	
Total: HDL cholesterol>4.5	1.00	0.569 (0.320-0.931)	0.308 (0.153-0.620)	0.603 (0.342-0.980)	

Abbreviations: OR: Odds Ratio; CI Confidence Interval

Adjusted for gender, Tanner stage, waist circumference, parental BMI, SES and birth weight.

* *P*-value derived from the non-parametric Kruskal-Wallis test.

† The cut-off values were adapted from NCEP guidelines for children and adolescents.

3.3. Determinants of behaviours related to children's health status (Papers I, VII and VIII).

Paper I

Several socio-economic and demographic factors that have been reported by previous studies as correlates of childhood overweight (154, 155) were also tested in paper I. Firstly, lower-medium annual family income of 12,000 to 20,000 was associated with an increased likelihood of childhood overweight/obesity compared to the higher annual family income level of >30,000 €, both at a bivariate and at a multivariate level. Furthermore, Greek nationality was also found to increase the likelihood of overweight and obesity in children. The vast majority of non-Greek children, whose parents came to Greece as economic immigrants during the period of the economic and political transition in the 1990's, were from Albania (75.6%) and from other eastern European countries (i.e. Russia, Ukraine, Poland, Serbia etc) (12,6%). In these countries Lobstein and Frelut (121) reported lower prevalence rates of childhood overweight in comparison to children living in non-eastern bloc European countries. The implication of this observation is that the lower prevalence of overweight also reported in the present study for non-Greek subjects, having mainly an eastern European nationality, could be attributed to a genetic predisposition for lower BMIs compared to Greek children. To our knowledge no evidence exists on this topic, therefore further research, including the study of certain genes and polymorphisms, is needed in order to shed more light to these differences.

Other important findings of Paper I were the inverse association observed between children's "popularity" score with overweight/obesity and the increased likelihood of overweight/obesity for children whose primary caregiver was their grandmothers. A potential explanation of the first association might be the fact that more athletic, normal-weight children are usually more popular amongst their peers. Previous studies have also confirmed the association observed in the present study between weight status and social acceptance in populations of preadolescents. Franklin et al (156) reported that overweight was an index of less social acceptance in girls aged 9-14 years. Furthermore, Latner and Stunkard (157) reported that overweight and obese 10-11 year-old children were significantly less popular than their normal-weight peers. Regarding the second association, a recent study showed that food consumption in children was adjusted according to the stimuli induced by the food served by their caregiver, instead of being regulated by the satiety and increased energy following the previously consumed meals (158). In most cases grandparents tend to believe that overweight signifies healthiness (159) or that young children may grow out of being overweight (160) thus tending to overfeed their grandchildren. Furthermore, Gordon-Larsen et al (161) in a study of girls aged 6-9 years observed that caregivers, including grandmothers, influenced their children in adopting a sedentary lifestyle, as they considered sedentary habits, such as TV viewing, an easier way of having control of their children. All abovementioned associations are summarised in *Table 11.*

The associations observed in the present study could have important future public health implications for the prevention of childhood obesity. Appropriate interventions and population-based strategies are needed, targeting lower income families, less popular children and probably families with both parents working and grandmothers as children's primary caregiver.

Table 11. Crude and adjusted Odds Ratios (OR) and 95% Confidence Intervals (95%C.I) for the association between overweight/obesity with several socio-economic and demographic factors in "full participants" (n=729). (Cells in bold indicate statistically significant Odds Ratios)

	Overweight/Obese		
	%	Crude OR (95% CI)	Adjusted OR† (95% CI)
Annual Family Income (€ per year)			
>30,000	38.5	1.00	1.00
<12,000	33.9	0.81 (0.54-1.27)	1.05 (0.61-1.86)
12,000-20,000	49.7	**1.56 (1.07-2.35)**	**1.61 (1.03-2.61)**
20,000-30,000	41.0	1.06 (0.70-1.79)	1.02 (0.62-1.67)
Parents' Nationality			
Non-Greek nationals	35.8	1.00	1.00
Greek nationals	44.1	**1.38 (1.03-1.96)**	1.04 (0.65-1.68)
Residence ownership			
No	34.4	1.00	1.00
Yes	46.7	**1.68 (1.22-2.25)**	1.58 (0.98-2.20)
Child's primary caregiver			
Parents	39.0	1.00	1.00
Grandfather	48.8	1.43 (0.74- 2.85)	1.66 (0.83- 3.19)
Grandmother	46.9	**1.38 (1.03 – 1.98)**	**1.53 (1.05 – 2.29)**
Nanny/ Someone else	45.9	1.34 (0.67- 2.63)	1.62 (0.82- 3.10)
"Popularity" score	-	**0.40 (0.17-0.97)**	1.09 (0.65-1.84)
SES of residence region			
Lower	38.6	1.00	
Medium	42.8	1.15 (0.81-1.62)	-
Higher	39.9	1.05 (0.72-1.58)	
Fathers' age categories			
Lower Tertile (<41 years old)	43.6	1.00	
Medium Tertile (41-46 years old)	37.9	0.80 (0.57-1.14)	-
Higher Tertile (>46 years old)	44.0	1.00 (0.66-1.48)	
Mothers' age categories			
Lower Tertile (<38 years old)	44.4	1.00	
Medium Tertile (38-42 years old)	40.1	0.83(0.57-1.18)	-
Higher Tertile (>42 years old)	40.1	0.81 (0.55-1.22)	
Paternal Educational level			
Low (≤ 9 years of education)	39.1	1.00	
Medium (> 9 and ≤ 14 years of education)	45.5	1.26 (0.78-1.92)	-
High (>14 years of education)	36.6	0.93 (0.59-1.46)	
Maternal Educational level			
Low (≤ 9 years of education)	38.6	1.00	
Medium (> 9 and ≤ 14 years of education)	42.7	1.17 (0.74-1.80)	-
High (>14 years of education)	42.3	1.13 (0.69-1.85)	
Parental marital status			
Core Families-Married Parents	41.6	1.00	
Single parent families-Not married Parents	53.3	1.50 (0.52-4.26)	-
Single parent families-Widowed Parent	53.8	1.61 (0.52-4.91)	
Single parent families-Divorced Parents	42.3	1.05 (0.58-1.89)	
Family size (no. of family members)	-	0.94 (0.77-1.11)	-

Papers VIII

Heath status indices (123) seem to follow a socioeconomic and demographic distribution. However, the available evidence on the relationship between health status in children and family socio-demographics is scarce. Paper VIII, revealed several significant positive univariate associations of IR in children, with female sex and non-Caucasian race. On the other hand, higher paternal educational level (i.e. >16 years) and higher annual family income (i.e. > 30,000 €/year) decreased the likelihood of IR. Although these associations have also been reported in earlier studies (162), in our study, most of these correlation lost their statistical significance when they were tested at a multivariable level. Female children and those of families with higher (12,000-30,000 € and >30,000 €) annual income were the two main socio-demographic indices that were independently associated with IR at a multivariable level (*Table 12*).

While the increased odds for IR in girls compared to boys could be attributed to their earlier pubertal development and the subsequent hormonal changes, still adjustments made for Tanner stage did not weaken the statistical significance of this association (163). The sex difference in insulin resistance could not be explained by differences in individual anthropometric variables, since compared with girls, boys had significantly higher BMI levels. Sex-linked genes may account for the intrinsic sex difference observed and such genes may have an important impact on the development of insulin resistance (164). Regarding the association reported in the current study between lower annual family income and IR in children, there is general consensus in social and health sciences of an increased risk for obesity and related metabolic disorders, including IR, in children of lower socio-economic status in several developed countries (165). Lower socio-economic status (SES) of the family most commonly defined as lower family income or parental educational level has been reported to be positively related to adverse health behaviors followed by children, including higher consumption of high energy and glycemic index foods and beverages, such as fats, sweets, sugar-sweetened beverages etc. (166-168). Furthermore, mothers of lower SES could be less aware of healthy practices during their child's early development, thus increasing the odds for smoking and excess weight gain during pregnancy, formula- instead of breast-feeding of their baby, etc.

Table 12. Adjusted‡ odds ratios (95% confidence intervals) for the association of family socio-economic and demographic factors with insulin resistance in children.

	Odds ratio (95% confidence interval)	
	Insulin resistance	P-value
Maternal menarche age (years)	1.01 (0.93-1.09)	0.864
Race		
Caucasians	1.00	
Non-Caucasians	1.68 (0.81-3.46)	0.161
Paternal education		
< 9 years	1.00	
9-12 years	0.76 (0.52-1.06)	0.088
12-16 years	0.99 (0.70-1.41)	0.964
> 16 years	0.78 (0.48-1.28)	0.329
Maternal education		
< 9 years	1.00	
9-12 years	1.22 (0.89-1.68)	0.224
12-16 years	1.15 (0.80-1.65)	0.449
> 16 years	1.14 (0.65-2.01)	0.638
Family income (€/year)		
< 12,000	1.00	
12,000-30,000	**0.71 (0.53-0.95)**	0.020
> 30,000	**0.68 (0.48-0.96)**	0.029
Gender		
Boy	1.00	
Girl	**1.67 (1.30-2.13)**	<0.001

Figures in bold indicate statistically significant odds ratios.
‡Adjusted for children's BMI , Tanner stage, parental weight status and certain perinatal factors.

Paper VII

Parents are very influential in shaping early dietary and physical activity patterns in their children and their ability to recognize the problem of increased adiposity in their own child has been reported as another important determinant of childhood overweight (169). In general it is very critical for parents to be able to make a distinction between normal versus abnormal levels of adiposity to ensure that appropriate preventive and corrective interventions are implemented. However, several studies have shown that a significant number of parents are unable to correctly perceive and report their child's weight status either verbally or visually (120, 170-182) or in both ways (183-185). This observation implies that public health messages regarding the prevention of childhood obesity may not always reach parents who often fail to identify excessive body weight in their children. The specific characteristics of parents that are more likely to misperceive their children's actual weight status have not yet been identified. The objectives of paper VII were to examine the accuracy of maternal ability to correctly classify their children's weight status using a verbal and a visual classification instrument and to assess the level of parental underestimation of overweight and obese children weight status .

Between the two different instruments used to assess maternal perceptions of their children's weight status, the verbal one was proved to be more accurate than the visual one in correctly classifying children's actual weight status. More specifically, 15% of mothers verbally underestimated their children's weight status, when they were asked to report their child's actual body weight and height. On the other hand, 41.3% of mothers visually underestimated their children's weight status, when they were asked to choose one photograph presenting body images of boys and girls in different BMI-for-age percentiles that most resembled their child's body shape. These underestimation rates, summarized in *Table 13,* were found to be even higher for overweight and obese children compared to normal weight ones, reaching 87.9% and 82.1%, respectively, in overweight and obese boys, whose weight status was visually assessed by their mothers. These findings were in agreement with those reported by other studies that examined parental perceptions of their children's weight status (120, 170-175, 177, 181-183).

Table 13. Maternal verbal and visual classification of children's weight status by children's actual weight status.

	Children's Actual Weight Status							
Primary Caregiver's classification of their children's weight status:	Underweight (n=56)		Normal-weight (n=1019)		Overweight (n=574)		Obese (n=209)	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
Verbal	%	%	%	%	%	%	%	%
Underweight	**61.3**	**42.9**	4.1[†]	4.8[*]	0.0	0.8	0.0	0.0
Normal-weight	35.5	57.1	**89.9**	**84.8**[*]	29.1[†]	21.2[*]	4.1[‡]	1.8[*]
Overweight	3.2	0.0	5.8[†]	9.7[*]	**67.7**[†]	**72.4**[*]	41.9	37.3
Obese	0.0	0.0	0.2	0.7	3.1	5.1	**54.1**	**60.9**[*]
Visual								
Underweight	**47.1**	**50.0**	11.2[†]	25.4[*]	0.3	0.7	1.2	0.0
Normal-weight	52.9	50.0	**87.3**	**72.9**[*]	67.1[†]	87.2[*]	20.9[‡]	48.0[*]
Overweight	0.0	0.0	1.1[†]	1.3[*]	**22.7**[†]	**9.7**[*]	30.2	34.1
Obese	0.0	0.0	0.4	0.4	9.8	2.4	**47.7**	**17.9**[*]

Percentages in bold indicate correct weight status classification.
[*]: *P*<0.001 for differences between verbal and visual classification of girls' weight status, based on the two-sample Z-test.
[†]: *P*<0.001 and [‡]: *P*<0.01 for differences between verbal and visual classification of boys' weight status, based on the two-sample Z-test.

3.4. Perinatal factors related to children's health status (Paper VIII)

Paper VIII

Insulin resistance (IR) represents a frequent clinical condition among children and adolescents (186). It reflects the impaired response of tissues to insulin-mediated cellular glucose uptake (187, 188). Although the end points for cardiometabolic risk are not seen in childhood, insulin resistance usually tracks from childhood to adulthood, indicating that the precursors of cardiovascular disease are present early in life (189). Therefore, identification of early life risk factors that predispose children to IR could be an important piece of information for researchers, clinicians, and public health policymakers for establishing appropriate initiatives that will facilitate early prevention. Following another recently published paper from the Healthy Growth study that examined the perinatal factors associated with childhood obesity (190), paper VIII aims to identify, among a wide range of perinatal factors, those independently associated with IR in late childhood, also taking into account certain family socio-demographic and parental characteristics.

This study revealed a relatively high prevalence of IR (28.4%) among 9-13 year-old schoolchildren. As displayed in *Table 14*, paper VIII identified small birth weight and female sex as the only perinatal factors independently associated with the occurrence of IR in late childhood, when examined at a multivariable level with a wide range of perinatal indices, as well as certain family socio-demographic and parental characteristics. Considering that foetal size at birth reflects the product of the nutritional and hormonal milieu in which the foetus develops, controlling the trajectory of foetal growth via optimization of maternal nutrition and health status during pregnancy seems fundamental (191).

Table 14. Adjusted‡ odds ratios (95% confidence intervals) for the association of perinatal factors with insulin resistance in children.

	Odds ratio (95% confidence interval)	
	Insulin resistance	P-value
Gender		
Boy	1.00	
Girl	**1.67 (1.30-2.13)**	<0.001
Mothers' pre-pregnancy weight status		
Normal-weight	1.00	
Underweight	0.79 (0.49-1.23)	0.360
Overweight	0.99 (0.69-1.43)	0.965
Obese	1.66 (0.90-3.04)	0.103
Maternal smoking during pregnancy		
Not Smoking	1.00	
Smoking 1st trimester	1.65 (0.77-3.53)	0.195
Smoking 2nd and 3rd trimester	0.57 (0.27-1.20)	0.136
Smoking 1nd and 2nd trimester	1.15 (0.35-3.76)	0.821
Smoking all trimesters	0.88 (0.61-1.26)	0.474
Use of folic acid: 2nd pregnancy trimester		
No	1.00	
Yes	0.86 (0.52-1.41)	0.550
Use of folic acid: 3rd pregnancy trimester		
No	1.00	
Yes	0.92 (0.55-1.53)	0.748
Birth weight for gestational age		
Appropriate (10th-89th percentile)	1.00	
Small (<10th percentile)	**1.41 (1.03-2.01)**	0.048
Large (>90th percentile)	**0.54 (0.34-0.87)**	0.012
Weight gain in the first 6 months		
Average (-1 to +1 z-score change)	1.00	
Poor (< -1 z-score change)	0.97 (0.65-1.44)	0.869
Rapid (> +1 z-score change)	1.08 (0.83-1.40)	0.563

Figures in bold indicate statistically significant odds ratios.
‡Adjusted for children's BMI, Tanner stage, parental weight status and family socio-demographics.

3.5. Development of easy to use tools to assist health professionals in the design of effective nutrition counselling and health education programs (Paper IX).

Papers IX

Unhealthy dietary and other lifestyle patterns have been reported as significant risk factors for both childhood obesity and ID (48). Considering the above, development of practical tools that will provide a quantitative measure of such patterns, also applicable in assessing the risk for both obesity and ID seem very promising from a public health perspective. The development of an index suitable for this purpose could be one such approach. Using the preliminary data from the Healthy Growth Study we have previously developed the Healthy Lifestyle-Diet Index (HLD-index) to evaluate the degree of adherence to existing dietary and lifestyle guidelines for school-aged children from the USDA's My Pyramid (www.mypyramid.gov) and reported significant associations with insulin resistance (192). The aim of paper IX was to revise the HDL-index in order to more accurately assess the degree of children's adherence to existing age-appropriate dietary and lifestyle guidelines based on USDA's Choose my Plate (www.choosemyplate.gov) and to examine the association of the revised HDL-index (R-HDL-index) with obesity and ID.

The scoring system of the revised HLD-index (R-HLD-index) is presented in *Table 15*.

Table 15. The scoring system of the revised HLD-index (R-HLD-index)

Index components	***Frequency***	***Score***
Fruit	Never or 1-6 servings per week containing <50% juice	0
	1-6 serving per week containing <50% juice or >3 servings per day >50% juice	1
	>3 servings per day containing <50% juice	2
	1-3 servings per day containing >50% juice	3
	1-3 servings per day containing <50% juice	4
	Never	0
Vegetables	1-6 servings per week	1
	1-2 servings per day or >4 servings per day	2
	3-4 servings per day	3
	2-3 servings per day	4
		0
Fish and seafood	Never or rarely	
	≥4 servings per week	1
	1-2 servings per week	2
	3-4 servings per week	3
	2-3 servings per week	4
Sweets	≥1 serving per day	0
	4-6 servings per week	1
	2-4 servings per week	2
	1-2 servings per week	3
	Never or rarely	4
Soft drinks	≥1serving per day	0
	4-6 servings per week	1
	2-4 servings per week	2
	1-2 servings per week	3
	Never or rarely	4
Grains	< 1 serving per day or 1-2 servings per day and <50% whole grains	0
	1-2 servings per day and 50% whole grains or >6 servings and <50% whole grains	1
	2-4 servings per day and <50% whole grains or >6 servings per day and >50% whole grains	2

	2-4 servings per day and >50% whole grains or 4-6 servings per day and <50% whole grains	3
	4-6 servings per day and > 50% whole grains	4
Milk/dairy	<1 serving per day or 1-2 servings per day and >50% low- or no-fat	0
	1-2 servings per day and >50% low- or no-fat, >4 servings per day and <50% low- or no-fat	1
	<4 servings per day and >50% low- or no-fat	2
	2-4 servings per day and <50% low- or no-fat	3
	2-4 servings per day and >50% low- or no-fat	4
Legumes	<1 serving per week	0
	>7 servings per week	1
	1-3 servings per week	2
	5-7 servings per week	3
	3-5 servings per week	4
Eggs	<1 serving per week	0
	>7 servings per week	1
	3-4 servings per week	2
	1-2 servings per week	3
	2-3 servings per week	4
Meat and meat products	<1 serving per week	0
	>4 servings per week	1
	1-2 servings per week	2
	3-4 servings per week	3
	2-3 servings per week	4
TV viewing	>4 h/day	0
	3-4 h/day	1
	2-3 h/day	2
	1-2 h/day	3
	<1 h/day	4
MVPA	0-15 min/day	0
	15-30 min/day	1
	30-45 min/day	2
	45-60 min/day	3
	>60 min/day	4

R-HDL-index, Revised Healthy Lifestyle-Diet index; MVPA, moderate to vigorous physical activity; TV, television.
The total score of R-HDL-Index was obtained summing the scores assigned to each component and this score range between 0 and 48.
Fruits: All fresh, frozen, canned and dried fruits and all fresh or packaged fruit juices with no added sugar or fat. The serving is equal to a medium fruit or ½ cup of fruit juice.
Vegetables: All vegetables with no added sugar or fats. The serving is equal to ½ cup.
Total grains: All whole-grain products and refined grain products and grains, such as breads, rice, pasta, cereals, crackers and oatmeal. The serving is equal to 1 slice of bread, ½ cup of cereals, pasta and rice.
Total dairy products: All milks, yogurts and cheeses. The serving is equal to 1 cup of milk or yogurt and 1.5 oz. of cheese.
Total meat: beef, pork, lamb, goat, meatballs, poultry, rabbits and legumes. The serving is equal to 2 oz.
Fish and seafood: All fishes and octopus, squid, cuttlefish, shrimps and crabs. The serving is equal to 2 oz.
Sweets: All chocolates and cakes, biscuits, cookies, ice cream, butter cookies, traditional sweets, doughnuts, waffles, candies, lollipops, fruits in syrup and jelly beans.
The serving is equal to 1 oz.

The total score derived from the R-HLD-index (higher scores are indicative of healthier diet and lifestyle) was inversely associated with obesity in children, highlighting the importance of the revised index as a useful screening tool to be used in the design of childhood obesity prevention initiatives. However, the R-HLD-index was not found to be significantly associated with ID. *Table 16* presents the associations between the R-HLD-index with childhood obesity as well as with ID.

Table 16. Associations between the R-HLD-index (independent variable), iron deficiency and obesity (dependent variables).

	Dependent variable: Iron deficiency[1]		Dependent variable: obesity[2]	
Independent variable	OR (95% CI)	P-value	OR (95% CI)	P-value
R-HDL-Index	1.020 (0.987-1.055)	0.233	0.940 (0.895-0.988)	0.014
Tertiles of R-HDL-Index				
1st tertile	Reference		Reference	
2nd tertile	1.263 (0.892-1.789)	0.356	0.648 (0.403-1.042)	0.073
3rd tertile	1.095 (0.760-1.578)	0.625	0.576 (0.330-0.973)	0.039

CI, confidence interval; R-HDL-Index, Revised Healthy Lifestyle-Diet Index; OR, odds ratio.
[1]Adjusted for age, sex, Tanner stage, total energy intake, body mass index category.
[2]Adjusted for age, sex, Tanner stage, total energy intake, birth weight, mother's body mass index category and moderate to vigorous physical activity.

4. Conclusions and perspectives

The results presented in the current PhD thesis confirm one of the highest prevalence of overweight and obesity (i.e. approximately 40%) for children in Greece compared to the prevalence rates reported for children in other developed countries worldwide. Furthermore, these results are supportive of the strong link between adiposity and related cardiometabolic complications, namely dyslipidaemias, IR and high blood pressure, while they are also indicative of the "double burden" paradox of obesity-related ID in children. In addition, the assessment and combined investigation in the current thesis of several behavioural indices either as lifestyle patters or as components of the "Healthy Diet and Lifestyle (HLD)" index, revealed several significant associations between these behaviours and all aforementioned clinical outcomes that could easily be translated into practical public health advice. More specifically, a lifestyle pattern comprising higher dairy consumption with adequate breakfast consumption; a second pattern characterized by increased consumption of high fibre foods; and a third pattern combining higher physical activity levels with frequent meals during the day were all found to be significantly associated with a lower likelihood of obesity and increased fat mass levels in children. The latter pattern and precisely engagement in more than 45 minutes of moderate-to-vigorous physical activity daily combined with at least five meals per day was also found to significantly decrease the odds of hypercholesterolemia in children. Moreover small size of infants at birth was the only perinatal factor that was found to significantly increase the likelihood of IR in late childhood when examined at a multivariate level with a wide range of other perinatal factors and after controlling for several possible socio-demographic and clinical confounders. The aforementioned findings were combined in the development of a Healthy Lifestyle and Diet index with higher scores in the behavioural components of this index (indicative of a healthier diet and lifestyle) being associated with lower odds for both obesity and IR in children.

Summarizing these findings, the current PhD thesis has managed to holistically examine a wide range of behavioural, anthropometric and biochemical indices in order to assess health status among children in Greece, as well as related behaviours and their determinants. The perspective derived from all aforementioned findings could be their combined use for developing effective public lifestyle and health recommendations. These recommendations can either be personalised at an individual level (i.e. according to an individual’s behavioural, anthropometric, biochemical, and clinical profile) or tailored to the specific needs of a population group at risk for obesity and related metabolic complications.

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PAPER I

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Social, economic and demographic correlates of overweight and obesity in primary-school children: preliminary data from the Healthy Growth Study

George Moschonis, Sofia Tanagra, Anastasia Vandorou, Aikaterini E Kyriakou, Vasiliki Dede, Paraskevi E Siatitsa, Alexandra Koumpitski, Odysseas Androutsos, Evangelia Grammatikaki, Maria Kantilafti, Anthi Naoumi, Aliki E Farmaki, Aikaterini Siopi, Eleni Z Papadopoulou, Elpida Voutsadaki, Fanouria Chlouveraki, Konstantina Maragkopoulou, Efstathoula Argyri, Aggeliki Giannopoulou and Yannis Manios*
Department of Nutrition & Dietetics, Harokopio University of Athens, 70 El. Venizelou Avenue, 176 71 Kallithea, Athens, Greece

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Abstract

Objective: To record the prevalence of overweight and obesity in urban primary-school children in relation to several socio-economic and demographic factors.
Design: Cross-sectional.
Setting/subjects: A representative sample of 729 schoolchildren (379 male and 350 female), aged 9–13 years, stratified by parental educational level, was examined in the urban region of Athens. Weight and height were measured using standard procedures. The International Obesity Task Force thresholds were used for the definition of overweight and obesity. Several socio-economic and demographic data and the child's 'popularity' score were also recorded with specifically designed standardized questionnaires.
Results: The prevalence of overweight and obesity was 29·6 % and 11·1 %, respectively. Annual family income of €12 000–20 000 (OR = 1·58), residence ownership (OR = 1·63) and the grandmother as the child's primary caregiver (OR = 1·38) were significantly associated with higher odds of childhood overweight and obesity. Non-Greek parental nationality (OR = 0·72) and higher 'popularity' scores of children (OR = 0·42) were significantly associated with lower odds of overweight and obesity. The grandmother as the child's primary caregiver and an annual family income of €12 000–20 000 remained significantly associated with childhood overweight and obesity after adding all significant correlates of childhood overweight and obesity observed at the bivariate level in a multivariate regression model (OR = 1·51 and 1·61, respectively).
Conclusions: Among family income, residence ownership, child's primary caregiver, parental nationality and popularity scores that were identified as significant correlates of childhood overweight and obesity at the bivariate level, lower family income and grandmother as the child's primary caregiver were the only factors that remained significantly associated with childhood overweight and obesity at a multivariate level.

Keywords
Body mass index
Overweight
Children
Socio-economic status

The prevalence of overweight and obesity among Greek children has increased over the past decades[1]. The rates of childhood overweight and obesity in Greece are similar or slightly higher to those reported in other European countries[2,3]. As with adults[4], the adoption of adverse obesogenic behaviours by children seem to have a socio-economic, demographic and cultural dimension[5,6]. Parental educational level, family income, ethnicity and the child's primary caregiver are some of the socio-economic and demographic factors that have been previously suggested to modify children's energy balance behaviours (i.e. dietary and physical activity patterns), thus having either a negative or positive impact on childhood obesity[6,7]. More specifically, previous studies in Greece and other developed countries have reported that paternal and/or maternal educational level was inversely associated with childhood obesity[8–10]. Furthermore, several indices that reflect the family's socio-economic status, such as annual family income, house or car ownership, house size, etc. have also been reported to be inversely

**Corresponding author:* Email gmoschi@hua.gr

related to childhood obesity[11]. Nationality has also been reported to exert a significant effect on childhood obesity, although the limited data available in the literature for European countries provide some contradictory findings on their exact association[10,11].

Clearly, family and other social factors influence children's eating patterns that may subsequently influence the onset of obesity. As children comprise the adult population of tomorrow, recording and understanding the prevalence of obesity in young children and the social, economic and demographic factors related to the phenomenon can facilitate the development of effective public health intervention policies in counteracting obesity and its health consequences later in life. It is known that early prevention is more effective in managing the epidemic of obesity in comparison with treating obesity later in life[12].

The aim of the present study was to record the prevalence of overweight and obesity among 9–13-year-old schoolchildren in the capital city of Athens, the largest urban area of Greece, in relation to several socio-economic and demographic characteristics of children and their families. To test our research hypothesis, the preliminary data from an urban sub-cohort of the 'Healthy Growth Study' were used in the present analyses.

Subjects and methods

Sampling

The 'Healthy Growth Study' is a cross-sectional study initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens. The population under study comprised schoolchildren aged 9–13 years attending the fifth and sixth grades from primary schools located in municipalities within the wider region of Athens. The sampling of municipalities and schools in the present study was random, multi-stage and stratified by the parental educational level and the total population of pre-adolescent students, thus yielding a representative sample of primary-school children from the wider urban region of Athens. More specifically, the municipalities in the county of Attica were divided into three groups based on the average educational level of their adult population (25–65-year-olds) that was estimated from data provided by the National Statistical Service of Greece (Census 2001). This procedure yielded two parental education cut-off points that allowed us to categorize municipalities into three categories of different socio-economic levels (SEL), i.e. higher, medium and lower SEL. Consequently, based on data from the National Statistical Service of Greece, a certain number of municipalities, proportional to the size of their pre-adolescent population (9–14-year-olds), was randomly selected from each of these three SEL groups. Finally, an appropriate number of schools was randomly selected from each of these municipalities in relation to the population of schoolchildren registered in the fifth and sixth grades in each municipality, based on data obtained from the Greek Ministry of Education.

Weight and height were measured in all pupils attending the fifth and sixth grades in the participating primary schools as part of a school-based health and nutrition education programme. Full medical examination (i.e. anthropometrics and body composition measurements, blood collection, clinical examination, etc.) and questionnaire data were obtained from a sub-group of pupils whose parents signed a relative consent form. More specifically, an extended letter explaining the aims of the present study and a consent form for conducting full measurements were provided to all parents or guardians having a child in these schools. Parents who responded positively had to sign the consent form and provide their contact details. Signed parental consent forms for full measurements were collected for 754 out of 1236 children (response rate: 61 %). From the total number of positive responses, complete medical examination data became available for 729 children who will be reported as 'study subjects' throughout the paper. Regarding data on weight and height, these were obtained from 1201 children in total (i.e. 729 'study subjects' and 472 'weight and height participants').

Anthropometry

'Study subjects' and 'weight and height participants' from each primary school underwent an examination by two trained members of the research team. The protocol and equipment used for all measurements were the same for all regions. The physical examination included basic anthropometric measurements, i.e. weight and height. Body weight was recorded to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany) and with children standing without shoes in the minimum clothing possible. Height was measured to the nearest 0·1 cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Oadby, UK) with the participants not wearing shoes, their shoulders in a relaxed position, their arms hanging freely and their head aligned in the Frankfort plane[13]. Weight and height were converted to BMI using Quetelet's equation (weight (kg)/height^2 (m^2)). The International Obesity Task Force (IOTF) cut-off points[14] were used to categorize participants as 'underweight', 'normal weight', 'overweight' and 'obese'.

Socio-economic and demographic variables

Data on the socio-economic and demographic background of the families having at least one child participating in the study were collected from the parents (most preferably from the mother) during scheduled face-to-face interviews at the school site. For those parents not able to attend the meetings (approximately 5 % of the total sample), data were collected via telephone interviews. All interviews were conducted by a research team of ten members who were rigorously trained to minimize interviewer's effect with the use of a standardized questionnaire. More specifically, the data collected by parents included (i) demographic characteristics, such as parent's age, educational level (years of education), origin and nationality; (ii) economic characteristics, such as family mean annual income over the past 3 years (€/year), family residence ownership; (iii) social characteristics, such as family size, i.e. the number of family members living in the family residence; the primary caregiver(s) of the child, i.e. parents, grandparents, nanny or someone else; family type based on parental marital status, i.e. core families and single-parent families with parents who were not married, widowed or divorced. Furthermore, a 'popularity' score among classmates was developed for each child by asking all pupils in the same class, including 'weight and height participants', to name at least one of their three best friends in their class. All votes were then summed to create 'popularity' scores for all 'study subjects'. These scores were then adjusted for class size by dividing them by the total number of boys and girls attending the same class for male and female 'study subjects', respectively. The test–retest reliability of the 'popularity score' index was checked in a representative sub-sample of 100 pupils and was found to be quite high, reaching a rate of 94·8 %.

Statistical analysis

Some of the independent variables used in the present analyses were continuous while others were categorical. Normality of the distribution of continuous variables was analysed using the Kolmogorov–Smirnov test. Normally distributed continuous variables were expressed as mean and SD, skewed variables were reported as median (25th and 75th interquartiles) and categorical variables were reported as frequencies (%). Associations between continuous and categorical variables were examined using Student's t test for normally distributed variables and the non-parametric Mann–Whitney U test for skewed variables, while the association among the categorical variables was assessed using the χ^2 test. In order to test the effect of the independent variables examined on being overweight and obese, univariate logistic regression analyses were conducted and crude OR with 95 % CI were computed. Furthermore, adjusted OR and 95 % CI were also estimated using multivariate logistic regression analysis, including all variables that were found to be significantly ($P < 0·05$) associated with childhood overweight and obesity at a univariate level. Moreover, since the sample of the present study is based on a cluster design (all children in primary schools enrolled in the study), this was taken into account in multiple logistic regression analysis using the option 'robust cluster'. All reported P values were based on two-sided tests. The level of statistical significance was set at $P < 0·05$. The Statistical Package for Social Sciences statistical software pakage version 13·0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses and the STATA statistical software package version 8 (STATA Corp., College Station, TX, USA) for testing the clustering effect of schools.

Results

Table 1 displays the prevalence of underweight, normal weight, overweight and obesity for all pupils (i.e. both 'study subjects' and 'weight and height participants') attending the fifth and sixth grades in the schools under study. Overall, the observed prevalence was 3·5 % for underweight, 57·7 % for normal weight, 27·9 % for overweight and 10·9 % for obesity. No significant differences were observed between 'study subjects' and 'weight and height participants' with respect to these prevalence rates ($P = 0·270$), thus reducing the chance of any potential bias deriving from non-participation in the full protocol of the present study. Table 1 also summarizes the differences in the prevalence of underweight, normal weight, overweight and obesity among male and female 'study subjects'. Specifically, the prevalence of obesity was found to be significantly higher in male than female subjects (15·1 % *v.* 6·6 %, $P < 0·001$). Although a higher prevalence of underweight (3·1 % *v.* 1·4 %) and normal weight (60·3 % *v.* 53·9 %) was observed in female than male subjects, this was not significantly differentiated between genders.

The socio-economic and demographic characteristics of the population of 'study subjects' are presented in Table 2. In summary, the mean age of children under study was 11·2 (SD 0·7) years. Approximately half of the children's population under study was living in medium SEL residential areas while their parents had a medium educational level (9–14 years). Nationality in the majority of subjects was

Table 1 Prevalence of overweight and obesity in study subjects and weight and height participants

	Study subjects (*n* 729)									
	Male (*n* 379)		Female (*n* 350)		Total (*n* 729)		Weight and height participants (*n* 472)		All subjects (*n* 1201)	
Weight groups	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Underweight	7	1·8	16	4·6	23	3·2	19	4·0	42	3·5
Normal weight	201	53·0	208	59·4	409	56·1	284	60·2	693	57·7
Overweight	113	29·8	103	29·4	216	29·6	119	25·2	335	27·9
Obese	58	15·1	23	6·6*	81	11·1	50	10·6	131	10·9

*$P < 0·001$ for differences between genders derived from the χ^2 test.

Table 2 Socio-economic status and demographic characteristics of study subjects

	Study subjects (n 729)	
Age (years)*	11·2	0·7
Popularity' score†	0·33	0·16–0·60
Family size (number of family members)	4·0	3·0–4·0
	n	%
SES of residential area		
Lower	254	34·7
Medium	314	43·0
Higher	161	22·3
Annual family income (€/year)		
<12 000	200	27·6
12 000–20 000	211	28·9
20 000–30 000	159	21·8
>30 000	159	21·8
Fathers' age categories		
Lower tertile (<41 years old)	251	34·5
Medium tertile (41–46 years old)	270	37·0
Higher tertile (>46 years old)	208	28·5
Mothers' age categories		
Lower tertile (<37 years old)	300	41·2
Medium tertile (37–42 years old)	239	32·8
Higher tertile (>42 years old)	190	26·0
Paternal educational level		
Low (≤9 years of education)	158	21·7
Medium (>9 and ≤14 years of education)	353	48·4
High (>14 years of education)	218	29·9
Maternal educational level		
Low (≤9 years of education)	128	17·5
Medium (>9 and ≤14 years of education)	382	52·4
High (>14 years of education)	219	30·0
Parents' nationality		
Greeks	516	70·9
Non-Greeks	213	29·1
Residence ownership		
No	268	36·6
Yes	461	63·4
Child's primary caregiver		
Parents	448	61·5
Grandfather	47	6·4
Grandmother	192	26·3
Nanny/someone else	42	5·8
Parental marital status		
Core families – married parents	641	87·9
Single parent families – not married parents	16	2·2
Single parent families – widowed parent	14	1·9
Single parent families – divorced parents	58	7·9

SES, socio-economic status.
*Data are presented as mean and SD.
†Data are presented as median and 25th–75th percentiles.

Greek (70·9%). Furthermore, most children (63·4%) lived in residences owned by their families. The grandmother was the child's primary caregiver in almost 25% of our population. An analysis per gender revealed that parent's non-Greek nationality and residence ownership were higher for girls than boys (32·5% v. 26%, $P<0·05$; 67·2% v. 59·7%, $P<0·05$, respectively), whereas the grandmother as the child's primary caregiver was more frequent for girls rather than boys (28·8% v. 23·5%, $P<0·05$), and the nanny or someone else appeared more frequently as the child's primary caregiver for boys than girls (8·3% v. 3·5%, $P<0·05$).

Table 3 summarizes the crude OR and 95% CI for being overweight and obese also controlling for the clustering effect of schools. An annual family income of €12 000–20 000 was related to an increased likelihood of childhood overweight and obesity (OR = 1·56; 95% CI 1·07, 2·35) compared to an annual family income of >€30 000. Furthermore, children whose residence was owned by their family were 1·68 times more likely (95% CI 1·22, 2·25) to be overweight and obese than children whose residence was not owned by their family. Moreover, in families in which the primary caregiver of the child was his/her grandmother, the risk of childhood overweight and obesity was 1·38 times higher (95% CI 1·03, 1·98) than families where the child's primary caregivers were his/her parents. In contrast, children whose parents' nationality was Greek were 1·38 times less likely (95% CI 1·03, 1·96) to be overweight and obese than children whose parents' nationality was non-Greek. Finally, for every 1° increase in the child's 'popularity' score, the likelihood of childhood overweight and obesity was found to decrease by 60% (95% CI 0·17, 0·97). Furthermore, Table 3 also presents the adjusted OR and 95% CI derived from the multivariate logistic regression analysis, including in the same model all variables that were found to be significantly related to childhood overweight and obesity ($P<0·05$) at a univariate level. Based on the results derived from the present analysis, those variables that remained significantly associated with childhood overweight and obesity were the grandmother as the child's primary caregiver (OR = 1·53; 95% CI 1·05, 2·29) and annual family income of € 12 000–20 000 (OR = 1·61; 95% CI 1·03, 2·61), respectively.

Discussion

The findings of the present study revealed a high prevalence of both overweight and obesity in a population of primary-school children living in a large urban area of Greece. Using the international cut-off points recommended by the IOTF[14] (Table 2), 29·6% of children were found to be overweight and 11·1% were found to be obese with the prevalence of obesity being significantly higher for boys than girls (15·1% v. 6·6%). Comparing the findings of the present study regarding the prevalence of overweight and obesity with those reported for other European countries[3], there seems to be a geographic variation. Precisely, the prevalence reported from studies conducted with children living in southern European countries surrounding the Mediterranean, including the present study, ranged between 20% and 41% and was considerably higher than the prevalence reported for the northern areas in Europe that showed prevalence rates in the range of 10–20%[3]. Several potential explanations have been proposed for these North–South trends in the prevalence of childhood overweight in Europe including higher stature and resting energy expenditure for children living in northern latitudes and a genetic predisposition towards weight gain in children living in southern Europe[3].

Table 3 Crude and adjusted OR and 95 % CI for the association between overweight and obesity with several socio-economic and demographic factors in the study subjects (*n* 729)

	Overweight and obese				
		Crude		Adjusted	
	%	OR	95 % CI	OR*	95 % CI
Annual family income (€/year)					
>30 000	38·5	1·00	Ref.	1·00	Ref.
<12 000	33·9	0·81	0·54, 1·27	1·05	0·61, 1·86
12 000–20 000	49·7	**1·56**	**1·07, 2·35**	**1·61**	**1·03, 2·61**
20 000–30 000	41·0	1·06	0·70, 1·79	1·02	0·62, 1·67
Parents' nationality					
Non-Greek	35·8	1·00	Ref.	1·00	Ref.
Greek	44·1	**1·38**	**1·03, 1·96**	1·04	0·65, 1·68
Residence ownership					
No	34·4	1·00	Ref.	1·00	Ref.
Yes	46·7	**1·68**	**1·22, 2·25**	1·58	0·98, 2·20
Child's primary caregiver					
Parents	39·0	1·00	Ref.	1·00	Ref.
Grandfather	48·8	1·43	0·74, 2·85	1·66	0·83, 3·19
Grandmother	46·9	**1·38**	**1·03, 1·98**	**1·53**	**1·05, 2·29**
Nanny/someone else	45·9	1·34	0·67, 2·63	1·62	0·82, 3·10
'Popularity' score (adjusted for class participation by gender)	–	**0·40**	**0·17, 0·97**	1·09	0·65, 1·84
SES of residence region					
Lower	38·6	1·00	Ref.	–	–
Medium	42·8	1·15	0·81, 1·62		
Higher	39·9	1·05	0·72, 1·58		
Fathers' age categories				–	–
Lower tertile (<41 years old)	43·6	1·00	Ref.		
Medium tertile (41–46 years old)	37·9	0·80	0·57, 1·14		
Higher tertile (>46 years old)	44·0	1·00	0·66, 1·48		
Mothers' age categories				–	–
Lower tertile (<38 years old)	44·4	1·00	Ref.		
Medium tertile (38–42 years old)	40·1	0·83	0·57, 1·18		
Higher tertile (>42 years old)	40·1	0·81	0·55, 1·22		
Paternal educational level				–	–
Low (≤9 years of education)	39·1	1·00	Ref.		
Medium (>9 and ≤14 years of education)	45·5	1·26	0·78, 1·92		
High (>14 years of education)	36·6	0·93	0·59, 1·46		
Maternal educational level				–	–
Low (≤9 years of education)	38·6	1·00	Ref.		
Medium (>9 and ≤14 years of education)	42·7	1·17	0·74, 1·80		
High (>14 years of education)	42·3	1·13	0·69, 1·85		
Parental marital status				–	–
Core families – married parents	41·6	1·00	Ref.		
Single parent families – not married parents	53·3	1·50	0·52, 4·26		
Single parent families – widowed parent	53·8	1·61	0·52, 4·91		
Single parent families – divorced parents	42·3	1·05	0·58, 1·89		
Family size (number of family members)	–	0·94	0·77, 1·11	–	–

Ref., referent category; SES, socio-economic status.
*All OR were adjusted for annual family income, parent's nationality, residence ownership, child's primary caregiver, popularity score, gender and for the clustering effect of schools.
Cells in bold indicate statistically significant OR.

Regarding Greece, the prevalence of overweight and obesity reported in the present study was comparable to the prevalence reported by other recent studies conducted in children of similar age living in urban and semi-urban areas from southern (29·8 % overweight and 14·9 % obesity)[1], northern (25·3 % overweight and 5·6 % obesity)[15], western (34·3 % overweight and 9·6 % obesity) [16] and eastern (30·3 % overweight and 6·7 % obesity)[1] parts of Greece. Similar to the present study, the prevalence of obesity was also found to be significantly higher in male than female subjects in almost all of the aforementioned studies. The IOTF cut-off points were used for screening children's overweight and obesity in all of these studies.

Certain socio-economic and demographic factors that have been reported by previous studies as correlates of childhood overweight[17,18] were also tested in the present study. A lower-medium annual family income of €12 000–20 000 was associated with an increased likelihood of childhood overweight and obesity compared to a higher annual family income level of € >30 000, both at the bivariate and multivariate levels. This finding is in line with those reported from other recent studies conducted in children in developed countries indicating that income, which is probably the most accurate index of socio-economic status, is inversely related to the consumption of energy-dense foods, such as fats and sweets, full-fat meat, dairy products, etc.[19–21]. In contrast, children from relatively lower-income families tend to have a lower intake of vegetables, fruits and whole-grain products[19]. It has been reported that the energy density of foods and their energy cost are inversely related, which means that the more energy-dense diets represent the lowest-cost dietary options to the consumer[22,23]. Provided that higher socio-economic status is probably reflected by residence ownership, then the finding of the present study regarding the association of this other index of socio-economic status with childhood overweight contradicts that observed for income. The increased likelihood of overweight observed in children whose parents owned their house could probably be ascribed to the fact that many parents reported their residence as privately owned, although their house was bought with a housing bank loan. In this case, residence ownership should not be considered as an accurate index of higher socio-economic status since a substantial amount of the family's budget pays off the monthly instalments of the bank loan, probably having a direct impact on children's nutrition. Moreover, residence ownership may not be a representative index of socio-economic status, when used as a single index[24].

The significant positive association observed in the present study between residence ownership and childhood overweight and obesity could also be attributed to the mediating role of the parent's nationality. More specifically, our findings showed that Greek nationals were 9·1 times (OR = 9·09; 95 % CI 6·18, 13·39) more likely to own their house and they were also 38 % more likely to be overweight or obese than non-Greek nationals. The vast majority of non-Greek subjects, whose parents came to Greece as economic immigrants during the period of the economic and political transition in the 1990s, were from Albania (75·6 %) and from other Eastern European countries (i.e. Russia, Ukraine, Poland, Serbia, etc.; 12·6 %). In these countries, Lobstein and Frelut[3] reported lower prevalence rates of childhood overweight in comparison to children living in the non-Eastern bloc European countries. The implication of this observation is that the lower prevalence of overweight also reported in the present study for non-Greek subjects, having mainly an Eastern European nationality, could be attributed to a genetic predisposition for lower BMI compared to Greek children. To our knowledge, no evidence exists on this topic, and therefore further research, including the study of certain genes and polymorphisms, is needed in order to shed more light on these differences.

Children's 'popularity' score was another index that was found to be inversely associated with childhood overweight when analysed at the bivariate level. A potential explanation for this finding might be the fact that children who are more athletic and of normal weight are usually more popular among their peers. Previous studies have also confirmed the association observed in the present study between weight status and social acceptance in populations of pre-adolescents. Reynolds *et al.*[25] have reported that overweight was an index of less social acceptance in girls aged 9–14 years. Furthermore, Latner and Stunkard[26] have reported that overweight and obese (10–11 years old) children were significantly less popular than their normal-weight peers. According to previous studies, children before and during puberty are very much concerned about their weight and body image[27]; thus, another way of explaining this association could be that children's 'popularity' concerns related to their body image may control the increase in their body weight.

Another important outcome of the regression analysis performed in the present study was that children whose primary caregiver was their grandmother had an increased likelihood of being overweight or obese both at the bivariate and multivariate levels. In a recent study, it was observed that food consumption in children was adjusted according to the stimuli induced by the food served by their caregivers or parents, instead of being regulated by the satiety and increased energy following the previously consumed meals[28]. In most cases, grandparents tend to believe that overweight signifies healthiness[29] or that young children may grow out of being overweight[30]. Another interpretation of this particular finding is probably based on the so-called 'post-World War II overfeeding syndrome'[31] referring to the post-war period in Greece when there was severely decreased accessibility to food. Children's grandmothers, who most likely experienced hunger and extreme difficulties in finding food as children during those years, tend to overfeed their grandchildren with affluent portions of foods, as an expression of their care and insecurity. Furthermore, Gordon-Larsen *et al.*[7], in a study of girls aged 6–9 years, observed that caregivers, including grandmothers, influenced their children in adopting a sedentary lifestyle, as they considered sedentary habits, such as television viewing, a more secure and easier way of having control of their children.

The present findings should be interpreted in the light of the limitations and strengths of the present study. Regarding the limitations, the cross-sectional design of the study does not allow the establishment of cause–effect relationships, but only generates hypotheses about the possible role of the social, economic and demographic factors examined in the present study on the development of overweight and obesity in children. Moreover, it should be noted that the present findings are the preliminary results of an ongoing study and thus should be interpreted with caution. However, the relatively large study sample and the random and multi-stage sampling of schools yielded in a representative sample of the wider region of Athens and this could be considered as a strength of the present study. Furthermore, the assessment of weight status in almost all children in the schools under study, combined with the fact that we observed no significant differences in the prevalence of overweight and obesity between 'study subjects' and 'weight and height participants' can also be considered as the strengths of the present study, indicating that our findings are unbiased and controlled for the parameter of will or motivation for participation in the study and its consequent possible confounding effect.

In conclusion, the present study showed that the considerably high prevalence of overweight and obesity among urban primary-school children in Greece also seems to have a socio-economic and demographic dimension. More specifically, lower family income and grandmother as the child's primary caregiver were the main risk factors for being overweight and obese among primary-school children living in a large urban area in Greece. The conclusion of the present study has several implications for the prevention of childhood obesity. Appropriate interventions and population-based strategies are needed, targeting lower-income families and probably those families in which both parents are working and the grandmother acts as the child's primary caregiver.

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PAPER II

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"Leaner and less fit" children have a better cardiometabolic profile than their "heavier and more fit" peers: The Healthy Growth Study

G. Moschonis [a], V. Mougios [b], C. Papandreou [a], C. Lionis [c], G.P. Chrousos [d], E. Malandraki [a], Y. Manios [a,*]

[a] Department of Nutrition and Dietetics, Harokopio University of Athens, 70, El.Venizelou Ave, 17671 Kallithea, Athens, Greece
[b] Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece
[c] Clinic of Social and Family Medicine, School of Medicine, Heraklion, University of Crete, Crete, Greece
[d] First Department of Pediatrics, Athens University Medical School, "Aghia Sophia" Children's Hospital, Athens, Greece

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Cardiometabolic risk;
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Abstract *Background and aims:* To examine differences in cardiometabolic risk factors between children of different BMI and fitness levels.
Methods and results: From a representative sample of 1222 boys and 1188 girls, aged 9–13 years, anthropometric, body composition, physical activity, cardiorespiratory fitness, biochemical and blood pressure data was collected. The prevalence of overweight and obesity was 29.9% and 11.8% respectively. In both genders, plasma HDL cholesterol concentration was higher in the 'leaner and less fit' group (lowest quartile of BMI and lowest quartile of fitness) compared to the 'heavier and more fit' (highest quartile of BMI and highest quartile of fitness) and intermediate (all other children) groups ($p < 0.05$). Furthermore, the 'leaner and less fit' groups in both genders had lower triacylglycerol concentration, total-to-HDL cholesterol ratio, HOMA-IR, insulin and systolic blood pressure levels compared to the 'heavier and more fit' and/or intermediate groups. Similar trends were observed for hypertension in boys and insulin resistance for both genders. Finally, the effect size of being 'leaner and less fit' on serum levels of cardiometabolic risk indices was mainly small to medium (i.e. Cohen's d 0.2–0.5).
Conclusion: Leaner and less fit boys and girls had better cardiometabolic risk profiles than their heavier and more fit peers, probably suggesting a higher importance of leanness over fitness in children from a cardiometabolic health benefit perspective.

* Corresponding author. Tel.: +30 210 9549156; fax: +30 210 9514759.
E-mail address: manios@hua.gr (Y. Manios).

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Introduction

The continuous increase in cardiovascular disease (CVD) mortality among adults in Greece and elsewhere is closely associated with risk factors that begin to develop in childhood. The shift from the traditional Mediterranean to a western-type diet, along with the transition from an active to a sedentary lifestyle over the past decades, is of major importance in relation to the occurrence of CVD in Greece [1].

Greece is one of the leading countries in Europe with respect to the prevalence of childhood obesity [2,3]. As adipose tissue accumulates in excessive amounts in the body several metabolic alterations begin to occur [4]. Research has shown that obese children are at higher risk for the development of adverse lipidemic and glycemic profiles compared to lean children [5]. This, is further related to an increase in fatty streaks within the arterial endothelium of children, which may lead to atheromatic plaques in adulthood [6].

While levels of obesity among Greek children are increasing, physical activity and consequently fitness levels are decreasing [7]. There is strong evidence for an inverse association of physical activity and fitness levels with cardiometabolic risk [8]. Several mechanisms have been proposed to mediate the protective effects of physical activity and fitness on cardiometabolic risk including (a) anti-inflammatory action, (b) increased insulin sensitivity, (c) increased non-insulin-dependent glucose uptake, (d) improved lipidemic profile and (e) improved function of hormones and enzymes involved in fat metabolism [9]. In adults, there is strong evidence supporting that low fitness is a stronger risk factor for CVD than obesity [10,11]. However, in children, there are few studies examining the combined effect of different levels of body weight and physical fitness on cardiometabolic risk factors [12]. Such studies are important from a public health perspective, since identification of the interacting or counterbalancing role of different body mass and fitness levels, especially for individuals who fall into the extremes of these health indices, could help public health professionals to develop more effective preventive measures.

Taking the above into consideration, the present study aimed to analyze differences in cardiometabolic risk factors among children after grouping them into quartiles of BMI and fitness levels.

Methods

Sampling

The 'Healthy Growth Study' was a cross-sectional epidemiological study initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens. The study population comprised schoolchildren attending the 5th and 6th grades of primary schools located in municipalities within the wider regions of Attica, Etoloakarnania, Thessaloniki and Iraklio. The sampling procedure is fully described elsewhere [13]. In brief, the sampling of schools was random, multistage and stratified by parental educational level and by the total population of students attending schools within the municipalities.

Anthropometry and physical examination

Participants were weighed to the nearest 0.1 kg in light clothing and without shoes by using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany). Height was measured to the nearest 0.1 cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Oadby, UK) with the participants barefoot, their shoulders in a relaxed position, their arms hanging freely and their heads in Frankfort horizontal plane. Two trained members of the research team carried out these measurements.

Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). The International Obesity Task Force thresholds were used to categorise children into underweight, normal-weight, overweight and obese [14,15].

Pubertal stage of children was assessed by paediatricians that classified children into five Tanner stages after visual inspection of breast development in girls and genital development in boys [16].

Body composition

Bioelectrical impedance analysis was used for the assessment of adiposity (Akkern BIA 101; Akkern Srl., Florence, Italy). Participants were instructed to abstain from any solid or liquid food and from any intensive exercise for 4 h prior measurement and not to wear any metal object during measurement. The assessment took place with the pupils lying on a non-conductive surface at neutral temperature. Fat mass, fat-free mass and lean tissue mass were calculated from the resistance and reactance values using valid equations [17].

Blood pressure

Blood pressure was recorded from the right arm while in a sitting position and after 5 min of rest. A valid automatic Omron M6 Blood Pressure Monitor (Omron Healthcare Europe BV, Hoofddorp, The Netherlands) [18] was used. The measurement was taken twice with a 2-min interval between readings. A third measurement was taken if there was a difference of over 10 mmHg between the previous measurements. The average value of the measurements was used in analysis. Systolic (SBP) and diastolic blood pressure (DBP) were recorded. Systolic or diastolic hypertension was defined as SBP or DBP above the 95th percentile for gender, age and height [19].

Biochemical measurements

Early morning blood samples were taken after a 12-hour overnight fast. The parents as well as the children were reminded on the previous day in order to ensure compliance with fasting. Serum triacylglycerols, total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and plasma glucose were measured enzymatically using commercially available kits (Roche Diagnostics, Basel, Switzerland). Low-density lipoprotein cholesterol (LDLC) was

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calculated as: LDLC = TC − (HDLC + triacylglycerols/5) [20]. The ratios of TC to HDLC and HDL to LDL were also calculated. The National Cholesterol Education Program cut-off points for blood lipids were used to define dyslipidemias [21]. Insulin was measured by a chemiluminescence assay (Kyowa Medex Ltd, Minami-Ishiki, Japan, for Siemens Diagnostics USA). The homeostatic model assessment (HOMA) was used to estimate insulin resistance (IR). The cut-off HOMA value used to define IR was 3.16 [22].

Physical activity assessment via questionnaire

Physical activity during leisure time was assessed using a standardized questionnaire completed by the children for two consecutive weekdays and one weekend day. Reported activities were assessed as moderate-to-vigorous physical activities (MVPA) provided that they were of intensity higher than 4 metabolic equivalents and included activities such as bicycling, rhythmic-gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, jumping rope and general participation in active outdoor games. Given the age group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity [23].

Physical activity assessment via step count

To assess step count as an objective estimate of physical activity, study participants were provided with and instructed to wear a waist-mounted pedometer (Yamax SW-200 Digiwalker, Tokyo, Japan) for one week, i.e. from Monday to Sunday. The pedometer was positioned according to the manufacturer's instructions on the right waistband, vertically aligned with the patella. Children were instructed to wear the pedometer from the time they woke up in the morning until the time they went to bed at night (except when taking a shower, bathing or swimming) and to record their total number of daily steps displayed by the pedometer in diary template before bedtime.

Cardiorespiratory fitness assessment

Cardiorespiratory fitness was estimated indirectly according to children's performance in the endurance 20-m shuttle run test (ERT). The ERT is a field test included in the European battery of physical fitness tests and recommended by the Committee of Experts on Sports Research [24]. Based on the test's instructions participants started running at a speed of 8.5 km/h and speed is increased in stages. Participants shuttle between two lines placed 20 m apart, at a pace dictated by a sound signal on an audiotape, which gets progressively faster (by 0.5 km/h every minute). Each stage of the test is made up of several shuttle runs and the score of the participant is the half-stage completed before the child drops out (thus, scores can be 0, 0.5, 1, 1.5, 2 etc). The higher the ERT score, the better the cardiorespiratory fitness. Prior to the test all children received clear and comprehensible instructions on rules and procedures, while during the test they were verbally encouraged by the researchers to succeed their maximal number of laps. The ERT is recommended for large groups of children, since it is reliable, valid, noninvasive, and requires limited facilities [25].

BMI and fitness categorization

Quartiles of BMI and ERT scores were estimated for boys and girls respectively as indicated in Table 1. Boys and girls in the first quartiles of BMI and ERT were characterized as 'leaner and less fit', while those in the fourth quartiles were characterized as 'heavier and more fit.' All other children were characterized as having intermediate BMI and ERT levels.

Statistical analysis

Categorical variables were reported as frequencies (%) and continuous variables as means and standard deviations. Student's *t*-test was used to assess differences in continuous variables between boys and girls. Two-sample *z*-test for proportions was used to assess differences in categorical variables between genders, BMI and fitness groups. Multilevel linear and logistic regression analyses were performed for the overall sample, with children nested within classes nested within schools nested within municipalities (four-level random intercept model) using the commands "xtmixed" and "xtmelogit" respectively [26]. In all analyses adjustments were made for children's Tanner stage and mean parental educational level. Finally, effect size was also estimated via the calculation of Cohen's *d*, which shows the magnitude of the difference in cardiometabolic risk indices between 'leaner and less fit' and 'heavier and more fit' groups (i.e. Cohen's *d* value of 0.2, 0.5 and 0.8 indicate small, medium and large effect size respectively) [27]. All reported *P*-values were based on two-sided tests. The level of statistical significance was set at $P < 0.05$. The STATA 11.0 (STATA Corp, College Station, TX, USA) statistical software was used for all statistical analyses.

Results

Full anthropometric, body composition, physical activity, fitness, biochemical and blood pressure data were

Table 1 Grouping of boys and girls into quartiles on the basis of their BMI and fitness levels.

	Reference values, boys	Reference values, girls
Quartiles	*BMI range (kg/m^2)*	
1st	13.2–17.4	10.5–17.2
2nd	17.5–19.8	17.3–19.4
3rd	19.9–22.6	19.5–22.2
4th	22.7–43.9	22.3–36.7
Quartiles	*Fitness range (no of stages at the ERT)*	
1st	0–1	0–1
2nd	1.5–2	1.5
3rd	2.5–3.5	2–2.5
4th	4–7	3–7

ERT, endurance run test; BMI, body mass index.

Table 2 Anthropometric characteristics, fitness and physical activity of the study population.

	Boys (n = 1222)	Girls (n = 1188)	Total (n = 2410)
Weight (kg)	45.6 ± 11.2	45.2 ± 11.0	45.4 ± 11.1
Height (m)	1.48 ± 0.07	1.49 ± 0.08[a]	1.48 ± 0.08
BMI (kg/m^2)	20.5 ± 3.7	20.1 ± 3.7[a]	20.3 ± 3.8
Weight groups			
Underweight (%)	2.6	3.9	3.2
Normal weight (%)	52.7	57.4	55.2
Overweight (%)	30.8	29.0	29.9
Obese (%)	14.0	9.6[b]	11.8
Fitness levels			
ERT score (no of stages)	2.9 ± 1.7	2.2 ± 1.2[a]	2.5 ± 1.5
Physical activity level indices			
MVPA (min/d)	79.2 ± 66.2	58.4 ± 58.3	69.0 ± 63.3
Steps/d	14,728 ± 5627	11,790 ± 4298	13,274 ± 5222

Values for continuous variables are expressed as the mean ± SD.
BMI, body mass index; ERT, endurance run test; MVPA, moderate-to-vigorous physical activity.
[a] Significantly different from boys (Student's t-test, $P < 0.001$).
[b] Significantly different from boys (two sample z-test for proportions, $P = 0.001$).

collected from 2410 children. Their anthropometric characteristics, fitness scores and physical activity data are presented in Table 2. Overall, the observed prevalence was 3.2% for underweight, 55.2% for normal weight, 29.9% for overweight and 11.8% for obesity. The prevalence of obesity was higher in boys ($P = 0.001$) and boys had also higher cardiorespiratory fitness levels ($P < 0.001$) compared to girls.

Table 3 displays adiposity and physical activity indices across the BMI and fitness groups by gender. Fat (presented in both kilograms and as percentage of body weight), fat-free and lean tissue mass differed significantly among groups in both boys and girls ($P < 0.001$), being higher in the 'heavier and more fit' group. The same trend was observed for MVPA ($P = 0.012$). Large effect sizes (|Cohen's d| > 1) were observed for fat, fat-free and lean tissue mass

Table 3 Body composition and physical activity indices across BMI and fitness groups presented by gender.

	Leaner and less fit	Intermediate groups	Heavier and more fit	P-value[a]	Effect size Cohen's d[d] Leaner and less fit vs. heavier and more fit
Boys	(n = 158)	(n = 957)	(n = 107)		
FM (kg)	7.9 ± 2.9[b]	14.4 ± 7.9	18.4 ± 5.3	<0.001	−2.46
FM (% of body weight)	21.2 ± 5.8	29.3 ± 9.4	34.1 ± 5.8[c]	<0.001	−2.22
FFM (kg)	28.2 ± 2.9[b]	31.7 ± 4.2	34.8 ± 4.0[c]	<0.001	−1.89
LTM (kg)	26.3 ± 2.8[b]	29.7 ± 4.0	32.5 ± 3.8[c]	<0.001	−1.86
MVPA (min/day)	60.7 ± 59.8[b]	80.3 ± 66.4	94.6 ± 69.0[c]	0.012	−0.52
Step count (no. of steps/day)	14,199 ± 4540	14,741 ± 5772	15,568 ± 5598	0.359	
Girls	(n = 231)	(n = 849)	(n = 108)		
FM (kg)	8.3 ± 2.8[b]	15.0 ± 7.5	19.2 ± 6.0[c]	<0.001	−2.33
FM (% of body weight)	22.9 ± 6.0[b]	30.7 ± 79.5	35.2 ± 6.6[c]	<0.001	−1.95
FFM (kg)	27.5 ± 3.7[b]	31.4 ± 4.7	34.4 ± 4.7[c]	<0.001	−1.63
LTM (kg)	26.2 ± 3.5[b]	29.7 ± 4.4	32.5 ± 4.3[c]	<0.001	−1.61
MVPA (min/day)	48.7 ± 52.0[b]	58.8 ± 58.1	74.1 ± 66.8[c]	0.012	−0.42
Step count (no. of steps/day)	11,756 ± 4137	11,695 ± 4390	12,520 ± 3830	0.781	

BMI, body mass index; FM, fat mass; FFM, fat-free mass; LTM, lean tissue mass; MVPA, moderate to vigorous physical activity.
Leaner and less fit: 1st quartile of BMI and 1st quartile of ERT score; Heavier and more fit: 4th quartile of BMI and 4th quartile of ERT score; Intermediate groups: all other children.
[a] Derived from a four-level random intercept linear regression analysis, after controlling for children's Tanner stage and parental educational level.
[b] $P < 0.05$ "Leaner and less fit" group vs. other two groups.
[c] $P < 0.05$ "Heavier and more fit" group vs. other two groups.
[d] Cohen's d indicates the effect size of "leaner and less fit" vs. "heavier and more fit" group.

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indices, whereas small to medium effect sizes were observed for MVPA in girls and boys (|Cohen's d| = 0.42 and 0.52, respectively).

The mean values of the examined cardiometabolic risk indices across the three groups of BMI and fitness levels are presented by gender in Table 4. In both genders HDLC levels were highest, while TC/HDLC ratio, insulin, HOMA-IR and SBP levels were lowest in the 'leaner and less fit' group ($P < 0.05$). Similarly, DBP levels were found to be lowest in the 'leaner and less fit' group only in boys ($P < 0.001$), while HDLC/LDLC ratio was found to be lowest in the "heavier and more fit" group only in girls ($P = 0.002$). Effect sizes in boys were small to medium for TC/HDLC ratio, HDLC, DBP, insulin and HOMA-IR levels (0.2 > |Cohen's d| < 0.5) and medium to large for SBP levels (|Cohen's d| = 0.75). In girls, effect sizes were small to medium for HDLC, SBP and triacylglycerols levels as well as for TC/HDLC and HDLC/LDLC ratios (0.2 > |Cohen's d| < 0.5), while large effect sizes were observed for insulin and HOMA-IR levels (|Cohen's d| > 0.8).

Based on the data summarized in Table 5 the percentage of children with IR was found to be lowest in the 'leaner and less fit' groups ($P < 0.05$). Finally, the percentage of boys with systolic hypertension and/or diastolic hypertension was also found to be lowest in the 'leaner and less fit' group ($P = 0.001$).

Discussion

The present large-scale epidemiological study examined a representative sample of 2410 preadolescents of whom 29.9% were overweight and 11.8% obese. The prevalence of obesity observed in the present study is comparable to that reported by other epidemiological studies in different parts of Greece with higher rates in boys than girls. However, when compared to other countries the overall prevalence of childhood obesity in Greece is two- or three-fold higher than the prevalence reported for children in central and northern European countries [2].

Although the existing literature supports a positive association of cardiometabolic risk with BMI [28] and a negative one with fitness levels [17], there are few studies examining the cardiometabolic profile in children, who are at the upper and lower extremes of body mass and fitness levels. A previous study on 375 7–9 year-old children

Table 4 Cardiometabolic risk profile indices across BMI and fitness groups presented by gender.

	Leaner and less fit	Intermediate groups	Heavier and more fit	P-value[a]	Effect size Cohen's d[d] Leaner and less fit vs. heavier and more fit
Boys	(n = 158)	(n = 957)	(n = 107)		
TC (mg/dl)	170.5 ± 30.5	167.3 ± 32.5	168.1 ± 33.4	0.500	
HDLC (mg/dl)	63.3 ± 15.3[b]	58.6 ± 15.2	57.6[c] ± 12.3	0.003	0.41
LDLC (mg/dl)	96.5 ± 25.7	96.6 ± 28.1	99.3 ± 30.5	0.196	
Triacylglycerols (mg/dl)	53.6 ± 23.6	60.8 ± 29.6	56.4 ± 24.0	0.049	
TC/HDLC	2.8 ± 0.7[b]	3.0 ± 0.8	3.02[c] ± 0.7	0.005	−0.31
HDLC/LDLC	0.7 ± 0.2	0.6 ± 0.3	0.6 ± 0.6	0.846	
Glucose (mg/dl)	92.7 ± 10.1	93.3 ± 10.2	93.8 ± 10.0	0.270	
Insulin (μU/ml)	8.4 ± 4.6[b]	11.3 ± 9.2	11.1[c] ± 6.1	0.002	−0.49
HOMA–IR	1.9 ± 1.1[b]	2.6 ± 2.2	2.5[c] ± 1.3	0.005	−0.49
SBP (mmHg)	114.2 ± 10.6[b]	121.3 ± 13.4	123.7[c] ± 14.4	<0.001	−0.75
DBP (mmHg)	65.8 ± 9.6[b]	69.3 ± 10.0	70.0[c] ± 9.3	<0.001	−0.44
Girls	(n = 231)	(n = 849)	(n = 108)		
TC (mg/dl)	164.2 ± 32.2	166.0 ± 30.9	162.4 ± 30.6	0.883	
HDLC (mg/dl)	60.5 ± 14.2[b]	57.3 ± 14.5	56.3 ± 14.0[c]	0.003	0.29
LDLC (mg/dl)	92.0 ± 25.6	92.7 ± 25.1	94.3 ± 25.6	0.682	
Triacylglycerols (mg/dl)	58.7 ± 18.6[b]	67.9 ± 29.7	66.7 ± 25.0[c]	0.001	−0.36
TC/HDLC	2.8 ± 0.5[b]	3.0 ± 0.8	3.0 ± 0.7[c]	0.005	−0.33
HDLC/LDLC	0.7 ± 0.2[b]	0.6 ± 0.3	0.6 ± 0.3[c]	0.002	0.39
Glucose (mg/dl)	91.1 ± 9.2	91.1 ± 9.3	90.8 ± 8.2	0.525	
Insulin (μU/ml)	9.7 ± 4.9[b]	14.0 ± 8.2	15.5 ± 7.7[c]	<0.001	−0.89
HOMA–IR	2.2 ± 1.1[b]	3.1 ± 1.7	3.4 ± 1.7[c]	<0.001	−0.84
SBP (mmHg)	117.0 ± 12.3[b]	121.3 ± 13.5	120.6 ± 13.3[c]	<0.001	−0.28
DBP (mmHg)	69.3 ± 9.7	71.3 ± 9.7	71.0 ± 9.8	0.214	

TC, Total cholesterol; HDLC, High-density lipoprotein cholesterol; LDLC, Low-density lipoprotein cholesterol; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

[a] Leaner and less fit: 1st quartile of BMI and 1st quartile of ERT score; Heavier and more fit: 4th quartile of BMI and 4th quartile of ERT score; Intermediate groups: all other children. Derived from a four-level random intercept linear regression analysis, after controlling for children's Tanner stage and parental educational level.

[b] $P < 0.05$ "Leaner and less fit" group vs. other two groups.

[c] $P < 0.05$ "Heavier and more fit" group vs. other two groups.

[d] Cohen's d indicates the effect size of "leaner and less fit" vs. "heavier and more fit" group.

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Table 5 Percentages of dyslipidemias, systolic and diastolic hypertension and insulin resistance across BMI and fitness groups presented by gender.

	Boys				Girls			
	Leaner and Less fit (n = 158)	Intermediate groups (n = 957)	Heavier and more fit (n = 107)	P-value[c]	Leaner and Less fit (n = 231)	Intermediate groups (n = 849)	Heavier and more fit (n = 108)	P-value[c]
HDLC < 40 mg/dl	4.4	7.6	4.7	0.959	5.6	10	8.3	0.903
Triacylglycerols >100 mg/dl	3.8	7.9	5.6	0.904	3.0	11.1	12.0	0.506
TC/HDLC > 4.5	1.9	5.7	2.8	0.884	0.9	4.2	2.8	0.849
Systolic hypertension	7.9[a,b]	24.7[b]	28.8[a]	0.001	18.0	25.1	31.0	0.121
Diastolic hypertension	3.9	7.0	3.6	0.936	5.7	8.9	8.0	0.874
Systolic and/or diastolic hypertension	9.9[a,b]	27.1[b]	29.7[a]	0.001	19.7	27.5	33.6	0.085
IR (HOMA-IR<3.16)	3.2[a,b]	13.9[b]	13.5[a]	0.022	6.1[a,b]	23.6[b]	31.5[a]	<0.001

TC: total cholesterol; HDLC: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment—insulin resistance.
Leaner and less fit: 1st quartile of BMI and 1st quartile of ERT score; Heavier and more fit: 4th quartile of BMI and 4th quartile of ERT score; Intermediate groups: all other children.
[a,b] Identical superscript letters denote statistically significant differences ($P < 0.05$) between BMI and ERT groups using the two-sample z-test for proportions.
[c] Derived from multilevel logistic regression analysis, after controlling for children's Tanner stage and mean parental educational level.

revealed that fitness attenuated the negative effect of BMI on insulin sensitivity, emphasizing the important role of fitness in reducing cardiometabolic risk in overweight and obese children [12]. The biological basis of these observations possibly lies on genetics, adipocytokines and mitochondrial function [29]. Furthermore, a study in adults showed that fit men in the highest quartile of fat mass had a lower risk of CVD mortality than did unfit, lean men [30] concluding that the health benefits of leanness are limited to fit men. Another recent systematic review that compared adults with high BMI and good aerobic fitness to individuals with normal BMI and poor fitness indicated lower cardiovascular mortality in the former group [31].

Contrary to the aforementioned studies, the findings of the present study indicate that the lipidemic and glycemic profiles were better in the 'leaner and less fit' than the 'heavier and more fit' groups in both genders. The interpretation of the diametrically opposed findings reported for children in the current study when compared to adults could have a developmental basis. More specifically, younger people have healthier tissues to begin with and their homeostatic mechanisms work better [32]. A young, lean and unfit person may deal better with the cacostatic load of unfitness than a middle-aged, lean and unfit person. The opposite may be true for young, fat but fit persons, on whom the cacostatic load of obesity may be higher compared to their middle-aged, fat and fit counterparts.

The present study also showed that the 'leaner and less fit' group exhibited the highest HDLC levels despite the fact that fitness and physical activity are known to be positively related to HDLC levels [33]. However, the effects of exercise training on blood lipid and lipoprotein levels of healthy children and adolescents are equivocal [34], with a meta-analysis reporting no positive effect of aerobic exercise on HDLC levels [35]. Furthermore, the present study reported better triacylglycerol levels, TC/HDLC and LDLC/HDLC ratios in the 'leaner and les fit' category than the 'heavier and more fit' one. Nonetheless, in subsequent analyses, which examined differences in the prevalence of dyslipidemias among the BMI and fitness categories (i.e. % of low HDLC levels, high triacylglycerols levels and TC/HDLC ratio), the statistical significance of these differences did not persist.

Regarding blood pressure, there is evidence for a positive association with BMI [36] and a negative one with physical fitness in children [37]. It is possible that the association between fitness and blood pressure weakens in the presence of increased body fat. Further to unfavorable blood pressure levels increased body fat is also an important risk factor of IR in children [38]. The present study confirmed this by showing higher mean serum HOMA-IR value and prevalence of IR in the 'heavier and more fit' group, thus indicating a more favorable effect of leanness vs. fitness on glycemic control in children.

The current findings should be interpreted on the basis of the study's limitations. The use of BMI to assess weight status is the first limitation, as BMI is not a direct measure of adiposity especially in children [39]. However, the results from the bioelectrical impedance analysis showed that children in the 'heavier and more fit' group had also the highest fat mass levels. A second limitation is that fitness levels were not assessed with direct measurement of peak oxygen uptake, which is considered the optimal method, but with ERT, which has been reported to introduce error. Nonetheless, in epidemiological studies of large cohorts conducted in community settings (i.e. schools in the present study) it is more practical to use indirect, simple and non-invasive methods to assess indices of health status. A third limitation could be the assessment of children's physical activity levels by a subjective self-report method,

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(i.e. questionnaire), which is known for its lower accuracy. However the questionnaire used in the present study was previously validated in children [40], while the parallel use of step counters, i.e. an objective method to assess physical activity levels confirmed the findings derived from the questionnaires.

Conclusions

The present study reported a lower cardiometabolic risk for 'leaner and less fit' children compared to their 'heavier and more fit' peers. These differences suggest that fatness may attenuate the benefits of fitness and physical activity on CVD risk factors in children and that, contrary to the findings in adults, leanness may be more important, from a cardiometabolic health benefit perspective, compared to fitness in children. An intriguing question, then, is at what age the situation is reversed, suggesting the need for future research.

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Disclosure statement

This manuscript represents an original work, which has not been or is not currently under consideration for publication elsewhere. Before submitting the final version of the manuscript all authors have read and approved it. None of the authors had any potential conflict of interest.

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Healthy Growth Study Group

1. Harokopio University Research Team/Department of Nutrition and Dietetics: Yannis Manios (Coordinator), George Moschonis (Project manager), Katerina P. Skenderi, Evangelia Grammatikaki, Odysseas Androutsos, Sofia Tanagra, Alexandra Koumpitski, Paraskevi-Eirini Siatitsa, Anastasia Vandorou, Aikaterini-Efstathia Kyriakou, Vasiliki Dede, Maria Kantilafti, Aliki-Eleni Farmaki, Aikaterini Siopi, Sofia Micheli, Louiza Damianidi, Panagiota Margiola, Despoina Gakni, Vasiliki Iatridi, Christina Mavrogianni, Kelaidi Michailidou, Aggeliki Giannopoulou, Efstathoula Argyri, Konstantina Maragkopoulou, Maria Spyridonos, Eirini Tsikalaki, Panagiotis Kliasios, Anthi Naoumi, Konstantinos Koutsikas, Katerina Kondaki, Epistimi Aggelou, Zoi Krommyda, Charitini Aga, Manolis Birbilis, Ioanna Kosteria, Amalia Zlatintsi, Elpida Voutsadaki, Eleni-Zouboulia Papadopoulou, Zoi Papazi, Maria Papadogiorgakaki, Fanouria Chlouveraki, Maria, Lyberi, Nora Karatsikaki-Vlami, Eva Dionysopoulou, Efstratia Daskalou.

2. Aristotle University of Thessaloniki/School of Physical Education and Sports Science: Vassilis Mougios, Anatoli Petridou, Konstantinos Papaioannou, Georgios Tsalis, Ananis Karagkiozidis, Konstantinos Bougioukas, Afroditi Sakellaropoulou, Georgia Skouli.

3. University of Athens/Medical School: George P. Chrousos, Maria Drakopoulou, Evangelia Charmandari, Panagiota Pervanidou.

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PAPER III

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Association of total body and visceral fat mass with iron deficiency in preadolescents: the Healthy Growth Study

George Moschonis[1], George P. Chrousos[2], Christos Lionis[3], Vassilis Mougios[4] and Yannis Manios[1]*
on behalf of the Healthy Growth Study group

[1]*Department of Nutrition and Dietetics, Harokopio University of Athens, 70, El. Venizelou Avenue, 17671 Kallithea-Athenes, Greece*
[2]*First Department of Pediatrics, Athens University Medical School, Aghia Sophia Children's Hospital, Athens, Greece*
[3]*Clinic of Social and Family Medicine, School of Medicine, Heraklion, University of Crete, Crete, Greece*
[4]*Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece*

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Abstract
The aim of the present study was to examine the associations of obesity, percentage body fat and visceral fat mass with body Fe status in a representative sample of 1493 schoolchildren aged 9–13 years. Anthropometric, body composition, biochemical, clinical (Tanner stage, age of menarche) and dietary intake data were collected. Fe deficiency (ID) was defined as transferrin saturation (TS) $< 16\,\%$; and Fe-deficiency anaemia (IDA) as ID with Hb < 120 g/l. Obese boys and girls and those in the highest quartiles of percentage body fat mass had significantly higher levels of serum ferritin ($P \leq 0{\cdot}05$) compared to their normal-weight peers and those in the corresponding lowest quartiles. Similarly, obese boys and girls and those in the highest quartiles of percentage body fat and visceral fat mass had significantly lower levels of TS ($P \leq 0{\cdot}05$) compared to normal-weight children and those in the corresponding lowest quartiles. The prevalence of ID and IDA was significantly higher in boys and girls in the highest quartiles of percentage body fat than in peers in the lowest quartile. Higher quartiles of percentage body fat and visceral fat mass were the main significant predictors of ID in boys, after controlling for other important confounders, with OR of 2·48 (95 % CI, 1·26, 4·88) and 2·12 (95 % CI, 1·07, 4·19), respectively. Similar significant associations were observed for girls. In conclusion, percentage body fat and visceral fat mass were positively associated with ID in both sexes of preadolescents. These associations might be attributed to the chronic inflammation induced by excess adiposity.

Key words: Iron deficiency: Obesity: Children: Adipose tissue

Many reports have indicated that the prevalence of obesity in childhood and adolescence has been increasing worldwide at an alarming rate[1,2]. On the basis of recent studies, the number of overweight children has doubled and the number of overweight adolescents has tripled since 1970[3]. Following the worldwide trends, obesity among Greek children and adolescents is also on the rise over the last 30 years[4]. It is well established that excess adiposity in both adults and children is strongly associated with several metabolic complications (i.e. metabolic syndrome, insulin resistance, dyslipidaemias)[5,6]. In addition, although contradictory at first sight, obesity has also been associated with nutritional deficiencies[7,8]. Specifically, overweight and obese individuals seem to be at higher risk of Fe deficiency (ID) than those having normal body weight[9–11]. These findings were consistent in both children[12–15] and adults[15–17].

Prevention of ID is crucial from a public health perspective, because it is associated with behavioural and cognitive delays in infancy and early childhood, such as impaired learning[18], decreased school achievements[19,20] and lower scores on tests of mental and motor development[21]. The aetiology of this phenomenon remains uncertain. Suggested contributing factors are poor Fe intake, repeated short-term restrictive diets, increases in blood volume when children enter adolescence, early onset of menstruation, limited physical activity, rapid growth and genetics[12,14]. However, these factors seem not to be significant predictors of the low serum Fe levels observed in overweight and obese individuals[16]. In contrast, ID could partially be explained by the fact that obesity is a low-grade chronic inflammatory state[22]. In particular, studies show that increased levels of inflammatory biomarkers, such as C-reactive protein, are inversely associated with serum Fe

Abbreviations: ID, Fe-deficiency; IDA, Fe-deficiency anaemia; TIBC, total Fe-binding capacity; TS, transferrin saturation; WC, waist circumference.

* **Corresponding author:** Dr Y. Manios, fax +30 210 9514759, email manios@hua.gr

levels in centrally obese adolescents[10]. The hypothesis that the association between ID and obesity is mediated by an obesity-induced low-grade chronic inflammation is strengthened by the higher levels of serum ferritin usually observed in overweight and obese individuals. Although ferritin serves as an index of Fe stores in the body, it is also an acute-phase protein that increases in inflammatory states, such as excess visceral fat accumulation[23].

The studies showing an inverse association between adiposity and Fe status in children and adolescents[10,12,14] have relied on BMI. However, BMI is not always a direct measure of adiposity, especially in children[24]. Furthermore, to our knowledge based on the available literature, no study so far has ever examined the relationship between more direct measures of adiposity and Fe status. Such studies may explore a more accurate association between obesity and ID in children and highlight further the discussion of dietary recommendations of Fe intake in them. The present study reports on the associations between central obesity, percentage body fat and visceral fat mass and ID in a representative sample of preadolescent Greek children.

Methods and procedures

Sampling

The Healthy Growth Study was a large-scale, cross-sectional, epidemiological study initiated in May 2007. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of Harokopio University of Athens. Approval to conduct the study was also granted by the Greek Ministry of National Education. The population under study comprised schoolchildren aged 9–13 years, attending the 5th and 6th grades of primary schools located in municipalities within the prefectures of Attica, Etoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multi-stage and stratified by parents' educational level and total population of students attending schools within these municipalities. Specifically, the municipalities in the prefectures under study were divided into three groups on the basis of average educational level of their adult population (25–65 years old) that was estimated from data provided by the National Statistical Service of Greece (2001 census). This procedure yielded two parents' education cut-off points that allowed us to categorise municipalities into three different socio-economic levels, i.e. higher, middle and lower. Consequently, again on the basis of data from the National Statistical Service, a certain number of municipalities, proportional to the size of their preadolescent population (9–13 years old), was randomly selected from each socio-economic level. Finally, a number of schools were randomly selected from each municipality, proportional to the population of schoolchildren registered in the 5th and 6th grades, according to data obtained from the Greek Ministry of Education.

All seventy-seven primary schools that were invited to participate in the study responded positively. Weight and height were measured in all pupils attending the 5th and 6th grades in these primary schools as part of a school-based health and nutrition education programme. Full medical examination (i.e. anthropometric and body composition measurements, blood collection, clinical examination etc.) and questionnaire data were obtained from a subgroup of pupils whose parents signed an informed consent form. Signed parental consent forms were collected for 2655 out of 4145 children (response rate 64·1 %). Still no significant differences with respect to parental educational level were observed between families that consented to data collection and those that did not.

Physical examination and anthropometry

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany). Pupils were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0·1 cm using a commercial stadiometer (Leicester Height Measure; Invicta Plastics, Oadby, UK), with the pupil standing barefoot, keeping shoulders in a relaxed position, arms hanging freely and head in Frankfurt horizontal plane. Weight and height were used to calculate BMI using Quetelet's equation (weight (kg)/height $(m)^2$). The International Obesity Task Force cut-off points[25,26] were used to categorise participants as 'normal weight', 'overweight' or 'obese'. Waist circumference (WC) was measured to the nearest 0·1 cm with the use of a non-elastic tape (Hoechstmass, Germany), with the pupil standing, at the end of a gentle expiration after placing the measuring tape on a horizontal plane around the trunk, at the level of umbilicus midway, between the lower rib margin and the iliac crest. The age- and sex-specific WC percentiles were used for the classification of central obesity ($\geq$90th percentile)[27]. One well-trained and experienced female paediatrician in each prefecture determined pubertal maturation (Tanner stage) after thorough visual inspection of breast development in girls and genital development in boys[28]. Finally, each girl was asked by the paediatrician about her menstruation status and age of menarche.

Assessment of percentage body fat and visceral fat mass

Bioelectrical impedance analysis was used for the assessment of percentage body fat (Akkern BIA 101; Akkern Srl., Florence, Italy) and for abdominal–visceral fat mass (Tanita Viscan AB-140, Kowloon, Hong Kong). In abdominal bioelectrical impedance analysis, an electric current is passed between the regions near the umbilicus and spinal cord at the umbilicus level, and the voltage generated in the lateral abdomen is recorded. Because the equipotential line that passes through visceral fat appears on the lateral abdominal surface, the amount of visceral fat can be estimated by measurement of the voltage generated at this location using a regression equation determined by computed tomography[29]. Participants were instructed to abstain from any food or liquid intake and from any intensive exercise for 4 h before measurement. They were also instructed not to wear any metallic object during measurement. The assessments took place with the pupils lying on a non-conductive surface at ambient room temperature. Percentage body fat was calculated from the resistance and reactance values using valid equations derived from a similar preadolescent population[30], while visceral fat mass was read directly from the instrument in a rating scale from 1 to 59 units, with 0·5 increments. On the basis of these data, children were categorised into four sex-specific quartiles of percentage body fat and visceral fat mass. As there were only two Tanita Viscan devices available, data on trunk–visceral fat mass were collected for a representative sub-sample of 1500 children.

Biochemical indices

Blood samples were obtained for biochemical and haematological screening tests between 08.30 and 10.30 hours after a 12 h overnight fast. Reminders were distributed the previous day to both parents and children in order to ensure compliance with fasting. Professional staff performed venepuncture, using two types of test tubes, one of which contained EDTA, to obtain a maximum of 10 ml blood. The EDTA-blood was transferred on the same day of collection to a local laboratory, where it was analysed in a CELL-DYN haematological autoanalyser (Abbott Diagnostics, Abbott Park, IL, USA) for the determination of haematological indices, including erythrocyte count, Hb and mean corpuscular volume. The remaining blood was collected in plain test tubes for the preparation of serum, which was divided into aliquots and stored at -80°C. When blood collection was completed in Aitoloakarnania, Thessaloniki and Iraklio, all serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where biochemical analyses and central storage of back-up samples at -80°C took place. Serum Fe and total Fe-binding capacity (TIBC) levels were determined by colorimetric assays (Roche Diagnostics SA, Basel, Switzerland). Transferin saturation (TS) was calculated by dividing serum Fe by TIBC and multiplying by 100. Finally, serum ferritin was measured by using a chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Fe deficiency (with or without anaemia) and Fe-deficiency anaemia (IDA) were defined using the following age- and sex-specific thresholds proposed by UNICEF and the WHO[31]: ID was defined as TS $<$ 16 %; IDA was defined as TS $<$ 16 % and Hb concentration $<$ 120 g/l, which is the threshold value for anaemia for children aged 9–13 years. The Mentzer Index (MCV (fl)/RBC (M/μl))[32] was also calculated for all pupils participating in the present study to differentiate beta-thalassaemia from ID. On the basis of this index, children with thalassaemia minor (eighteen cases) were excluded from further analysis.

Dietary assessment

Dietary intake data were obtained by trained dieticians and nutritionists via morning interviews with the children at school-site for two consecutive weekdays and one weekend day, using the 24 h recall technique. Specifically, all study participants were asked to describe the type and amount of foods, as well as all beverages consumed during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts where appropriate. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimise the interviewer's effect, reviewed the collected data with the respondent in order to clarify entries, servings and possible forgotten foods. Food intake data were analysed using the Nutritionist V diet analysis software (version 2.1, 1999; First Databank, San Bruno, CA, USA), which was extensively amended to include traditional Greek recipes, as described[33]. Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

Socio-economic and demographic variables

Family socio-economic and demographic data (i.e. total years of education for the father and mother and annual family income) were collected during scheduled interviews at the school with the parents (mainly with the mother). For those parents not able to attend (approximately 5 % of the total sample), data were collected via telephonic interviews. All interviews were conducted with the use of a standardised questionnaire by a research team that was rigorously trained to minimise the interviewer's effect.

Statistical analysis

Statistical analysis was conducted for the sub-sample of children with full anthropometric, biochemical, dietary, body composition and socio-economic data and no thalassaemia minor (*n* 1493). Continuous variables were expressed as mean values and standard deviations and categorical variables were reported as frequencies (%). Comparisons between levels of the continuous variables were conducted using Student's *t* test or ANOVA, using the Bonferroni correction for *post hoc* multiple comparisons among groups. Comparisons between levels of the categorical variables were conducted using the χ^2 test or the Fisher's exact test, as appropriate. The two-sample *Z*-test was also used to perform pair-wise comparisons of the prevalence of ID and IDA between quartiles of percentage body fat and visceral fat mass. In order to test the effect of the independent variables examined on ID, multivariate logistic regression analysis was conducted and adjusted OR with 95 % CI were computed. All reported *P* values were based on two-sided tests. The level of statistical significance was set at $\alpha = 0{\cdot}05$. Statistical analysis was conducted using STATA (Stata Corporation, College Station, TX, USA) for the two-sample *Z*-test and SPSS version 17.0 (SPSS, Inc., TX, USA) for all other tests.

Results

The sample consisted of 1493 children attending the 5th and 6th grades of primary school. Table 1 presents the main descriptive characteristics of the study population. The age of the study participants was 11·2 (SD 0·7) years. No significant differences were found between boys and girls with respect to nationality and socio-economic characteristics (i.e. socio-economic level of school region, paternal and maternal educational level and family income). Similarly, no significant sex differences were found with respect to the prevalence of ID. On the other hand, more girls than boys were found to be at Tanner stages 3–5 ($P<0·05$), while the prevalence of obesity was higher in boys than in girls (12·5 *v.* 10·2 %, $P<0·05$).

Table 1. Descriptive characteristics of the study population

	Percentage of boys (*n* 740)	Percentage of girls (*n* 753)	Total percentage (*n* 1493)
Age (years)			
9–11	38·7	39·1	38·9
11–13	61·3	60·9	61·1
SEL of school			
Lower	25·9	24·8	25·4
Medium	28·5	30·8	29·7
Higher	45·5	44·4	44·9
Grade			
5th	47·0	47·4	47·2
6th	53·0	52·6	52·8
Nationality			
Greek	89·7	87·8	88·7
Other	10·3	12·2	11·3
Tanner stage			
1	44·8	21·6*	32·8
2	43·4	37·6*	40·4
3	10·6	26·6*	18·8
4	1·1	10·9*	6·2
5	0·1	3·3*	1·8
Menarche			
Yes	–	77·4	–
No	–	22·6	–
Paternal education (years)			
< 9	25·0	27·0	26·0
9–12	40·0	37·0	38·5
> 12	34·9	36·0	35·5
Maternal education (years)			
< 9	20·7	24·1	22·4
9–12	39·7	37·6	38·7
> 12	39·6	38·3	38·9
Family income (€/year)			
< 12 000	18·3	21·9	20·1
12 000–20 000	24·8	24·9	24·9
20 000–30 000	24·1	24·2	24·2
30 000–40 000	15·9	15·6	15·7
40 000–50 000	8·4	6·2	7·3
> 50 000	8·4	7·2	7·8
Weight groups			
Normal-weight	57·0	61·3	59·2
Overweight	30·5	28·6	29·5
Obese	12·5	10·2*	11·3
ID			
Normal	85·2	85·4	85·3
ID (TS < 16 %)	14·8	14·6	14·7

SEL, socio-economic level; ID, Fe deficiency; TS, transferin saturation.
* Values were significantly different from boys ($P<0·05$, derived from the two-sample *Z*-test for proportions).

Table 2 displays the biochemical indices of Fe status across weight groups in both boys and girls. In boys, serum Fe and TS differed significantly among weight groups ($P<0·05$), being lower in obese and overweight than in normal-weight ones. In contrast, serum ferritin was lowest in normal-weight boys ($P<0·05$). In girls, TS exhibited lower values in obese than in normal-weight girls. On the contrary, ferritin was higher in obese than in normal-weight girls; and TIBC exhibited higher values in obese and overweight than in normal-weight girls. Moreover, the prevalence of ID was higher in obese boys and girls compared to their normal-weight peers ($P=0·009$ in boys and $P=0·017$ in girls). Similarly, the prevalence of IDA was higher in obese than in overweight or normal-weight girls ($P=0·002$). Table 3 also presents the biochemical indices of Fe status in the categories of normal WC and central obesity. TS in centrally obese boys was significantly lower ($P=0·033$) compared to peers of normal WC, while the prevalence of IDA was significantly higher in centrally obese boys than in boys of normal WC ($P=0·006$). Furthermore, centrally obese girls exhibited higher prevalence of both ID and IDA than girls of normal WC ($P=0·001$ and 0·042, respectively).

Table 4 summarises the biochemical indices of Fe status across quartiles of percentage body fat. As far as boys were concerned, serum Fe and TS were found to differ significantly among quartiles, being lowest in the highest quartile. On the contrary, serum ferritin was lowest in the lowest quartile ($P=0·001$). Furthermore, the prevalence of ID and IDA was significantly higher for boys in the highest quartile of percentage body fat compared to the middle and lowest ones ($P=0·005$ and 0·010, respectively). In girls, serum Fe and TS exhibited lower values in the highest compared to the lowest quartile, whereas TIBC and ferritin exhibited higher values in the highest compared to the lowest quartile. Furthermore, the prevalence of ID and IDA was significantly higher for girls in the highest quartile of percentage body fat than for those in the lowest quartile ($P=0·027$ and 0·046, respectively). Additionally, Table 5 also presents the same indices of Fe status across quartiles of visceral fat mass. In boys, serum Fe and TS differed significantly among quartiles ($P<0·05$), being lower in the highest than in the lowest quartile. On the other hand, TIBC and ferritin differed significantly among quartiles ($P<0·05$), but were higher in the highest than in the lowest quartile. As far as girls were concerned, TIBC was higher and TS was lower in the highest compared to the lowest quartile of visceral fat mass ($P<0·05$).

The logistic regression analysis showed that overweight boys and obese girls were 2·13 (95 % CI, 1·27, 3·60) and 2·25 (95 % CI 1·14, 4·46) times more likely to be Fe deficient than normal-weight boys after controlling for several important covariates (Table 6). Furthermore, centrally obese girls were 2·23 (95 % CI, 1·30, 3·83) times more likely to be Fe deficient in comparison with girls of normal WC. Boys in the highest quartiles of percentage body fat and visceral fat mass were 2·48 (95 % CI, 1·26, 4·88) and 2·12 (95 % CI, 1·07, 4·19) times more likely to be Fe deficient than boys in the lowest quartiles (Table 6). Similarly, girls in the highest quartiles of percentage body fat and visceral fat mass were 2·12 (95 % CI, 1·07, 4·20)

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Table 2. Biochemical and dietary indices of iron status across BMI groups in prepubertal children

(Mean values and standard deviations)

	Boys							Girls						
	Normal-weight (*n* 421)		Overweight (*n* 226)		Obese (*n* 93)			Normal-weight (*n* 461)		Overweight (*n* 215)		Obese (*n* 77)		
BMI groups	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**
Biochemical serum indices														
Fe (μg/l)	905†‡	329	836†	354	816‡	304	0·024	920	349	926	340	835	361	0·166
TIBC (μg/l)	3338	534	3372	515	3462	430	0·160	3422†‡	535	360·2†	610	3600‡	943	0·002
TS (%)	27·3†‡	9·5	25·2†	10·6	23·5‡	8·2	0·002	27·2‡	10·2	26·1	9·7	23·7‡	10·3	0·032
Ferritin (ng/ml)	30·6†‡	20·7	35·3†	22·9	35·8‡	19·1	0·021	27·3‡	15·5	30·5	19·4	31·6‡	14·4	0·036
Dietary indices														
Fe intake (mg/4184 kJ (mg/1000 kcal))	5·8	1·9	6·3	2·2	5·9	2·9	0·246	6·0	4·4	6·7	3·6	6·4	3·9	0·176
Fe status categories (% of total)														
ID	10·7§¶		20·7§		16·0¶		0·009‖	12·9¶		13·9		26·6¶		0·017‖
IDA	0·9		2·8		3·9		0·113‖	1·6¶		2·2**		9·4¶**		0·002‖

TIBC, total Fe-binding capacity; TS, transferrin saturation; ID, Fe deficiency with or without anaemia; IDA, Fe-deficiency anaemia.
* Derived from ANOVA.
† Mean values were significantly different between normal-weight and overweight children after *post-hoc* multiple comparisons ($P < 0·05$, Bonferroni rule).
‡ Mean values were significantly different between normal-weight and obese children after *post-hoc* multiple comparisons ($P < 0·05$, Bonferroni rule).
§ Values were significantly different between normal-weight and overweight children ($P < 0·05$, two-sample *Z*-test).
‖ Derived from the Pearson χ^2 test.
¶ Values were significantly different between normal-weight and obese children ($P < 0·05$, two-sample *Z*-test).
** Values were significantly different between over-weight and obese children ($P < 0·05$, two-sample *Z*-test).

Table 3. Biochemical and dietary indices of iron status across waist circumference (WC) groups in prepubertal children

(Mean values and standard deviations)

	Boys					Girls				
	Normal WC (*n* 613)		Central obesity* (*n* 127)			Normal WC (*n* 614)		Central obesity* (*n* 139)		
WC groups	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†
Biochemical serum indices										
Fe (μg/l)	892	340	769	299	0·165	933	349	820	330	0·267
TIBC (μg/l)	3343	526	3468	461	0·285	3474	563	3583	811	0·153
TS (%)	27·0	9·9	22·3	8·3	0·033	27·2	10·1	23·4	9·7	0·304
Ferritin (ng/ml)	32·3	21·5	34·5	19·9	0·683	28·3	16·8	30·0	16·1	0·799
Dietary indices										
Fe intake (mg/4184 kJ (mg/1000 kcal))	5·9	3·4	6·2	3·2	0·483	6·2	4·1	6·5	4·2	0·563
Fe status categories (% of total)										
ID	13·5		20·2		0·093‡	12·4		25·2		0·001‡
IDA	1·0		5·8		0·006‡	2·0		5·4		0·042‡

TIBC, total Fe-binding capacity; TS, transferrin saturation; ID, Fe deficiency with or without anaemia; IDA, Fe-deficiency anaemia.
* WC ≥ 90th age- and sex-specific percentile.
† Derived from Student's *t* test.
‡ Derived from Pearson χ^2.

and 2·17 (95 % CI, 1·09, 4·34) times more likely to be Fe deficient than girls in the lowest quartiles.

Discussion

The present study is among the first to examine the association of several total and regional body fat indices with biochemical indices of Fe status in a considerably large and representative sample of preadolescents. Overall, the prevalence of ID was remarkably high for obese and centrally obese children and for children in the highest quartiles of percentage body fat and visceral fat mass, ranging from 16 to 27 %. These values are similar or higher than those reported for overweight Swiss and Israeli children and adolescents (i.e. 20 and 12 %, respectively)[14,34]. In these two studies, as well as in the present one, the definition of ID was based on reliable biochemical markers of Fe status, such as serum transferrin receptor concentration, TS and free erythrocyte protoporphyrin concentration. However, the prevalence of ID reported by the present and the two aforementioned studies was considerably higher compared to similar data from the USA[12], since, according to the 3rd National Health and Nutrition Examination Survey, the prevalence of ID in obese children and adolescents was 2·4 and 9·1 %, respectively. The use of serum ferritin as one of the diagnostic criteria for ID could probably provide an explanation for the lower prevalence of ID observed in the American population. Ferritin is an acute-phase protein and its serum levels are plausibly elevated in states of chronic or acute inflammation[35]. Considering that excess total and, especially, visceral fat mass represents a chronic inflammatory state, the increased levels of serum ferritin in overweight and obese children could have contributed to an underestimation of the prevalence of ID in this cohort. This hypothesis is strengthened by a more recent study that also used NHANES data to examine the incidence of ID among overweight female adolescents[10]. In this study, serum ferritin was excluded from the definition of ID, resulting in a prevalence value of 30·8 %.

Consistent with other studies that have reported a positive association between serum ferritin levels and body fat indices, mainly in adults[23,36,37], the present study has found significantly higher serum ferritin in boys in the highest quartiles of percentage body fat and visceral fat mass, as well as in girls in the highest quartile of percentage body fat, compared to the corresponding lowest quartiles (Tables 2–6). By contrast, the values of the other biochemical indices of Fe status were indicative of depleted body Fe stores for children in the highest quartiles of percentage body fat and visceral fat mass. In particular, boys and girls in the highest quartiles of percentage body fat and visceral fat mass had significantly lower TS than their peers in the lowest quartiles. This was also true for serum Fe in most cases. In addition, TIBC of girls in the highest quartiles of percentage body fat and visceral fat mass were significantly higher compared to girls in the lowest quartiles. These findings are consistent with recent studies showing that obese children and adults had

Table 4. Biochemical and dietary indices of iron status across quartiles of percentage body fat mass in prepubertal children

(Mean values and standard deviations)

	Boys							Girls						
	Lower quartile (*n* 183)		Middle quartile (*n* 374)		Higher quartile (*n* 183)			Lower quartile (*n* 189)		Middle quartile (*n* 371)		Higher quartile (*n* 193)		
Percentage body fat mass	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**
Biochemical serum indices														
Fe (μg/l)	935†	339	883‡	341	782†‡	299	0·001	961†	361	914	338	862†	351	0·048
TIBC (μg/l)	3388	578	3323	497	3421	493	0·144	3393†	558	3510	665	3564†	569	0·046
TS (%)	27·8†	9·6	26·8‡	9·9	23·2†‡	9·1	0·001	28·7†	10·9	26·4	9·6	24·5†	9·8	0·001
Ferritin (ng/ml)	27·3§†	16·0	33·6§	21·8	36·3†	23·9	0·001	26·3†	15·1	28·7	17·8	31·0†	16·5	0·050
Dietary indices														
Fe intake (mg/4184 kJ (mg/1000 kcal))	5·6	1·7	5·9	2·2	6·4	5·7	0·107	6·5	1·4	5·9	2·3	6·8	4·1	0·079
Fe status categories (% of total)														
ID	11·0¶		12·2‖		22·6¶‖		0·005**	10·7¶		13·5		21·1¶		0·027**
IDA	1·4¶		0·7‖		4·8¶‖		0·010**	1·3¶		2·0		5·3¶		0·046**

TIBC, total Fe-binding capacity; TS, transferrin saturation; ID, Fe deficiency with and without anaemia; IDA, Fe-deficiency anaemia.
* Derived from ANOVA.
† Mean values were significantly different between lower and higher quartiles after *post- hoc* multiple comparisons ($P<0·05$, Bonferroni rule).
‡ Mean values were significantly different between middle and higher quartiles after *post-hoc* multiple comparisons ($P<0·05$, Bonferroni rule).
§ Mean values were significantly different between lower and middle quartiles after *post-hoc* multiple comparisons ($P<0·05$, Bonferroni rule).
‖ Values were significantly different between middle and higher quartiles ($P<0·05$, two-sample *Z*-test).
¶ Values were significantly different between lower and higher quartiles ($P<0·05$, two-sample *Z*-test).
** Derived from Pearson χ^2.

Table 5. Biochemical and dietary indices of iron status across quartiles of visceral fat mass in prepubertal children

(Mean values and standard deviations)

	Boys							Girls						
	Lower quartile (*n* 217)		Middle quartile (*n* 346)		Higher quartile (*n* 177)			Lower quartile (*n* 188)		Middle quartile (*n* 381)		Higher quartile (*n* 184)		
Visceral fat mass	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**
Biochemical serum indices														
Fe (μg/l)	918†	330	875	349	807†	311	0·013	958	379	912	336	873	338	0·105
TIBC (μg/l)	3354	583	3326‡	487	345·3‡	474	0·049	3380†	515	3481‡	554	3642†‡	783	0·001
TS (%)	27·5†	9·1	26·6‡	10·5	23·6†‡	8·8	0·001	28·5†	10·8	26·6	9·8	24·5†	9·8	0·003
Ferritin (ng/ml)	29·1†	17·8	33·7	22·1	34·8†	22·9	0·028	27·9	16·7	27·9	16·6	31·2	16·7	0·110
Dietary indices														
Fe intake (mg/4184 kJ (mg/1000 kcal))	5·8	1·9	5·8	2·1	6·5	5·7	0·096	5·8	2·8	6·4	4·8	6·3	3·5	0·348
Fe status categories (% of total)														
ID	10·2		15·6		18·8		0·089§	11·0		13·8		20·1		0·068§
IDA	1·7		1·1		3·5		0·223§	1·3¶		1·9		5·4¶		0·047§

TIBC, total Fe-binding capacity; TS, transferrin saturation; ID, Fe deficiency with and without anaemia; IDA, Fe-deficiency anaemia.
* Derived from ANOVA.
† Mean values were significantly different between lower and higher quartiles after *post-hoc* multiple comparisons ($P<0·05$, Bonferroni rule).
‡ Mean values were significantly different between middle and higher quartiles after *post-hoc* multiple comparisons ($P<0·05$, Bonferroni rule).
§ Derived from the Pearson χ^2 test.
¶ Values were significantly different between lower and higher quartiles ($P<0·05$, two-sample *Z*-test).

lower levels of serum Fe and TS than their normal-weight counterparts[10,13,16].

The mechanism linking obesity with ID has not yet been elucidated. One proposed such mechanism is low dietary Fe intake by overweight individuals. Still, the available literature in this regard remains controversial. Although a study on Greek adolescents reported low Fe intakes by overweight pupils[38], other recent studies did not find any significant differences in Fe intake between obese and non-obese children and adolescents[10,16,34]. Similarly, the present study did not show any significant differences in dietary Fe intake between the weight and WC groups and among quartiles of total and visceral fat mass (Tables 1–5). Differences in the dietary intakes of specific nutrients that either enhance (vitamin C) or inhibit (Ca, dietary fibre) intestinal non-haeme Fe absorption[39] could also provide an explanation for the depleted Fe stores observed in children with increased adiposity. However, Aeberli *et al.*[34] reported no significant associations between intakes of bioavailable Fe or factors enhancing Fe absorption and overweight in Swiss children and adolescents.

Furthermore, the bioavailability of Fe may also be modulated by other physiological factors mainly linked to the chronic inflammation induced by excess adiposity. The significant positive associations observed in the present study, between higher percentage body fat and visceral fat mass and ID (Table 6), could strengthen the aforementioned link. More specifically, increased total and visceral fat mass accumulation stimulates the production of inflammatory cytokines[22], that have been reported to be inversely associated with serum Fe levels[10]. The hypothesis, that the significant association observed between ID and obesity is mediated by a low-grade chronic inflammation, is also supported by the higher levels of serum ferritin observed among overweight and obese children in the present study. Moreover, serum levels of hepcidin, which is the main regulatory hormone for Fe absorption and recirculation, have been reported by other studies to be significantly higher in overweight children, adolescents[13,34] and adults[10,40] than in normal-weight peers. Adipose-derived cytokines (such as IL-6 and leptin) produced in response to obesity-related inflammation activate hepcidin gene transcription[41]. This results in increased serum hepcidin levels that lead to the sequestration of Fe within the reticuloendothelial system and to decreased dietary Fe absorption from the intestine by controlling the expression of ferroportin 1 at the basolateral membranes of the enterocytes[42].

The present study has certain strengths and limitations. Regarding strengths, the Healthy Growth Study is a large-scale, epidemiological study covering the central, northern, southern and western parts of the Greek territory. Furthermore, to our knowledge, this is the first study reporting associations between visceral fat mass and Fe status in children. One other strength of the present study relates to the fact that detailed data on diet, blood and anthropometry as well as important confounders (e.g. socio-economic status and pubertal status) were collected. Regarding limitations, additional to its cross-sectional design, the present study has been based on a sub-sample of 1493 pupils with full body

Table 6. Risk for iron deficiency with and without anaemia among children with different BMI, waist circumference (WC), percentage of body fat and visceral fat mass, controlling for other important covariates

(Adjusted odds ratios and 95 % confidence intervals)

	Boys (*n* 740)					Girls (*n* 753)				
		Model 1*		Model 2†			Model 1*		Model 2†	
	n	Adjusted OR	95 % CI	Adjusted OR	95 % CI	*n*	Adjusted OR	95 % CI	Adjusted OR	95 % CI
BMI groups										
Normal-weight	421	1·00		1·00		461	1·00		1·00	
Overweight	226	2·14	1·28, 3·54	2·13	1·27, 3·60	215	1·09	0·65, 1·84	1·11	0·64, 1·94,
Obese	93	1·64	0·80, 3·34	1·62	0·78, 3·37	77	2·43	1·29, 4·58	2·25	1·14, 4·46
WC groups										
Normal	613	1·00		1·00		614	1·00		1·00	
Central obesity	127	1·62	0·93, 2·84	1·64	0·92, 2·95	139	2·39	1·44, 3·96	2·23	1·30, 3·83
Body fat quartiles										
Lower quartile	183	1·00		1·00		189	1·00		1·00	
Medium quartiles‡	374	1·24	0·65, 2·35	1·19	0·62, 2·30	371	1·31	0·71, 2·43	1·12	0·59, 2·15
Higher quartile	183	2·48	1·28, 4·81	2·48	1·26, 4·88	193	2·23	1·16, 4·27	2·12	1·07, 4·20
Visceral fat quartiles										
Lower quartile	217	1·00		1·00		188	1·00		1·00	
Medium quartiles‡	346	1·81	0·98, 3·35	1·71	0·92, 3·20	381	1·27	0·70, 2·32	1·30	0·69, 2·45
Higher quartile	177	2·16	1·11, 4·23	2·12	1·07, 4·19	184	2·06	1·08, 3·93	2·17	1·09, 4·34

* Unadjusted.
† Adjusted for dietary Fe, Ca, vitamin C and fibre intake, maternal educational level, Tanner stage and menarche (girls only).
‡ Quartiles 2 and 3 in each case were combined to the 'medium' quartiles.

composition data out of the 2655 pupils with parental consent to participate in the study. Still, no statistically significant differences were observed between pupils with full body composition data (including visceral fat mass levels; i.e. *n* 1493) and the rest 1162 participating pupils with no visceral fat mass data with regards to parental educational level and weight status (data not shown). Another limitation was the use of only three 24 h recalls, which may not be sufficient to assess children's habitual dietary intake. Nonetheless, this is of particular concern for foods consumed only occasionally (whereas foods providing Fe are consumed on a more regular basis) while 24 h recalls are most feasible in larger samples of children such as in the present study.

In conclusion, the present study showed significant positive associations of higher percentage body fat and visceral fat mass with ID in both sexes. These associations may have important public health implications, since they point to the need to also consider excess adiposity as a non-traditional risk factor for ID and IDA. Higher total and visceral fat mass levels should be taken into account when assessing children's body Fe status and should probably be treated before providing dietary recommendations to correct ID or IDA. Future prospective studies could help highlight the basis of the association between obesity and ID, thus providing better insight into how to most effectively tackle the important public health issues of excess adiposity and ID as early in life as possible.

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Healthy Growth Study Group

Harokopio University Research Team/Department of Nutrition and Dietetics: Yannis Manios (Coordinator), George Moschonis (Project Manager), Katerina P. Skenderi, Evangelia Grammatikaki, Odysseas Androutsos, Sofia Tanagra, Alexandra Koumpitski, Paraskevi-Eirini Siatitsa, Anastasia Vandorou, Aikaterini-Efstathia Kyriakou, Vasiliki Dede, Maria Kantilafti, Aliki-Eleni Farmaki, Aikaterini Siopi, Sofia Micheli, Louiza Damianidi, Panagiota Margiola, Despoina Gakni, Vasiliki Iatridi, Christina Mavrogianni, Kelaidi Michailidou, Aggeliki Giannopoulou, Efsthoula Argyri, Konstantina Maragkopoulou, Maria Spyridonos, Eirini Tsikalaki, Panagiotis Kliasios, Anthi Naoumi, Konstantinos Koutsikas, Katerina Kondaki, Epistimi Aggelou, Zoi Krommyda, Charitini Aga, Manolis

Birbilis, Ioanna Kosteria, Amalia Zlatintsi, Elpida Voutsadaki, Eleni-Zouboulia Papadopoulou, Zoi Papazi, Maria Papadogiorgakaki, Fanouria Chlouveraki, Maria Lyberi, Nora Karatsikaki-Vlami, Eva Dionysopoulou, Efstratia Daskalou.

Aristotle University of Thessaloniki/School of Physical Education and Sports Sciences: Vassilis Mougios, Anatoli Petridou, Konstantinos Papaioannou, Georgios Tsalis, Ananis Karagkiozidis, Konstantinos Bougioukas, Afroditi Sakellaropoulou, Georgia Skouli.

University of Athens/Medical School: George P. Chrousos, Maria Drakopoulou, Evangelia Charmandari, Neni Pervanidou.

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PAPER IV

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RESEARCH PAPER

The double burden of obesity and iron deficiency on children and adolescents in Greece: the Healthy Growth Study

Y. Manios,* G. Moschonis,† G. P. Chrousos,† C. Lionis,‡ V. Mougios,§ M. Kantilafti,* V. Tzotzola,* K. P. Skenderi,* A. Petridou,§ G. Tsalis,§ A. Sakellaropoulou,§ G. Skouli§ & C. Katsarou*

*Department of Nutrition and Dietetics, Harokopio University of Athens, Athens, Greece
†First Department of Pediatrics, Athens University Medical School, 'Aghia Sophia' Children's Hospital, Athens, Greece
‡Clinic of Social and Family Medicine, School of Medicine, University of Crete, Crete, Greece
§Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece

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Correspondence
Y. Manios, Department of Nutrition and Dietetics, Harokopio University of Athens, 70, El.Venizelou Ave, 17671 Kallithea, Athens, Greece.
Tel.: +30 210 9549156
Fax: +30 210 9514759
E-mail: manios@hua.gr

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Abstract

Background: Some small cohort studies have noted that obesity co-exists with lower serum iron levels. The present study aimed to examine the association between being overweight and iron deficiency (ID) in a large cohort of Greek children and adolescents.
Methods: A representative sample of 2492 primary schoolchildren aged 9–13 years old was examined. Anthropometric, biochemical, clinical, dietary intake and physical activity data were collected.
Results: The prevalence of ID and iron deficiency anaemia (IDA) was higher in obese boys and girls compared to their normal-weight peers ($P < 0.05$). Serum ferritin was higher in obese compared to normal-weight boys ($P = 0.024$) and higher in obese compared to normal-weight and overweight girls ($P = 0.001$). By contrast, a negative association was found between transferrin saturation and adiposity in both boys and girls ($P = 0.001$ and $P = 0.005$). Furthermore, obese girls had significantly higher fibre intake than normal-weight girls ($P = 0.048$) and also overweight and obese boys and girls recorded significantly fewer pedometer steps than their normal-weight peers ($P < 0.001$). Finally, obesity more than doubled the likelihood of ID in both boys (odds ratio = 2.83; 95% confidence inteval = 1.65–4.85) and girls (odds ratio = 2.03; 95% confidence interval = 1.08–3.81) after controlling for certain lifestyle and clinical indices as potential confounders.
Conclusions: The present study shows that obese children and adolescents were at greater risk for ID and IDA than their normal-weight peers. Low grade inflammation induced by excessive adiposity may be a reason for the observed low iron levels. This is also strengthened by the elevated serum ferritin levels, comprising an acute phase protein that is plausibly increased in inflammation.

Introduction

Wenzel *et al.* (1962) were the first to report lower serum iron levels in obese compared to non-obese adolescents. Subsequently, this association has received little attention. However, some recent studies have shown that overweight children and adolescents are at higher risk for iron deficiency (ID) compared to normal-weight counterparts (Pinhas-Hamiel *et al.*, 2003; Nead *et al.*, 2004; del Giudice *et al.*, 2009). The exact aetiology of this double burden (i.e. obesity and ID) remains uncertain. Suggested contributing factors include a poor dietary iron intake, probably as a result of repeated short-term restrictive diets, in overweight individuals and genetics (Pinhas-Hamiel *et al.*, 2003; Nead *et al.*, 2004). Still, the exact aetiology of obesity-related hypofaerremia in children remains uncertain (Menzie *et al.*, 2008).

Considering that obesity represents a low-grade chronic inflammatory state, the hypofaerremia associated with obesity could be attributed to certain metabolic and molecular adaptations induced by inflammation. This hypothesis is supported by the significant inverse correlations observed in overweight and obese individuals between serum iron concentration and the concentrations of a variety of adipocytokines and pro-inflammatory cytokines after controlling for iron intake, iron needs and iron losses (Chung *et al.*, 2007; Tussing-Humphreys *et al.*, 2009). Furthermore, certain acute-phase peptides (e.g. hepcidin) that are usually elevated in obesity have also been found to regulate iron haemostasis, mainly via their iron-binding effects (Menzie *et al.*, 2008; del Giudice *et al.*, 2009).

Impaired iron status in children and adolescents could have several important clinical implications for future physical and cognitive development and maturation. Thus, identifying overweight/obese children as a high-risk group for ID could be very important from a public health perspective for designing and implementing tailored-made dietary intervention approaches to correct both excess body weight and poor iron status, even in developed countries. The present study aimed to examine the association between being overweight and iron status in children and adolescents in Greece and to identify the main risk factors for ID.

Materials and methods

Sampling

The Healthy Growth Study was a large scale cross-sectional epidemiological study, initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethical Committee of Harokopio University of Athens. The population under investigation comprised schoolchildren aged 9–13 years old, who were attending the fifth and sixth grades of primary schools located in municipalities within the prefectures of Attica, Aitoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multistage and stratified by parents' educational level and the total population of students attending schools within these municipalities, as described in more detail elsewhere (Moschonis *et al.*, 2010). The multistage, random sampling procedure yielded 77 primary schools that responded positively when they were invited to participate in the study. Weight and height were measured in all pupils attending the fifth and sixth grades in these primary schools as a part of a school-based health and nutrition education programme. Full medical examination (i.e. anthropometric and body composition measurements, blood collection, clinical examination, etc.) and questionnaire data were obtained from a subgroup of pupils whose parents provided their written informed consent form. Signed parental consent forms were collected for 2655 out of 4145 children (a response rate of 64.1%).

Physical examination and anthropometry

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770; SECA, Hamburg, Germany). Pupils were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0.1 cm using a commercial stadiometer (Leicester Height Measure; Invicta Plastics, Oadby, UK) with the pupil standing barefoot, keeping the shoulders in a relaxed position, arms hanging freely and head in Frankfurt horizontal plane. Weight and height were used to calculate body mass index (BMI) using Quetelet's equation [weight (kg)/height (m)^2]. The International Obesity Task Force cut-off points (Cole *et al.*, 2007, 2000) were used to categorise participants as 'underweight', 'normal weight', 'overweight' or 'obese' (cut-off points for 'Overweight' ranged from 19.10 to 21.91 for boys and 19.07 to 22.58 for girls; cut-off points for 'Obesity' ranged from 22.77 to 26.84 for boys and 22.81 to 27.76 for girls). Furthermore, one well-trained and experienced female paediatrician in each prefecture determined pubertal maturation (Tanner stage) after a thorough visual inspection of breast development in girls and genital development in boys (Tanner, 1955). Finally, each girl was asked by the paediatrician about her menstruation status and age of menarche.

Haematological and biochemical indices

Blood samples were obtained for biochemical and haematological screening tests between 08.30 h and 10.30 h after a 12-h overnight fast. Professional staff performed venipunctures to obtain a maximum of 10 mL of blood. A CELL-DYN haematological auto analyser (Abbott Diagnostics, Abbott Park, IL, USA) was used to assess red blood cell count (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The remaining blood was centrifuged to collect serum, divided into aliquots of 2 mL and stored at

−80 °C. All serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where biochemical analyses took place. Serum iron and total iron binding capacity (TIBC) were determined by colorimetric assays (Roche Diagnostics SA, Basel, Switzerland). Transferrin saturation (TSAT) was calculated by dividing serum iron by TIBC and multiplying by 100. Finally, serum ferritin was measured using a chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

ID (with or without anaemia) and iron deficiency anaemia (IDA) were defined using the age- and sex-specific thresholds proposed by UNICEF and the World Health Organization (WHO, 2001): ID was defined as TSAT <16%; IDA was defined as ID with blood haemoglobin concentration <12 g dL^{-1}, which is the threshold value for anaemia for children aged 9–13 years. The Mentzer Index [MCV(fL)/RBC (M μL^{-1})] (Mentzer, 1973) of all the children and adolescents with anaemia was also calculated to differentiate thalassemia minor from IDA. On the basis of this index, children and adolescents with thalassemia minor (18 cases) were excluded from further analysis.

Dietary assessment

Dietary intake data were obtained for two consecutive weekdays and one weekend day, using the 24-h recall technique. Specifically, all study participants were asked to describe the type and amount of foods, as well as all beverages consumed during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts where appropriate. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimise the interviewer's effect, reviewed the collected data with the respondent to clarify entries, servings and possibly forgotten foods. Food intake data were analysed using NUTRITIONIST V diet analysis software, version 2.1 (First Databank, San Bruno, CA, USA), which was extensively amended to include traditional Greek recipes, as described previously (Trichopoulou, 2004). Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

Physical activity assessment

To assess physical activity, study participants were provided with and instructed to wear a waist-mounted pedometer (Yamax SW-200 Digiwalker; Yamax Corporation, Tokyo, Japan) for 1 week (i.e. from Monday to Sunday). The pedometer was positioned in accordance with the manufacturer's instructions on the right waistband, vertically aligned with the patella. The pedometer used in the present study displayed the cumulative number of steps from the time it was worn in the morning until the time it was removed at bedtime. Participants were provided with a diary template and instructed to record the total number of daily steps displayed by the pedometer at bedtime and then zero the pedometer. Participants were also instructed to take off the pedometers when bathing or swimming and to record these activities in the diary. Finally, in cases where some children or adolescents forgot to wear the pedometer for a whole day, the relevant cells in the diary were left blank and the calculation for total mean steps per day was made using the appropriate number of days.

Socio-economic and demographic variables

Data on parents' educational level (i.e. total years of education for the father and mother) were collected from the parents (mainly the mother) during scheduled interviews at school. For those parents not being able to attend (approximately 5% of the total sample), data were collected via telephone interviews. All interviews were conducted with the use of a standardised questionnaire by researchers that were rigorously trained to minimise the interviewer's effect.

Statistical analysis

Normality of the distribution of continuous variables was tested using the Kolmogorov–Smirnov test and homogeneity of variances was tested using Levene's test. Continuous variables are expressed as the mean (SD), and categorical variables are reported as frequencies (%). Comparisons between levels of the continuous variables were conducted using one-way analysis of variance for normally distributed variables and the nonparametric Kruskal–Wallis test for variables that remained non-normally distributed, even though logarithmic transformations were made. Comparisons between levels of the categorical variables were conducted using the chi-squared test or the Fisher's exact test, as appropriate. The two-sample *Z*-test was also used to make pairwise comparisons on the prevalence of ID and IDA between weight groups. To test the effect of the independent variables examined on ID and IDA, multivariate logistic regression analysis was conducted and adjusted odds ratios (OR) with 95% confidence intervals (CI) were computed. All reported *P*-values refer to two-sided tests. The level of statistical significance was set at $\alpha = 0.05$. Statistical analysis was conducted using SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Voluntary participation did not affect the representativeness of the population under study because there were no significant differences observed between respondents and nonrespondents with regard to children's weight status and parental educational level (data not shown). Data were available for 2492 out of 2655 participating children and adolescents in the fifth and sixth grades of primary school. Table 1 presents the characteristics of the study population. The mean (SD) age of the study participants was 11.2 (0.7) years. No significant differences were found between boys and girls with respect to nationality and socio-economic characteristics (i.e. socio-economic level of school region, paternal and maternal educational level and family income). Similarly, no significant sex differences were found with respect to the prevalence of ID, anaemia and IDA. On the other hand, more girls than boys were found to be at Tanner stages 3–5 ($P < 0.05$), whereas the prevalence of obesity was higher in boys compared to girls (13.3% versus 9.6; $P < 0.05$).

Furthermore, regarding children and adolescents with ID and IDA, there were no significant differences either within or between groups in terms of dietary intake of iron, calcium, vitamin C and fibre, steps count, Tanner stage and prevalence of being overweight and obesity. Nevertheless, BMI was found to differ significantly between ID and IDA study participants (20.6 versus 22.7 kg m^{-2}; $P < 0.001$; data not shown).

Table 2 displays the haematological and biochemical indices of iron status across weight groups in both boys and girls. In boys, MCH, MCHC, serum iron and TSAT differed significantly among weight groups ($P < 0.05$), being lower in obese compared to normal-weight groups. By contrast, serum ferritin and TIBC were lowest in normal-weight boys ($P < 0.05$). In girls, significant differences across weight groups were found in nine haematological and biochemical indices of iron status ($P \leq 0.05$). Of those, haemoglobin, MCV, serum iron and TSAT exhibited lower values in obese than normal-weight girls; MCH and MCHC were lower in obese and overweight girls compared to their normal-weight peers. By contrast, RBC was higher in obese and overweight compared to normal-weight girls; ferritin was higher in obese compared to overweight or normal-weight girls; and TIBC exhibited higher values in overweight compared to normal-weight girls (in states of iron depletion, the iron-binding capacity of transferin, and thus TIBC levels, increases). Moreover, the prevalence of ID was higher in obese boys and girls compared to their overweight and normal-weight peers ($P < 0.001$ in boys and $P = 0.002$ in girls). Similarly, the prevalence of IDA was higher in obese compared to normal-weight boys ($P = 0.029$) and in obese compared to overweight or normal-weight girls ($P = 0.003$).

Table 1 Characteristics of the study population

	Boys % (*n* = 1241)	Girls % (*n* = 1251)	Total % (*n* = 2492)
Age (years)			
9–11	41.4	41.9	41.6
11–13	58.6	58.1	58.4
SEL of school			
Lower	26.7	25.1	25.9
Medium	39.2	32.6	35.9
Higher	34.1	42.3	38.2
Grade			
5th	47.6	49.2	48.5
6th	52.4	50.8	51.5
Nationality			
Greek	85.8	83.1	84.4
Other	14.2	16.9	15.6
Tanner stage			
1	44.4	21.0*	32.7
2	40.0	39.6	39.8
3	11.2	26.4*	18.8
4	3.9	10.5*	7.2
5	0.5	2.5*	1.5
Menarche			
Yes	–	77.1	–
No	–	22.9	–
Paternal education (years)			
<9	26.0	26.9	26.3
9–12	38.7	37.8	38.1
>12	35.3	35.3	35.6
Maternal education (years)			
<9	19.2	23.9	21.6
9–12	40.8	38.1	39.4
>12	40.0	38.0	39.0
Family income (€/year)			
<12 000	21.1	24.1	22.6
12 000–20 000	26.9	26.5	26.7
20 000–30 000	23.3	23.9	23.6
30 000–40 000	14.5	13.7	14.1
40 000–50 000	8.0	5.6	6.8
>50 000	6.3	6.1	6.2
Weight group			
Normal-weight	55.2	60.0	57.6
Overweight	31.5	30.4	30.9
Obese	13.3	9.6*	11.4
Iron status groups			
Normal iron status	73.6	76.4	75.0
Iron deficiency (TSAT < 16%)	16.7	14.0	15.3
Anemia (Hgb <12 g dL^{-1})	7.4	6.7	7.1
Iron deficiency anemia (TSAT < 16% and Hgb < 12 g dL^{-1})	2.4	2.9	2.6

*$P < 0.05$, significantly different from boys.
Hgb, haemoglobin; SEL, socio-economic level; TSAT, transferring saturation.

Table 2 Haematological and biochemical indices of iron status across weight groups in children and adolescents

	Boys				Girls			
	Normal-weight (n = 685)	Overweight (n = 391)	Obese (n = 165)	P-value	Normal-weight (n = 751)	Overweight (n = 380)	Obese (n = 120)	P-value*
Haematological indices, mean (SD)								
RBC (M μL^{-1})	4.88 (0.42)	4.93 (0.38)	4.94 (0.38)	0.219*	4.82 (0.35)^{a,b}	4.90 (0.41)^{b}	4.93 (0.45)^{a}	0.002*
Haemoglobin (g dL^{-1})	13.2 (0.87)	13.1 (0.95)	13.0 (0.74)	0.069*	13.1 (0.85)^{a}	13.1 (0.85)	12.9 (0.91)^{a}	0.028*
Haematocrit (%)	39.0 (2.3)	39.1 (2.3)	38.9 (2.0)	0.717*	39.1 (2.1)	39.3 (2.1)	39.0 (2.4)	0.646*
MCV (fL)	80.2 (6.1)	79.6 (5.4)	79.1 (5.0)	0.094*	81.5 (5.5)^{a}	80.6 (6.1)	79.6 (6.1)^{a}	0.002*
MCH (pg)	27.0 (2.5)^{a}	26.8 (2.3)	26.5 (2.1)^{a}	0.020*	27.4 (2.3)^{a,b}	26.9 (2.6)^{b}	26.3 (2.5)^{a}	0.001*
MCHC (g dL^{-1})	33.8 (1.3)^{a}	33.6 (1.4)	33.4 (1.2)^{a}	0.011*	33.6 (1.3)^{a,b}	33.3 (1.4)^{b}	33.1 (1.2)^{a}	0.001*
Biochemical serum indices, mean (SD)								
Iron (μg dL^{-1})	89.4 (35.6)^{a}	84.0 (34.1)	76.0 (30.9)^{a}	0.001*	91.4 (37.4)^{a}	90.0 (36.3)	81.6 (37.4)^{a}	0.050*
TIBC (μg dL^{-1})	339.1 (54.1)	348.0 (54.0)	351.2 (48.4)^{a}	0.013*	348.5 (55.2)^{b}	364.6 (57.1)^{b}	357.3 (81.6)	0.001*
TSAT (%)	26.7 (10.4)^{a,b}	24.5 (10.1)^{b,c}	21.6 (8.1)^{a,c}	0.001*	26.7 (12.4)^{a}	25.0 (9.9)	23.2 (10.7)^{a}	0.005*
Ferritin (ng mL^{-1})	31.1 (21.0)^{a}	33.0 (20.3)	36.5 (20.7)^{a}	0.024*	27.4 (16.1)^{a}	30.3 (19.3)^{b}	35.6 (21.5)^{a,b}	0.001*
Iron status categories (% of total)								
ID	15.5^{a,b}	20.3^{b,c}	28.6^{a,c}	<0.001†	14.3^{a}	18.2^{b}	28.9^{a,b}	0.002†
IDA	1.4^{a}	2.8	5.3^{a}	0.029†	2.0^{a}	3.3^{b}	8.2^{a,b}	0.003†

Values marked with the same superscript letters are significantly different ($P < 0.05$ after post-hoc multiple comparisons using the Bonferroni rule or after two-sample Z-test, as appropriate).

*Derived from analysis of variance.

†Derived from the Pearson chi-squared test.

ID, iron deficiency (with or without anaemia); IDA, iron deficiency anaemia; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell count; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

Tables 3 and 4 summarise the intakes of nutrients that affect iron status, adjusted for energy intake, and the walking/running activity across weight groups in both sexes. No significant differences among weight groups in either boys or girls were found in iron, calcium or vitamin C intakes. Nevertheless, obese girls had a higher intake of fibre compared to normal-weight girls ($P = 0.048$). Overweight and obese boys and girls recorded significantly fewer steps than their normal-weight peers ($P < 0.001$).

Based on the results of the multivariate logistic regression analysis shown in Table 5, obesity was the only factor that significantly increased the likelihood of ID and IDA in both boys and girls. Furthermore, a higher maternal educational level decreased the likelihood of ID in boys, whereas boys with higher walking/running activity (fourth quartile of number of steps) were more likely to be iron deficient. Finally, a high stage of pubertal maturation in girls (Tanner stage 4) significantly increased the likelihood of ID.

Discussion

The present study showed a considerably high prevalence of ID and IDA in obese male (28.6% and 5.3%, respectively) and female (28.9% and 8.2%, respectively) children and adolescents. This prevalence was comparable to the prevalence of ID reported for overweight Swiss and Israeli children and adolescents (i.e. 20% and 12%, respectively; Pinhas-Hamiel *et al.*, 2003; Aeberli *et al.*, 2009). In the latter studies, the definition of ID was based on certain biochemical markers of iron status, such as serum transferrin receptor concentration, free erythrocyte protoporphyrin levels and TSAT, which was the sole index used in the present study to assess ID. However, the prevalence of ID reported by the present and the two studies cited above is considerably higher compared to data reported from the USA (Nead *et al.*, 2004). Specifically, the prevalence of ID reported for obese children and adolescents in the Third National Health and Nutrition Examination Survey (NHANES) was 2.4% and

Table 3 Dietary intake and physical activity levels across weight status categories in male children and adolescents

	Boys			
	Normal-weight (*n* = 685)	Overweight (*n* = 391)	Obese (*n* = 165)	*P*-value
Iron (mg/4184 kJ[§] day^{-1})	5.9 (2.5)	6.1 (4.2)	5.8 (2.6)	0.680[†]
Calcium (mg/4184 kJ[§] day^{-1})	571.8 (181.4)	594.5 (179.8)	572.1 (206.4)	0.131[†]
Vitamin C (mg/4184 kJ[§] day^{-1}) *	52.3 (48.6)	56.0 (44.3)	51.4 (42.5)	0.238[‡]
Fibre (g/4184 kJ[§] day^{-1}) *	7.8 (3.5)	8.2 (3.8)	7.4 (3.4)	0.077[‡]
Steps (number of steps day^{-1})	15745 (5202)[a,b]	13362 (4912)[b]	12842 (6591)[a]	<0.001[‡]

Values marked with the same superscript letters are significantly different ($P < 0.05$) after post-hoc multiple comparisons using the Bonferroni rule.
*Parameters were log-transformed.
[†]Derived from Kruskal–Wallis test.
[‡]Derived from analysis of variance.
[§]4184 kJ is equal to 1000 kcal.

Table 4 Dietary intake and physical activity levels across weight status categories in female children and adolescents

	Girls			
	Normal-weight (*n* = 751)	Overweight (*n* = 380)	Obese (*n* = 120)	*P*-value
Iron (mg/4184 kJ[§] day^{-1})	6.0 (3.8)	6.7 (3.8)	6.3 (3.4)	0.064[†]
Calcium (mg/4184 kJ[§] day^{-1})	576.8 (200.1)	610.8 (372.2)	576.2 (195.0)	0.577[†]
Vitamin C (mg/4184 kJ[§] day^{-1})*	59.7 (48.7)	56.2 (47.9)	55.6 (43.6)	0.228[‡]
Fibre (g/4184 kJ[§] day^{-1})*	7.5 (3.6)[a]	8.0 (3.6)	8.2 (4.3)[a]	0.048[‡]
Steps (number of steps day^{-1})	12299 (4027)[a,b]	11263 (4467)[b]	10281.4 (5335)[a]	<0.001[‡]

Values marked with the same superscript letters are significantly different ($P < 0.05$) after post-hoc multiple comparisons using the Bonferroni rule.
*Parameters were log-transformed.
[†]Derived from Kruskal–Wallis test.
[‡]Derived from analysis of variance.
[§]4184 kJ is equal to 1000 kcal.

Table 5 Adjusted odds ratios (95% confidence intervals) for iron deficiency and iron deficiency anaemia among children and adolescents

	Boys (n = 1241)		Girls (n = 1251)	
	ID	IDA	ID	IDA
Weight group				
Normal-weight	1.00	1.00	1.00	1.00
Overweight	1.48 (0.99–2.18)	1.90 (0.69–5.21)	1.30 (0.88–1.94)	1.29 (0.51–3.26)
Obese	**2.46 (1.52–3.96)**	**3.13 (1.01–9.71)**	**2.05 (1.19–3.51)**	**3.28 (1.15–9.33)**
Tanner stage				
Stage 1	1.00	1.00	1.00	1.00
Stage 2	1.49 (0.99–2.17)	1.20 (0.44–3.24)	1.57 (0.94–2.62)	1.10 (0.32–3.82)
Stage 3	1.39 (0.79–2.42)	2.51 (0.76–8.29)	1.55 (0.88–2.73)	1.70 (0.48–6.06)
Stage 4	1.06 (0.28–3.92)	2.29 (0.24–21.7)	**3.03 (1.51–6.09)**	3.12 (0.73–13.3)
Stage 5	–	–	0.30 (0.36–2.46)	–
Mother's education (years)				
<9	1.00	1.00	1.00	1.00
9–12	1.05 (0.65–1.70)	0.79 (0.27–2.34)	1.29 (0.81–2.05)	1.04 (0.36–3.02)
>12	**0.56 (0.36–0.88)**	0.38 (0.09–1.47)	0.93 (0.59–1.47)	1.43 (0.54–3.80)
Steps quartiles (number of steps day^{-1})				
1st Quartile	1.00	1.00	1.00	1.00
2nd Quartile	1.22 (0.74–2.02)	1.13 (0.34–3.72)	1.20 (0.73–1.97)	0.79 (0.28–2.24)
3rd Quartile	1.57 (0.96–2.57)	1.20 (0.35–4.06)	0.97 (0.58–1.61)	0.80 (0.28–2.31)
4th Quartile	**1.85 (1.13–2.04)**	1.09 (0.31–3.86)	1.41 (0.86–2.31)	0.56 (0.17–1.78)

All odds ratios were adjusted for dietary iron, calcium, vitamin C and fibre, paternal educational level and menarche (girls only).
Bold values indicate statistical significance.
ID, iron deficiency; IDA, iron deficiency anaemia.

9.1%, respectively (Nead *et al.*, 2004). The use of serum ferritin as one of the diagnostic criteria for ID in this previous study on USA children and adolescents could probably provide an explanation for this discrepancy. Ferritin is an acute-phase protein whose serum levels are plausibly elevated in states of chronic or acute inflammation (Wisse, 2004). Considering that obesity represents such a state, the increased levels of serum ferritin in overweight and obese children could have led to an underestimation of the true magnitude of ID in this cohort. This hypothesis is supported by a more recent study that also used NHANES data to examine the incidence of ID among overweight female adolescents (Tussing-Humphreys *et al.*, 2009). In that study, serum ferritin was not included in the definition of ID, leading to a prevalence rate of 30.8%. As a confirmation of the above, the present study showed that the prevalence of ID and IDA was lower when using serum ferritin as a diagnostic criterion compared to using TSAT (i.e. 15.1% for ID and 2.0% for IDA using serum ferritin versus 18.5% for ID and 2.7% for IDA using TSAT).

The prevalence of ID and IDA reported by the present study was found to be significantly higher in obese boys and girls compared to normal-weight children rather than overweight ones (Table 2). In most cases, the prevalence of ID and IDA in overweight children and adolescents did not differ significantly compared to their normal-weight counterparts. Similar to the prevalence of ID and IDA, the values of several haematological and biochemical indices of iron status differed significantly between obese and normal-weight children and adolescents (Table 2). These differences are consistent with those reported for children and adolescents in similar studies examining the association between iron and weight status (Pinhas-Hamiel *et al.*, 2003; Tussing-Humphreys *et al.*, 2009).

Differences between obese and normal-weight children and adolescents in dietary intakes of iron and nutrients that either enhance (i.e. vitamin C) or reduce (i.e. calcium and fibre) iron bioavailability could provide an explanation for the double burden of ID and obesity. However, the present study, in line with other recent studies (Menzie *et al.*, 2008; Aeberli *et al.*, 2009; Tussing-Humphreys *et al.*, 2009), did not detect such differences (Tables 3 and 4), with the exception of dietary fibre intake, which was higher in obese compared to normal-weight girls. Still, at a multivariate analysis level (Table 5), dietary fibre intake in girls was not significantly associated with either ID or IDA. Similarly, as shown by multivariate analyses, physical activity, assessed in the present study as number of steps measured by pedometers, was not associated with the prevalence of ID in girls or IDA in both sexes, in accordance with another study (Tussing-Humphreys *et al.*, 2009). On the other hand, boys with higher walking/running activity (fourth quartile of number of steps) were more likely to be iron deficient than their peers in the first quartile of number of steps. Although ID is a common disorder in professional athletes (Peeling *et al.*, 2008), the exact aetiology of this association in children is hard to interpret.

Another important finding of the present study was the positive association observed between Tanner stage and ID in girls only (Table 5). Although more girls than boys were found to be at a higher level of sexual maturation, as indicated by Tanner stage, when comparing the prevalence of ID and IDA between boys and girls, no statistically significant differences were observed (Table 1). This could probably indicate that although iron losses as a result of menstruation increases the risk of ID in girls, this might not add any further risk of impaired iron status in relation to boys.

The positive associations found in the present study between obesity and either ID or IDA in both sexes (Table 5) support the hypothesis that the bioavailability of iron may be modulated by factors linked to chronic inflammation induced by excess adiposity. In particular, the increased levels of inflammatory cytokines found in obese individuals (Subramanian & Ferrante, 2009) have been inversely associated with serum iron levels (Tussing-Humphreys *et al.*, 2009). Low iron bioavailability as a result of increased sequestration by high amounts of ferritin has also been proposed as a factor contributing to the hypoferraemia of obesity (Zafon *et al.*, 2009). Furthermore, serum levels of hepcidin, which is the main regulatory hormone of iron absorption and recirculation, were higher in overweight children, adolescents (Aeberli *et al.*, 2009; del Giudice *et al.*, 2009) and adults (Tussing-Humphreys *et al.*, 2009) compared to their normal-weight peers. Adipose tissue-derived cytokines (such as interleukin-6 and leptin), produced in response to obesity related-inflammation, promote hepcidin gene transcription (Verga Falzacappa *et al.*, 2007). This results in increased serum hepcidin levels that lead to the sequestration of iron within the reticuloendothelial system and to decreased dietary iron absorption from the intestine by controlling the expression of ferroportin 1 at the basolateral membranes of the enterocytes. Consequently, there is hypoferraemia and diminished availability of iron for erythropoiesis (Munoz *et al.*, 2009).

The cross-sectional design of the present study is its main limitation because it cannot provide cause-and-effect relationships. Another limitation could be the use of BMI as a measure of adiposity because it is not a reliable index of body fat and fat-free mass, especially in children (Taylor *et al.*, 2002; Reilly *et al.*, 2010). Nevertheless, BMI remains an acceptable and easy-to-use screening tool for identifying excess adiposity in the general population (Duncan *et al.*, 2009). The use of pedometers could be an additional limitation because it is not considered as the gold standard method for estimating physical activity levels in children and adolescents (Sirard & Pate, 2001). However, the sample size (n = 2492) could not permit the use of accelerometers in terms of cost to assess participants' physical activity levels. Besides, previous studies have shown that the use of pedometers is a valid objective method for assessing physical activity levels in children and adolescents (McNamara *et al.*, 2010).

Furthermore, the use of TSAT and serum ferritin for the definition of ID instead of multiple indicators of iron status (e.g. soluble transferin receptor levels) could have possibly affected the true magnitude of ID in the present study. Finally, there were no inflammatory marker data (i.e. C-reactive protein and/or interleukin-6) available in the present study, thus making the understanding of the mechanistic relationship between iron status and body fat rather difficult.

In conclusion, the present study reports significant positive associations of obesity with ID and IDA in children and adolescents of both sexes. These associations may have important public health implications because they indicate the need to take into account children and adolescents' weight status when providing recommendations for dietary iron intake or when assessing their iron status.

Conflict of interests, source of funding and authorship

The authors declare that there are no conflicts of interest. This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program 'Education and Lifelong Learning' of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracletus II. Investing in knowledge society through the European Social Fund.
YM, GPC, CL and GM contributed to the study design. YM, VM, MK, KS, GT, AF and GM contributed to data collection. AS and AP contributed to biochemical analyses and blood management, respectively. YM, VT and GM contributed to data management and analysis. YM, GPC, CL, VM, CK and GM contributed to the interpretation of results and the writing of the paper. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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PAPER V

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ORIGINAL CONTRIBUTION

Increased physical activity combined with more eating occasions is beneficial against dyslipidemias in children. The Healthy Growth Study

George Moschonis · Christina Mavrogianni · Kalliopi Karatzi · Vasiliki Iatridi · George P. Chrousos · Christos Lionis · Yannis Manios

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Abstract
Purpose To identify lifestyle patterns associated with blood lipid levels in children.
Methods A representative sample of 2,043 schoolchildren (9–13 years) participated in a cross-sectional epidemiologic study conducted in 77 primary schools in four large regions in Greece. Dietary intakes, breakfast patterns and eating frequency, physical activity levels, sleep duration, anthropometric and physical examination data, biochemical indices and socioeconomic information (collected from parents) were assessed in all children. Principal component analysis was used to identify the lifestyle patterns.
Results A lifestyle pattern of more screen time, shorter sleep duration and higher sugar-sweetened beverage consumption was inversely associated with HDL cholesterol ($\beta = -0.077$; $P < 0.001$) and positively associated with total/HDL cholesterol ratio ($\beta = 0.049$; $P = 0.025$). Furthermore, a lifestyle pattern of more eating occasions and higher moderate-to-vigorous physical activity (MVPA) levels was inversely associated with total cholesterol ($\beta = -0.064$; $P = 0.006$), LDL (low-density lipoprotein) cholesterol ($\beta = -0.065$; $P = 0.004$) and total/HDL (high-density lipoprotein) cholesterol ratio ($\beta = -0.043$; $P = 0.049$) in multivariate models. Finally, children with MVPA levels and eating frequency higher than that corresponding to the second quartile of this lifestyle pattern (i.e., > 44.8 min of MVPA per day and > 4.7 meals per day) were 29.7, 32.6 and 43.1 % less likely of having abnormal levels of total cholesterol, LDL and total/HDL cholesterol ratio, respectively, according to the National Cholesterol Education Program (NCEP) cutoff points.
Conclusions A lifestyle pattern of more than approximately 45 min of MVPA and 5 eating occasions per day was significantly associated with reduced likelihood of dyslipidemias in schoolchildren (9–13 years).

Keywords Children · Serum lipids · Lifestyle patterns · Diet

This study is on behalf of the "Healthy Growth Study" group. The details of the "Healthy Growth Study" are given in the "Appendix."

G. Moschonis · C. Mavrogianni · K. Karatzi · V. Iatridi · Y. Manios (✉)
Department of Nutrition and Dietetics, Harokopio University of Athens, 70, El.Venizelou Ave, 17671 Kallithea, Athens, Greece
e-mail: manios@hua.gr

G. P. Chrousos
First Department of Pediatrics, Athens University Medical School, Aghia Sophia Children's Hospital, Athens, Greece

C. Lionis
Clinic of Social and Family Medicine, School of Medicine, Heraklion, University of Crete, Heraklion, Greece

Introduction

Recent data have clearly demonstrated that serum lipid and lipoprotein levels can be measured above optimal values during early childhood [1]. Increased concentration of triglycerides, total and LDL (low-density lipoprotein) cholesterol and decreased levels of HDL (high-density lipoprotein) cholesterol in children are significantly associated with the development of arterial atherosclerotic lesions [2]. Likewise, there is a significant association of abnormal values of LDL, HDL, triglycerides and LDL/HDL ratio in childhood with intima media thickness (IMT), a marker of preclinical atherosclerosis in adulthood [3]. Accordingly, blood lipid levels in children and adolescents

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are important indices of atherosclerosis and risk of cardiovascular diseases later in life [2].

The study of dietary patterns in nutritional epidemiology is common. Nutrients are rarely eaten in isolation, and nutrient-only investigations underestimate the possible interactions between multiple nutrients or between single foods and other dietary components [4]. Since food is usually consumed as meals with a variety of foods, simple consideration of nutrient intake ignores the complex, additive, synergistic or antagonistic effects of food combinations. Dietary patterns represent the types and amounts of foods consumed and focus on the entire diet rather than just a single food or nutrient [4].

Several epidemiologic studies in children and adults have produced important information on the possible relationship of common dietary patterns with blood lipid levels [5, 6]. These studies suggested that varying patterns of nutrition may affect measurable cardiovascular risk factors, including blood lipids, while other lifestyle factors, like physical activity and sleep duration, may also influence such relations. Physical activity in childhood has been inversely related to cardiovascular risk factors through several mechanisms, including an improved ratio between HDL and LDL concentration and decreased levels of triglycerides and diastolic blood pressure [7]. Short sleep duration in children 4–10 years was associated with increased health risks and specifically increased circulating LDL concentrations [8].

Therefore, the purpose of the current study was to examine not just dietary but lifestyle patterns associated with blood lipid levels in children, offering valuable information in the field and possibly in cardiovascular risk reduction strategies.

Methods

Sampling

The Healthy Growth Study was a large-scale cross-sectional epidemiologic study initiated in May 2007 and completed in June 2009. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens and was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki. The study population comprised of schoolchildren 9–13 years, attending the 5th and 6th grades of primary schools located in municipalities within the counties of Attica, Aitoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multistage and stratified by parents' educational level and total population of students attending schools within municipalities of these counties, as described in more detail elsewhere [6]. The study population was representative of the 9–13-year-old school children living in the four counties under study. Nonetheless, these counties are scattered throughout the Greek territory, covering the northern (i.e., Thessaloniki), central (i.e., Attica), western (i.e., Aitoloakarnania) and southern (i.e., Iraklio-Crete) parts of Greece. This combined with the random, multistage and stratified sampling procedures followed to recruit our sample is indicative of the representativeness of our population. The sampling procedure yielded 77 primary schools, representative of the total number of schools in the counties under study, which responded positively when they were invited to participate in the study. An extended letter explaining the aims of the study and a consent form for taking full measurements were provided to all parents or guardians having a child in these schools. Parents who agreed to the participation of their children in the study had to sign the consent form and provide their contact details. Signed parental consent forms were collected for 2,656 out of 4,145 children (response rate 64.1 %).

Dietary intake

Dietary intake data were obtained by trained dieticians and nutritionists via morning interviews with the children at the school site for two consecutive weekdays and 1 weekend day, using the 24-h recall technique. Specifically, all study participants were asked to describe the type and amount of foods and beverages consumed, during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimize the interviewer's effect, reviewed the collected data with the respondent in order to clarify entries, servings and possible forgotten foods. Food intake data were analyzed using the Nutritionist V diet analysis software (version 2.1, 1999, First Databank, San Bruno, California, USA), which was extensively amended to include traditional Greek recipes [9]. Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

The food-grouping scheme was designed for all foods or entries (core and recipe) appearing in Nutritionist V. In total, 47 food groups were initially established, based on similar source characteristics and nutrient content. Composite food items, such as recipes, were analyzed and were assigned to food groups according to primary ingredients. A similar methodology for the extraction of food groups was previously reported in studies with a smaller sample size, but with only one 24-h recall available [10]. Examples of foods included in the food groups were documented previously [11]. In total, 18 large food categories (wholegrain bread and cereals, refined bread and cereals, dairy products, vegetables, fruits, red meat, processed meat, eggs, potatoes, legumes, fish, other seafood, white meat, fat oils, fresh fruit juices, sugared beverages, namely regular soft beverages and sugared fruit juices, salty snacks and sweets) were created, and the relevant values were calculated as consumption in grams per day.

Breakfast and eating frequency patterns

Although there is no widely acceptable definition for breakfast, a more balanced breakfast should include as the first meal of the day choices from the following three food groups: milk and milk-derived products, cereals and fruits (fresh fruits or natural juices; no sugar) [12]. Using data from the 24-h recalls and on the basis of the aforementioned definition of breakfast, a categorical variable was developed to assess the adequacy of children's breakfast consumption. More specifically, the following categories were created: 0 = salty, sweet or other savory snacks, 1 = zero portions of dairy products, cereals and fruits, 2 = one portion of dairy products or cereals or fruits, 3 = one portion of dairy products and one portion of cereals or fruits and 4 = one or more portions of dairy products, cereals and fruits. Data from 24-h dietary recalls were also used to define eating frequency. More specifically, eating frequency was assessed by summing the number of snacks or meals in which any portion of food was consumed.

Physical activity levels

Physical activity during leisure time was assessed using a standardized activity interview, based on a questionnaire completed by the participants in the presence of a member of the research team. Further details regarding the reliability and validity of the questionnaire are provided elsewhere [13]. Respondents reported the time spent on various physical activities on 2 weekdays and 1 weekend day, most preferably Sunday. Reported activities were grouped by a member of the research team into moderate-to-vigorous physical activities (MVPA) (intensity higher than 4 metabolic equivalents, METs), including activities such as brisk walking, bicycling, gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, jumping rope and general participation in active outdoors games. Given the age-group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity, rather than the strict aerobic definition of 20 continuous minutes appropriate for adults. The other level of physical activity that was assessed in the present study was "Light" (i.e., intensity lower than 4 METs). Physical activity levels that have been proposed to positively affect cardiorespiratory function and to produce desirable health outcomes (i.e., better weight, glycemic, lipidemic control, etc.) are those higher than 4 METs, which corresponds to the level of moderate, vigorous and very vigorous physical activity [14, 15]. Finally, the average weekdays and weekend time (h/day) spent on sedentary activities and more specifically on TV/DVD/video watching, video games and recreational computer usage was characterized as screen time.

Anthropometry and physical examination

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany). Students were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0.1 cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics, Oadby, UK) with the participant standing barefoot, keeping shoulders in a relaxed position, arms hanging freely and head in Frankfurt horizontal plane. Weight and height were used to calculate body mass index (BMI) using Quetelet's equation (weight (kg)/height (m)^2). The International Obesity Task Force (IOTF) cutoff points [16, 17] were used to categorize participants as "underweight," "normal weight," "overweight" or "obese." Waist circumference (WC) was measured to the nearest 0.1 cm with the use of a non-elastic tape (Hoechstmass, Germany) with the student standing at the end of a gentle expiration. The measuring tape was placed around the trunk, at the level of umbilicus midway, between the lower rib margin and the iliac crest. Furthermore, one well-trained and experienced female pediatrician in each prefecture determined pubertal maturation (Tanner stage) after thorough visual inspection of breast development in girls and genital development in boys [18].

Biochemical indices

Blood samples were obtained for biochemical and hematological screening tests between 08.30 and 10.30 after a 12-h overnight fast. Reminders were distributed the previous day to both parents and children to ensure compliance with fasting. Professional staff performed venipuncture to obtain a maximum of 23 ml blood. Part of the blood was collected in test tubes with no added anticoagulant, where it was allowed to clot for approximately

2 h, as this was designated for serum separation. Clotted blood was centrifuged at 3,000 rpm for 15 min, and the collected serum was divided into aliquots and stored at −80 °C. All serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where central storage of backup samples at −80 °C took place.

Total cholesterol (TC), HDL and triglycerides (TG) were determined in duplicate using commercially available enzymatic colorimetric assays (Roche Diagnostics SA, Vasilia, Switzerland) on an automated analyzer (Roche/Hitachi Modular). LDL was calculated by the Friedewald equation [19]. The LDL/HDL and the TC/HDL ratios were also estimated. The National Cholesterol Education Program (NCEP) cutoff points for blood lipids were used to define dyslipidemias [20].

Socioeconomic, perinatal and other information collected from the parents

Data on the socioeconomic background of the families having at least one child participating in the study were collected from the parents (most preferably from the mother) during scheduled face-to-face interviews at school. For those parents not able to attend the meetings (approximately 5 % of the total sample), data were collected via telephone interviews. All interviews were conducted by research team members who were rigorously trained to minimize interviewer's effect by using a standardized questionnaire. The socioeconomic data collected from parents included parental educational level (years of education), home square meters (m^2), number of family members living at home, number of cars owned by the family and home property. All aforementioned data were grouped, scored and combined for the development of a Socio-Economic Status index (SES index) as follows: Scores 0, 1, 2 and 3 were assigned to <9, 9–12, 12–16 and >16 years of maternal and paternal education, respectively; scores 0, 1, 2 and 3 were assigned to <20, 20–25, 25–30 and >30 home m^2 per family member, respectively; scores 0, 1, 2 and 3 were assigned to 0, 1, 2 and ≥3 cars owned by the family, respectively; and scores 0 and 1 were assigned to rented or owned home, respectively. The total score of the SES index was obtained by summing the scores to each index component. The values of the total SES index score were ranging between 0 and 13, with higher values indicating higher SES of the family. The self-reported weight and height of the mother and the father were also recorded.

Pediatric records were used to obtain data about children's birth weight. Parents were also asked to report the time their children usually go to bed at night and wake up in the morning on weekdays and weekend days, respectively. This information was used for the calculation of children's night sleep duration.

Statistical analysis

Lifestyle component derivation

Principal component analysis (PCA) was used to identify lifestyle patterns. The Kaiser–Meyer–Olkin (KMO) criterion was applied, and it was equal to 0.6. The orthogonal rotation (varimax option) was used to derive optimal non-correlated components (lifestyle patterns). To decide the number of components to retain, Kaiser criterion (eigenvalues > 1) was used. Factor loadings represent the correlations of each habit with the lifestyle pattern score. Higher absolute values of factor loadings indicate that the habit predictor contributes most to the construction of this particular component. The lifestyle components (patterns) were named according to the factor loadings of those behaviors correlated most with the component (factor loadings > |0.4|). When a behavior had a factor loading value >|0.4| in more than one component, then this behavior was used to name the component for which it had the higher factor loading value.

Descriptive and other statistical analyses

Categorical variables were presented as frequencies. Associations between categorical variables were examined by using the chi-square test and the two-sample z test for proportions whenever appropriate. Multiple linear regression analysis was used to evaluate the associations between blood lipids and lifestyle patterns derived from PCA, which are treated as continuous variables. More specifically, 3 different models were applied: model 1, unadjusted; model 2, adjusted for sex and Tanner stage; and model 3, adjusted for sex, Tanner stage, waist circumference, mean parental BMI, SES index score and birth weight. The results from the linear regression models are presented as standardized beta coefficients, and the level of significance was defined at $P < 0.05$. Moreover, participants' lifestyle pattern scores were categorized into quartiles, so that for each lifestyle component, quartile 4 consisted of persons whose dietary and physical activity behaviors were most adherent to that particular pattern. Based on the statistical significant associations provided by the linear regression models, logistic regression analysis was performed to evaluate the association between the quartiles of each lifestyle component and the probability of dyslipidemias. The results of logistic regression models were presented as odds ratios (OR) and 95 % confidence interval (CI). Data were analyzed using Statistical Package for Social Sciences software (version 13.0, 2004, SPSS Inc, Chicago, IL).

Results

Full socioeconomic, demographic, parental BMI, perinatal, clinical, biochemical and dietary data, as well as information on the time spent on physical and sedentary activities and on sleep duration, were collected for 2,043 children (50.2 % females and 49.8 % males). Regarding the descriptive characteristics of the study sample, Table 1 illustrates that the total prevalence of overweight and obesity was 29.7 and 11.3 %, respectively, with a significantly higher prevalence of obesity in boys than in girls (13.3 vs. 9.3 %, $P < 0.05$). Furthermore, Table 1 shows that the prevalence of hypertriglyceridemia (i.e., TG > 100 mg/dL) was significantly higher in girls than in boys.

Table 2 summarizes the loadings of the factors retained from the PCA. The value of the KMO criterion indicates that the lifestyle variables entered in the analysis were strongly intercorrelated and that PCA could be correctly used for assessing "healthy" or "unhealthy" lifestyle patterns. The PCA analysis indicated five lifestyle components explaining 52.7 % of the total variance with regard to the examined variables. These components were characterized as follows: higher dairy consumption and more adequate breakfast (component 1); higher consumption of high fiber foods (component 2); more screen time, shorter sleep duration and higher consumption of sugared beverages (component 3); more time spent on MVPA and more eating occasions (component 4); higher red meat and lower fish consumption (component 5).

Table 3 presents the associations derived from the three different models of the linear regression analyses between serum lipids (i.e., TC, HDL, LDL, TG), total/HDL cholesterol and HDL/LDL cholesterol ratio with each one of the five lifestyle components. Total cholesterol, LDL cholesterol and total/HDL cholesterol ratio were found to be inversely associated with lifestyle component 4 ($\beta = -0.064$, $P = 0.006$; $\beta = -0.065$, $P = 0.004$; and $\beta = -0.043$; $P = 0.049$, respectively), even after adjusting for several potential confounding factors in model 3. Furthermore, lifestyle component 3 was inversely associated with HDL cholesterol ($\beta = -0.077$; $P < 0.001$) and positively associated with total/HDL cholesterol ratio ($\beta = 0.049$; $P = 0.025$). Several other significant associations between certain lifestyle components and serum lipids were also observed in models 1 and 2; however, these became statistically insignificant after controlling for several potential confounding factors in model 3.

The logistic regression analysis summarized in Table 4 showed that children at the second quartile of lifestyle component 4 were 29.7, 32.6 and 43.1 % less likely of having total cholesterol levels > 170 mg/dL, LDL cholesterol levels > 110 mg/dL and total/HDL cholesterol ratio > 4.5 compared to children at the first quartile, after controlling for several potential confounding factors. Furthermore, children at the second quartile of lifestyle component 4 were found to spend more time on MVPA and to have more eating occasions than children in the first quartile (i.e., 44.8 ± 28.6 vs. 20.0 ± 22.6 min/day and 4.7 ± 0.7 vs. 4.1 ± 0.8 eating occasions/day). Similar findings were also observed in children at the third and fourth quartiles.

Table 1 Prevalence of overweight, obesity and dyslipidemias† in preadolescents

	Boys (n = 1,018) %	Girls (n = 1,025) %	Total (n = 2,043) %
Weight status			
Normal weight	56.0	62.1	59.0
Overweight	30.7	28.6	29.7
Obese	13.3*	9.3	11.3
Total cholesterol (mg/dL)			
<170[a]	55.7	57.9	56.8
170–199[b]	30.7	29.2	30.0
≥200[c]	13.6	13.0	13.3
HDL cholesterol (mg/dL)			
≥35[a]	96.6	95.6	96.1
<35[d]	3.4	4.4	3.9
LDL cholesterol (mg/dL)			
<110[a]	71.5*	75.5	73.5
110–129[b]	18.1	16.0	17.0
≥130[c]	10.4	8.5	9.4
Triglycerides (mg/dL)			
<75[a]	80.3*	72.8	76.5
75–99[b]	13.0*	16.7	14.8
≥100[c]	6.8*	10.5	8.7

* $P < 0.05$ for differences between sexes based on the chi-square test and the two-sample z test for proportions

† Adapted from NCEP guidelines for children and adolescents [20]. Letter superscripts indicate categorization of children based on the proposed thresholds of serum lipids, that is, [a] acceptable levels; [b] borderline-high levels; [c] high levels, [d] low levels

Discussion

During the past decades, the prevalence of dyslipidemia, a major cardiovascular risk factor, has been rapidly increasing in Greek children [21], while dyslipidemia in childhood tracks into adulthood and represents an increasing risk of cardiovascular diseases later in life [3]. Accordingly, several studies need to identify factors that may influence abnormal lipid profile in children, specifically lifestyle factors including diet, physical activity and sleep pattern.

Table 2 Factor loadings derived from principal component analysis regarding certain behaviors in preadolescents

Predictors (behaviors)	Component				
	1	2	3	4	5
Dairy consumption[a]	0.793[g]	0.154	−0.113	0.099	0.002
Fruit consumption	0.051	0.620[g]	−0.006	0.149	0.000
Vegetable consumption	−0.050	0.739[g]	0.113	−0.037	0.038
Whole-grain food consumption[b]	0.108	0.501[g]	−0.223	−0.090	−0.110
Red meat consumption	0.014	−0.055	0.066	0.116	0.719[g]
Fish consumption	−0.013	−0.011	0.069	0.114	−0.753[g]
Sugared beverages consumption[c]	−0.171	−0.154	0.560[g]	0.362	0.114
Adequate breakfast consumption	0.760[g]	−0.088	−0.016	−0.130	0.034
Eating frequency	0.446[g]	0.352	0.123	0.476[g]	−0.035
Screen time[d]	0.040	−0.062	0.680[g]	−0.214	−0.018
Time spent on physical activity[e]	−0.039	−0.004	−0.124	0.822[g]	−0.001
Sleep duration[f]	0.033	−0.072	−0.580[g]	0.008	0.033
Explained variance (%)	12.1	11.4	10.2	9.7	9.3

[a] Consumption of milk, yogurt and cheese (g/day)

[b] Consumption of whole-grain bread and cereals (g/day)

[c] Consumption of regular soft beverages and sugared fruit juices (g/day)

[d] Time spent on watching TV/DVD/video and/or using games consoles/computer for fun (h/day)

[e] Time spent on moderate-to-vigorous physical activity (min/day)

[f] Night sleep duration on weekdays (h/day)

[g] Habit with the highest factor loading (>|0,4|) within the component

The results of the present study in terms of anthropometric and biochemical indices, concerning prevalence of overweight and obesity, shown in Table 1, are in accordance with the proportional results from previous studies in Greek children [22–24], introducing obesity levels around 8–12 % and overweight levels around 25–30 %. Additionally, our data related to the prevalence of dyslipidemia are consistent with similar studies conducted in Greece [21] and the United States [25]. However, gender-specific prevalence of hypertriglyceridemia has been rarely investigated in children and adolescents and has produced inconsistent results [26, 27], precluding comments on our findings for higher prevalence in girls.

Statistical analysis revealed predominantly five lifestyle components. Out of these, only component 3 comprising a greater screen time, shorter sleep duration and higher consumption of sugared beverages and component 4 comprising more time spent on MVPA and more eating occasions were significantly associated with blood lipid profile after adjustment for sex, Tanner stage, waist circumference, parental BMI, SES index and birth weight.

Consumption of low-nutrient, energy-dense foods, including sugared sweetened beverages, is very popular among children and adolescents, especially those who eat most of their meals away from home [28]. Indeed, beverages containing sugar were related to cardiovascular risk factors in childhood, both independently and through development of obesity leading to dyslipidemia [29]. In adulthood, increased soft drink intake was associated with risk of metabolic syndrome, while daily consumption of more than 1 soft drink/day was associated with reduced HDL cholesterol levels and increased triglycerides [30]. Likewise, decreased sleep duration and increased time spent on watching TV and playing video games for fun were associated, respectively, negatively or positively with blood lipid levels. Thus, in adolescent girls, short sleep duration is correlated with risk of hypercholesterolemia in adulthood [31], while obese children having short sleep duration seem to have significant association with elevated levels of LDL [8]. Additionally, screen time at the age of 7–13 years also seems as an important behavior affecting lipid profile [32]. In adolescents, screen time is dose dependent correlated with the risk of developing metabolic syndrome and subsequently, among others, of introducing elevated TG and decreased HDL levels [33]. Therefore, our results concerning strong association of sleep duration, screen time and sugar beverages consumption with blood lipids in children are in accordance with previous relevant studies, indicating predominant lifestyle patterns possibly capable of modifying lipid profile in children.

Component 4 was also inversely associated with TC, LDL and total/HDL cholesterol ratio. Physical activity is a matter of great importance, as it seems that there is a remarkable decline in its levels between the ages of

Table 3 Single (model 1) and multiple (models 2 and 3) linear regression analysis models, exploring the association of lifestyle components with serum lipids levels ($n = 2{,}043$)

Components	Model 1 (unadjusted)		Model 2		Model 3	
	β	*P* value	β	*P* value	β	*P* value
Total cholesterol (mg/dL)						
Lifestyle component 1	−0.001	0.955	−0.004	0.855	−0.006	0.779
Lifestyle component 2	0.048	0.029	0.047	0.034	0.040	0.080
Lifestyle component 3	−0.052	0.019	−0.047	0.036	−0.038	0.098
Lifestyle component 4	−0.062	0.005	−0.069	0.002	−0.064	0.006
Lifestyle component 5	0.025	0.262	0.023	0.312	0.015	0.521
HDL cholesterol (mg/dL)						
Lifestyle component 1	0.052	0.018	0.050	0.025	0.024	0.264
Lifestyle component 2	0.056	0.012	0.054	0.015	0.029	0.187
Lifestyle component 3	−0.104	<0.001	−0.097	<0.001	−0.077	<0.001
Lifestyle component 4	0.007	0.744	0.004	0.857	−0.007	0.756
Lifestyle component 5	0.017	0.445	0.009	0.678	0.013	0.538
LDL cholesterol (mg/dL)						
Lifestyle component 1	−0.025	0.258	−0.028	0.210	−0.022	0.343
Lifestyle component 2	0.030	0.174	0.030	0.183	0.031	0.169
Lifestyle component 3	−0.015	0.448	−0.013	0.558	−0.009	0.695
Lifestyle component 4	−0.063	0.004	−0.074	0.001	−0.065	0.004
Lifestyle component 5	0.024	0.282	0.022	0.326	0.012	0.592
Triglycerides (mg/dL)						
Lifestyle component 1	−0.027	0.228	−0.023	0.304	0.003	0.895
Lifestyle component 2	−0.021	0.334	−0.022	0.311	<0.001	0.983
Lifestyle component 3	0.061	0.006	0.056	0.012	0.032	0.136
Lifestyle component 4	−0.068	0.002	−0.041	0.067	−0.026	0.221
Lifestyle component 5	−0.020	0.368	−0.003	0.885	−0.012	0.587
Total/HDL cholesterol ratio						
Lifestyle component 1	−0.045	0.040	−0.046	0.038	−0.021	0.331
Lifestyle component 2	−0.015	0.512	−0.014	0.526	0.003	0.887
Lifestyle component 3	0.066	0.003	0.061	0.006	0.049	0.025
Lifestyle component 4	−0.058	0.009	−0.059	0.010	−0.043	0.049
Lifestyle component 5	−0.004	0.858	0.002	0.938	−0.010	0.648
HDL/LDL cholesterol ratio						
Lifestyle component 1	0.024	0.275	0.022	0.315	0.012	0.605
Lifestyle component 2	0.015	0.501	0.014	0.530	−0.002	0.929
Lifestyle component 3	−0.026	0.246	−0.023	0.305	−0.019	0.395
Lifestyle component 4	0.047	0.034	0.049	0.031	0.040	0.077
Lifestyle component 5	−0.007	0.746	−0.012	0.597	−0.006	0.774

β Standardized beta coefficient

Model 1, unadjusted; Model 2, adjusted for Tanner stage and sex; Model 3, adjusted for sex, Tanner stage, waist circumference, parental BMI, SES index and birth weight

Lifestyle component 1: higher dairy consumption and more adequate breakfast

Lifestyle component 2: higher consumption of high fiber food (i.e., fruits, vegetables and whole-grain food)

Lifestyle component 3: more screen time, shorter sleep duration and higher consumption of sugared beverages

Lifestyle component 4: more time spent on physical activity and more eating occasions

Lifestyle component 5: higher red meat consumption and lower fish consumption

Table 4 Mean levels of behaviors comprising lifestyle components 3 and 4 and likelihood of dyslipidemias across quartiles of lifestyle components 3 and 4

Behaviors of component 3	Quartiles of lifestyle component 3 (more screen time, shorter sleep duration and higher consumption of sugared beverages)				
	1st quartile Mean ± SD	2nd quartile Mean ± SD	3rd quartile Mean ± SD	4th quartile Mean ± SD	*P* value
Screen time (h/day)	1.35 ± 0.69	1.85 ± 0.83	2.42 ± 0.97	3.61 ± 1.68	<0.001
Sleep duration (h/day)	9.42 ± 0.58	8.98 ± 0.52	8.73 ± 0.55	8.35 ± 0.73	<0.001
Sugared beverages consumption (g/day)	21.9 ± 57.8	44.2 ± 82.7	82.5 ± 117.1	221.9 ± 222.4	<0.001*
Serum lipids		OR (95 % CI)	OR (95 % CI)	OR (95 % CI)	
HDL cholesterol < 35 mg/dl[†]	1.00	0.650 (0.309–1.368)	1.025 (0.530–1.983)	1.480 (0.802–2.731)	
Total/HDL cholesterol > 4.5[†]	1.00	1.056 (0.563–1.979)	1.229 (0.671–2.252)	0.929 (0.490–1.764)	
Behaviors of component 4	**Quartiles of lifestyle component 4 (more time spent on physical activity and more eating occasions)**				
	1st quartile Mean ± SD	2nd quartile Mean ± SD	3rd quartile Mean ± SD	4th quartile Mean ± SD	*P* value
Time spent on MVPA (min/day)	20.0 ± 22.6	44.8 ± 28.6	72.4 ± 37.7	142.0 ± 67.7	<0.001*
Meal frequency (no. of meals/day)	4.10 ± 0.84	4.70 ± 0.74	5.05 ± 0.75	5.22 ± 0.72	<0.001
Serum lipids		OR (95 % CI)	OR (95 % CI)	OR (95 % CI)	
Total cholesterol > 170 mg/dl[†]	1.00	0.703 (0.554–0.909)	0.705 (0.544–0.914)	0.619 (0.476–0.805)	
LDL cholesterol > 110 mg/dl[†]	1.00	0.674 (0.507–0.896)	0.695 (0.522–0.925)	0.667 (0.499–0.890)	
Total/HDL cholesterol > 4.5	1.00	0.569 (0.320–0.931)	0.308 (0.153–0.620)	0.603 (0.342–0.980)	

OR odds ratio, *CI* confidence interval

Adjusted for sex, Tanner stage, waist circumference, parental BMI, SES index and birth weight

* *P* value derived from the nonparametric Kruskal–Wallis test

[†] The cutoff values were adapted from NCEP guidelines for children and adolescents [20]

4–13 years in both sexes [34]. Additionally, findings from several epidemiologic studies have shown significant associations between higher physical activity levels and cardiovascular disease risk factors in children, mainly through an improved ratio of HDL/LDL cholesterol, attributed to increased lipoprotein lipase activity [7]. Alongside, eating frequency is a very popular dietary pattern investigated in many recent relevant studies [6]. However, most data concern adults rather than children and adolescents. In adulthood, increasing number of eating occasions from 3 to 6 or more for several weeks leads to a reduction in total and LDL cholesterol levels, possibly due to a relevant reduction in endogenous cholesterol synthesis or the enhancement of the reverse cholesterol transport pathway [35]. Additionally, a nibbling meal pattern caused significant decreases in total, LDL cholesterol and triglycerides compared to gorging [36]. In another recent study in diabetic children, skipping meals was correlated with higher levels of LDL cholesterol compared with having a steady number of meals daily [37].

Our results further amplify these findings as children being in the highest quartile of eating frequency and time spent on MVPA were less likely to have an abnormal lipid profile, implying a possible dose-dependent correlation. Recent recommendations for children and adolescents concerning physical activity (60 min/day) [38] and eating occasions (at least 4/day) [39] focus on general health and prevention of overweight and obesity, which is a possible explanation for the slight diversification of our findings (45 min/day of physical activity and 5 eating occasions/day) that concentrate more on dyslipidemias.

Components 1 and 2 were also associated with blood lipids after adjustment for only sex and Tanner stage, but the associations lost their significance when adjusted for several confounders. These findings were well described previously, as it was reported that dietary patterns characterized by high fiber consumption were inversely correlated with abnormal lipid profile in children and adults [5, 6], while skipping breakfast was associated with elevated levels of total and LDL cholesterol in both children and adults [40].

The current study has both strengths and limitations. The Healthy Growth Study was a large-scale epidemiological study conducted in a representative sample of children from four prefectures within the wider region of Greece. Furthermore, the adjustment for specific confounders resulted in the extraction of more accurate results. However, as the study has a cross-sectional design, it is not suitable for interpreting causal relationships. Finally, although all statistical conditions for performing PCA analysis were kept in the current study, still the use of variables measured in different scales should be considered as another limitation.

The importance of healthy food choices in the prevention of cardiovascular diseases is now unequivocal. As we continue to explore the roles of nutrients and other compounds in our foods and their complex interrelations in influencing cardiovascular risk, it is now of great importance to identify lifestyle patterns that might also relate to future risk of developing cardiovascular diseases. In the present study, a pattern consisting of more than approximately 45 min of MVPA and 5 eating occasions per day was found to be significantly associated with reduced likelihood of dyslipidemias in schoolchildren (9–13 years). This pattern can be physiologically explained and employed in practical public health advice and individual counseling for reducing the future risk of cardiovascular diseases later in life.

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Conflict of interest None of the authors has any conflict of interest to declare.

Appendix

Healthy Growth Study Group

1. *Harokopio University Research Team/Department of Nutrition and Dietetics:* Yannis Manios (Coordinator), George Moschonis (Project manager), Katerina P. Skenderi, Evangelia Grammatikaki, Odysseas Androutsos, Sofia Tanagra, Alexandra Koumpitski, Paraskevi-Eirini Siatitsa, Anastasia Vandorou, Aikaterini-Efstathia Kyriakou, Vasiliki Dede, Maria Kantilafti, Aliki-Eleni Farmaki, Aikaterini Siopi, Sofia Micheli, Louiza Damianidi, Panagiota Margiola, Despoina Gakni, Vasiliki Iatridi, Christina Mavrogianni, Kelaidi Michailidou, Aggeliki Giannopoulou, Efstathoula Argyri, Konstantina Maragkopoulou, Maria Spyridonos, Eirini Tsikalaki, Panagiotis Kliasios, Anthi Naoumi, Konstantinos Koutsikas, Katerina Kondaki, Epistimi Aggelou, Zoi Krommyda, Charitini Aga, Manolis Birbilis, Ioanna Kosteria, Amalia Zlatintsi, Elpida Voutsadaki, Eleni-Zouboulia Papadopoulou, Zoi Papazi, Maria Papadogiorgakaki, Fanouria Chlouveraki, Maria Lyberi, Nora Karatsikaki-Vlami, Eva Dionysopoulou, Efstratia Daskalou.

2. *Aristotle University of Thessaloniki/School of Physical Education and Sports Science:* Vassilis Mougios, Anatoli Petridou, Konstantinos Papaioannou, Georgios Tsalis, Ananis Karagkiozidis, Konstantinos Bougioukas, Afroditi Sakellaropoulou, Georgia Skouli.

3. *University of Athens/ Medical School:* George P. Chrousos, Maria Drakopoulou, Evangelia Charmandari, Neni Pervanidou.

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PAPER VI

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Public Health Nutrition

Identification of lifestyle patterns associated with obesity and fat mass in children: the Healthy Growth Study

George Moschonis[1], Andriana C Kalliora[1], Vassiliki Costarelli[2], Christopher Papandreou[1], Dimitris Koutoukidis[1], Christos Lionis[3], George P Chrousos[4] and Yannis Manios[1,*] on behalf of the Healthy Growth Study Group

[1]Department of Nutrition and Dietetics, Harokopio University of Athens, 70 El Venizelou Avenue, 17671 Kallithea, Athens, Greece: [2]Department of Ecology and Home Economics, Harokopio University of Athens, Athens, Greece: [3]Clinic of Social and Family Medicine, School of Medicine, University of Crete, Heraklion, Crete, Greece: [4]First Department of Pediatrics, Aghia Sophia Children's Hospital, Athens University Medical School, Athens, Greece

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Abstract

Objective: To investigate possible associations of lifestyle patterns with obesity and fat mass in children.
Design: Cross-sectional epidemiological study. Principal component analysis was used to identify lifestyle patterns.
Setting: Primary schools from four regions in Greece.
Subjects: A total of 2073 schoolchildren (aged 9–13 years).
Results: Children in the fourth quartile of the lifestyle pattern combining higher dairy foods with more adequate breakfast consumption were 39·4 %, 45·2 % and 32·2 % less likely to be overweight/obese and in the highest quartile of sum of skinfold thicknesses and fat mass, respectively, than children in the first quartile of this pattern. Similarly, children in the fourth quartile of a lifestyle pattern comprising consumption of high-fibre foods, such as fruits, vegetables and wholegrain products, were 27·4 % less likely to be in the highest quartile of sum of skinfold thicknesses than children in the first lifestyle pattern quartile. Finally, children in the fourth quartile of a lifestyle pattern characterized by more time spent on moderate-to-vigorous physical activity and more frequent meals were 38·0 %, 26·3 % and 29·5 % less likely to be overweight, centrally obese and in the highest quartile of fat mass, respectively, than their peers in the first quartile of this lifestyle pattern (all $P < 0.05$).
Conclusions: The current study identified three lifestyle patterns (i.e. one pattern comprising higher dairy consumption with a more adequate breakfast; a second pattern characterized by increased consumption of high-fibre foods; and a third pattern combining higher physical activity levels with more frequent meals), which were all related with lower odds of obesity and/or increased fat mass levels. From a public health perspective, promotion of these patterns among children and their families should be considered as one of the components of any childhood obesity preventive initiative.

Keywords
Children
Adiposity indices
Lifestyle patterns
Diet
Physical activity

In developed countries the prevalence of childhood obesity has reached epidemic proportions[1]. Childhood obesity has been associated with hypertension, dyslipidaemia and insulin resistance[2]. Most importantly, childhood obesity often tracks into adulthood[2,3], where it becomes associated with an increased risk of mortality from chronic non-communicable diseases[4]. In recent years marked lifestyle changes have occurred resulting in increased sedentary behaviours, decreased time spent in physical activities and increased consumption of energy-dense foods[1].

Although childhood obesity has strongly been associated with several energy balance-related behaviours, the actual aetiology of increased adiposity in children requires further investigation due to its complex and multifactorial nature. In this context, it is very important to examine the possible synergistic role of several lifestyle factors on adiposity in children[5]. A common approach to examine the possible synergy of such factors is the development of specific dietary and lifestyle patterns. Principal component analysis (PCA) is frequently used for this purpose, since it enables the grouping of several strongly associated behaviours into patterns.

Recent epidemiological studies have highlighted certain lifestyle patterns that when combined can significantly

*Corresponding author: Email manios@hua.gr

increase the risk of childhood obesity[6,7]. More specifically, certain eating behaviours such as infrequent meals, breakfast skipping, lower adherence to the Mediterranean diet and higher consumption of sugar-sweetened beverages, fruit juice, salty snacks and French fries, as well as lower consumption of dairy products, fruits and vegetables, have consistently been reported as the most important factors positively associated with childhood obesity[8]. Additionally, increased time spent in front of screens (i.e. television, games console and computer), less time spent on physical activity and shorter sleep duration represent other important behavioural factors that have also been implicated in the aetiology of childhood obesity[9,10]. From a public health perspective, these associations are very important, since identification of lifestyle patterns that are strongly related to an increased risk of obesity could potentially facilitate the formation of practical public health advice specifically targeted at the prevention and management of childhood obesity. Several previous studies have investigated the association between dietary patterns and childhood obesity; however, to the best of our knowledge, no previous study has set out to examine, concurrently, the possible role of specific lifestyle and dietary behavioural patterns in the rise of childhood prevalence.

Therefore the aim of the current study was to concurrently examine, for the first time, specific lifestyle and dietary patterns of behaviour associated with increased adiposity in children.

Methods

Sampling

The Healthy Growth Study was a large-scale cross-sectional epidemiological study which was started in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens, and the study was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki. The study population comprised schoolchildren aged 9–13 years old, attending the 5th and 6th grades of primary schools located in municipalities within the counties of Attica, Aitoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multistage and stratified by parents' educational level and total population of students attending schools within municipalities of these counties, as described in more detail elsewhere[11]. The study population was representative of the 9–13-year-old schoolchildren living in the four counties included in the study. Nevertheless, these counties are scattered throughout the Greek territory, covering the northern (i.e. Thessaloniki), central (i.e. Attica), western (i.e. Aitoloakarnania) and southern (i.e. Iraklio, Crete) parts of Greece. This, combined with the random, multistage and stratified sampling procedures followed to recruit the sample, are indicative of the representativeness of the study population. The sampling procedure yielded seventy-seven primary schools, representative of the total number of schools in the counties under study, which responded positively when they were invited to participate in the study. An extended letter explaining the aims of the study and a consent form for taking full measurements were provided to all parents or guardians having a child in these schools. Parents who agreed to the participation of their children in the study had to sign the consent form and provide their contact details. Signed parental consent forms were collected for 2656 out of 4145 children (response rate 64·1 %).

Dietary intake

Dietary intake data were obtained by trained dietitians and nutritionists via three 24 h recall morning interviews (i.e. two consecutive weekdays and one weekend day) conducted with children at the school site. Specifically, all study participants were asked to describe the type and amount of foods and beverages consumed during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts. At the end of each interview, the interviewers, who were dietitians rigorously trained to minimize the interviewer effect, reviewed the collected data with the respondent in order to clarify entries, servings and possible forgotten foods. Food intake data were analysed using the Nutritionist V diet analysis software version 2·1, which was extensively amended to include traditional Greek recipes[12]. Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

The food-grouping scheme was designed for all foods or entries (core and recipe) appearing in Nutritionist V. In total, forty-seven food groups were initially established, based on similar source characteristics and nutrient content. Composite food items, such as recipes, were analysed and were assigned to food groups according to primary ingredients. A similar methodology for the extraction of food groups was previously reported in studies with a smaller sample size, but with only one 24 h recall available[13]. Examples of foods included in the food groups were documented previously[14]. In total, eighteen large food categories (wholegrain bread and cereals; refined bread and cereals; dairy products; vegetables; fruits; red meat; processed meat; eggs; potatoes; legumes; fish; other seafood; white meat; fats and oils; fresh fruit juices; sugared beverages (i.e. soft drinks and packed fruit juices); salty snacks; and sweets) were created and the relevant values were calculated as consumption in grams per day.

Breakfast and meal frequency patterns

Although there is still no widely acceptable definition for breakfast, it has been proposed that an ideal breakfast is the one that would typically include as the first meal of the day choices from the following three food groups: milk and milk-derived products; cereals (preferably whole grain); and fruits (fresh fruits or natural juices; no sugar)[15]. Using data from the 24 h recalls and on the basis of the aforementioned definition of an ideal breakfast, a categorical variable was developed to assess the adequacy of children's breakfast consumption. More specifically, the following categories were created: 0 = salty, sweet or other savoury snacks; 1 = zero portions of dairy products, cereals and fruits; 2 = one portion of dairy products or cereals or fruits; 3 = one portion of dairy products and one portion of cereals or fruits; and 4 = one or more portions of dairy products, cereals and fruits. Data from 24 h dietary recalls were also used to define meal frequency. In turn, meal frequency (i.e. number of meals per day) was assessed by summing the number of meals in which any portion of food was consumed.

Physical activity levels

Physical activity during leisure time was assessed using a standardized activity interview, based on a questionnaire completed by the participants in the presence of a member of the research team. Further details regarding the reliability and validity of the questionnaire are provided elsewhere[16]. Respondents reported the time spent on various physical activities on two weekdays and one weekend day, most preferably Sunday. Reported activities were grouped by a member of the research team into moderate-to-vigorous physical activity (MVPA; intensity higher than 4 MET (metabolic equivalents)), including activities such as brisk walking, bicycling, gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, skipping and general participation in active outdoors games. Given the age group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity, rather than the strict aerobic definition of twenty continuous minutes appropriate for adults. The other level of physical activity that was assessed in the present study was defined as 'light' (i.e. intensity lower than 4 MET). Physical activity levels that have been proposed to positively affect cardiorespiratory function and to produce desirable health outcomes (i.e. better weight, glycaemic, lipidaemic control, etc.) are those being higher than 4 MET and correspond to MVPA[17]. Finally, the average weekdays and weekend time (h/d) spent on sedentary activities and more specifically on watching television/DVD/videos and/or recreational usage of games consoles/computer was defined as screen time.

Anthropometry

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. Weight was measured to the nearest 10 g using a digital scale (Seca Alpha, model 770; Seca, Hamburg, Germany). Children were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0·1 cm using a commercial stadiometer (Leicester Height Measure; Invicta Plastics, Oadby, UK) with the child standing barefoot, keeping shoulders in a relaxed position, arms hanging freely and head in the Frankfurt horizontal plane. Weight and height were used to calculate BMI using Quetelet's equation: weight (kg)/[height (m)]^2. The International Obesity Taskforce cut-off points[18,19] were used to categorize participants as 'underweight', 'normal weight', 'overweight' or 'obese'. Waist circumference (WC) was measured to the nearest 0·1 cm with the use of a non-elastic tape (Hoechstmass, Sulzbach, Germany) with the child standing, at the end of a gentle expiration, after placing the measuring tape on a horizontal plane around the trunk, at the level of the umbilicus, midway between the lower rib margin and the iliac crest. The age- and sex-specific WC percentiles were used for the classification of central obesity (≥90th percentile)[20]. Furthermore, one well-trained and experienced female paediatrician in each prefecture determined pubertal maturation (Tanner stage) after thorough visual inspection of breast development in girls and genital development in boys[21].

The thickness of four skinfolds (triceps, biceps, subscapular and suprailliac) was measured to the nearest 0·1 mm to the right side of the body with a Lange skinfold calliper (Cambridge Industries, Cambridge, MD, USA). Each skinfold was grasped gently, in order to avoid causing any unnecessary discomfort to the child. Triceps and biceps skinfold thickness was measured with the right arm hanging relaxed at the side of the body. The skinfold was picked up about 1 cm bellow the midpoint mark over the triceps and biceps muscle, respectively. Measurement of the subscapular skinfold thickness was performed while the child stood with shoulders relaxed and after identifying the inferior angle of the scapula. The skinfold was picked up 1 cm below the subscapular mark. Suprailliac skinfold was measured just above the iliac crest, along the axis of the anterior line. In each case the calliper was applied to the 'neck' of the fold just above the finger and thumb, for two repeated measurements.

Assessment of percentage body fat and visceral fat mass

Bioelectrical impedance analysis (BIA) was used for the assessment of percentage body fat (Akkern BIA 101; Akkern Srl, Florence, Italy) and for abdominal/visceral fat mass (Tanita Viscan AB-140, Kowloon, Hong Kong). In abdominal BIA an electric current is passed between the regions near the umbilicus and spinal cord at the umbilicus level and the voltage generated in the lateral abdomen is recorded. Because the equipotential line that passes through visceral fat appears on the lateral abdominal surface, the amount of visceral fat can be estimated by measurement of the voltage generated at this location using a regression equation determined by computed tomography[22]. Participants were instructed to abstain from any food or liquid intake and from any intensive exercise for 4 h before measurement. They were also instructed not to wear any metal object during measurement. The assessments took place with the study participants lying on a non-conductive surface at ambient room temperature. Percentage body fat was calculated from the resistance and reactance values using valid equations derived from a similar pre-adolescent population[23], while percentage trunk fat mass and visceral fat rating (rating scale from 1 to 59 units, with 0·5 increment) were read directly from the instrument. As there were only two Tanita Viscan devices available, data on trunk-visceral fat mass were collected for a representative sub-sample of 1500 children.

Socio-economic, perinatal and other information collected from parents

Data on the socio-economic background of the families having at least one child participating in the study were collected from the parents (most preferably from the mother) during scheduled face-to-face interviews at school. For those parents not being able to attend the meetings (approximately 5 % of the total sample), data were collected via telephone interviews. All interviews were conducted by members of the research team who were rigorously trained to minimize the interviewer effect by using a standardized questionnaire. The socio-economic data collected from parents included: parental educational level (years of education); home size (m^2); number of family members living at home; number of cars owned by the family; and home property ownership. All aforementioned data were grouped, scored and combined for the development of a socio-economic status (SES) index as follows: scores of 0, 1, 2 and 3 were assigned to <9, 9–12, 12–16 and >16 years of maternal and paternal education, respectively; scores of 0, 1, 2 and 3 were assigned to <20, 20–25, 25–30 and >30 home m^2 per family member, respectively; scores of 0, 1, 2 and 3 were assigned to 0, 1, 2 and ≥3 cars owned by the family, respectively; and scores of 0 and 1 were assigned to rented or owned home, respectively. The total score of the SES index, obtained by summing the scores from each index component, had values ranging between 0 and 13 and with higher values indicating higher SES of the family. Data on self-reported weight and height of the mother and the father were also recorded. Furthermore, during the interviews parents were also asked to bring with them their child's birth certificate and medical record from which birth date (this was used for estimation of the exact age of each child) and birth weight were recorded. Finally, parents were also asked to report their children's nightly sleep duration, which was calculated by asking parents the time their children usually go to bed at night and the time they usually wake up in the morning on weekdays and on weekend days, separately.

Statistical analysis

Lifestyle component derivation

PCA was used to identify the lifestyle patterns. PCA is a multivariate technique that can evaluate intercorrelations between individual predictor variables and results in the extraction of uncorrelated components (i.e. lifestyle patterns). The Kaiser–Meyer–Olkin (KMO) criterion and Bartlett's test of sphericity were used to assess the suitability of the data for PCA. An overall KMO criterion close to unity means that the data set is suitable for PCA. The orthogonal rotation (varimax option) was used to derive optimal non-correlated components (lifestyle patterns). The number of components that can be extracted is equal to the number of predictor variables. To decide the number of components to retain, the Kaiser criterion and the scree plot (eigenvalues >1) were used. Factor loadings represent the correlations of each predictor with the lifestyle pattern score. Higher absolute values of factor loadings indicate that the predictor contributes most to the construction of this particular component (Table 2). The lifestyle components (patterns) were named according to the factor loadings of dietary, physical activity or sleep behaviours correlated most with the component (factor loadings $>|0{\cdot}4|$).

Descriptive and other statistical analyses

Categorical values were presented as frequencies. Associations between categorical variables were examined by using the χ^2 test and the two-sample z test for proportions whenever appropriate. Multiple linear regression analysis was used to evaluate the associations between body fat mass indices and lifestyle patterns derived from PCA, which were treated as continuous variables after testing for linearity (Table 4). More specifically, three different models were applied: Model 1 was unadjusted; Model 2 adjusted for sex and Tanner stage; and Model 3 adjusted for sex, Tanner stage, mean parental BMI, SES index score and birth weight. The results from the linear regression models are presented as standardized beta coefficients (β). Moreover, participants' lifestyle component scores were categorized into quartiles so that, for each lifestyle component, the fourth quartile consisted of persons whose dietary, physical activity and sleep behaviours were adherent most to that particular pattern. Based on the statistically significant relationships provided by the linear regression models tested, logistic regression analysis was further performed to evaluate the association between the quartiles of each lifestyle component and the probability of being overweight or of having increased fat mass levels. The results of logistic regression models were presented as odds ratios and 95 % confidence intervals (Table 5).

Table 1 Descriptive characteristics of the study population: schoolchildren aged 9–13 years (*n* 2073) participating in the Healthy Growth Study

	Boys (*n* 1032)		Girls (*n* 1041)		Total (*n* 2073)	
	%		%		%	
Weight status						
Underweight	2·2		3·7		2·9	
Normal weight	53·7†		58·2		56·0	
Overweight	30·9		28·8		29·9	
Obese	13·2†		9·3		11·2	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	11·2	0·7	11·2	0·7	11·2	0·7
Weight (kg)	45·3	11·0	45·0	10·9	45·2	10·9
Height (cm)	148·2*	7·4	149·1	8·1	148·7	7·8
BMI (kg/m^2)	20·5*	3·9	20·1	3·7	20·3	3·8
Waist circumference (cm)	69·9*	9·9	67·5	9·0	68·7	9·6
Sum of skinfold thicknesses (mm)	53·8*	24·7	56·0	21·0	54·9	23·0
Fat mass (%)	28·6*	9·2	29·7	9·1	29·2	9·2
Trunk fat (%)‡	20·0*	7·0	25·3	7·9	22·7	7·9
Visceral trunk fat rating‡	5·6*	3·7	4·0	2·2	4·8	3·2

*Mean values were significantly different from those of girls (Student's *t* test): $P<0.05$.
†Proportions were significantly different from those of girls (based on the χ^2 test and the two-sample *z* test): $P<0.05$.
‡Trunk and visceral fat mass data were available for 1211 children.

Data were analysed using the statistical software package SPSS version 13·0. In all statistical analyses, the level of significance was set at $P<0.05$.

Results

A complete set of socio-economic, demographic, parental BMI, family status, perinatal, clinical, anthropometric and body composition data were collected for 2073 children (49·8 % boys and 50·2 % girls) from the 2665 children whose parents had provided signed consent forms. The attrition was attributed to the inclusion in the parental questionnaire of questions relating mainly to certain components of the SES index (i.e. number of cars, home m^2 and home property ownership) during the course of the study. However, the sample of 2073 children participating in the current study was adequate to provide the required statistical power needed for the analysis of the data. Table 1 illustrates that the total prevalence of overweight and obesity was 29·9 % and 11·2 %, respectively, with a significantly higher prevalence of obesity in boys than in girls (13·2 % *v.* 9·3 %, $P<0.05$). Furthermore, BMI, WC and visceral trunk fat rating were higher in boys than in girls ($P<0.05$). On the other hand, height, sum of skinfold thicknesses, percentage fat mass and percentage trunk fat mass were significantly higher in girls than in boys ($P<0.05$).

Table 2 summarizes the factor loadings of the factors retained from the PCA. From the initial eighteen food groups, seven were included in the PCA as these were strongly intercorrelated with each other. The value of the KMO criterion was equal to 0·6 and the *P* value for Bartlett's test of sphericity was <0·001, indicating that the lifestyle variables entered in the analysis were strongly intercorrelated and that PCA could be correctly used for assessing 'healthy' or 'unhealthy' patterns. PCA analysis indicated five different lifestyle components explaining 52·7 % of the total variance with regard to the examined variables. These components were defined as follows: higher dairy consumption and more adequate breakfast (component 1); higher consumption of high-fibre foods (component 2); more screen time, less sleep time and higher consumption of sugared beverages (component 3); more time spent on MVPA and more frequent meals (component 4); higher red meat and lower fish consumption (component 5).

Table 3 displays the descriptive characteristics of the dietary and lifestyle variables included in the PCA for the total sample and by gender. Regarding gender differences, boys were found to have significantly higher consumption of dairy products and sugared drinks as well as more time spent on MVPA compared with girls ($P<0.05$). No other statistically significant gender differences were observed.

Table 4 presents the associations, derived from the three different models of the linear regression analyses, between anthropometric (i.e. BMI, WC and sum of skinfold thicknesses) as well as BIA fat mass indices and each one of the five lifestyle components. In the case of the anthropometric indices, lifestyle components 1 and 4 were negatively associated with BMI and WC, even after adjusting for several potential confounders in Model 3 ($\beta=-0.057$, $P=0.007$ and $\beta=-0.049$, $P=0.024$ respectively for BMI; $\beta=-0.058$, $P=0.007$ and $\beta=-0.055$, $P=0.012$ respectively for WC). The sum of skinfold thicknesses was also negatively associated with lifestyle component 1 ($\beta=-0.084$, $P<0.001$) and lifestyle component 2 ($\beta=-0.069$,

Table 2 Factor loadings derived from principal component analysis conducted with dietary and lifestyle variables available for schoolchildren aged 9–13 years (*n* 2073) participating in the Healthy Growth Study

	Component				
Predictor	1	2	3	4	5
Dairy consumption‡	0·79⫴	0·15	−0·11	0·10	0·002
Fruit consumption	0·05	0·62⫴	−0·01	0·15	0·000
Vegetable consumption	−0·05	0·74⫴	0·11	−0·04	0·04
Wholegrain products consumption§	0·11	0·50⫴	−0·22	−0·09	−0·11
Red meat consumption	0·01	−0·06	0·07	0·12	0·72⫴
Fish consumption	−0·01	−0·01	0·07	0·11	−0·75⫴
Sugared drinks consumption‖	−0·17	−0·15	0·56⫴	0·36	0·11
Adequate breakfast consumption	0·76⫴	−0·09	−0·02	−0·13	0·03
Meal frequency	0·45⫴	0·35	0·12	0·48⫴	−0·04
Screen time¶	0·04	−0·06	0·68⫴	−0·21	−0·02
Time spent on physical activity‡‡	−0·04	−0·004	−0·12	0·82⫴	−0·001
Sleep time§§	0·03	−0·07	−0·58⫴	0·01	0·03
Explained variance (%)	12·1	11·4	10·2	9·7	9·3

‡Consumption of milk, yoghurt and cheese.
§Consumption of wholegrain bread and cereal.
‖Consumption of regular soft drinks and sugared fruit juices.
¶Time spent watching television/DVD/videos and/or using games consoles/computer for fun.
‡‡Time spent on moderate-to-vigorous physical activity.
§§Nightly sleep time on weekdays.
⫴Predictor with the highest factor loading (>|0·4|) within the component.

Table 3 Descriptive characteristics of the dietary and lifestyle variables included in the principal component analysis

	Boys (*n* 1032)		Girls (*n* 1041)		Total (*n* 2073)	
	Median	P25–P75	Median	P25–P75	Median	P25–P75
Dairy consumption (g/d)‡	387·8*	258·0–536·0	349·3	223·5–516·0	373·9	244·6–526·1
Fruit consumption (g/d)	59·0	0·0–138·0	59·0	0·0–138·0	59·0	0·0–138·0
Vegetable consumption (g/d)	56·8	7·6–126·7	56·8	12·6–113·5	56·8	10·0–120·1
Wholegrain consumption (g/d)§	10·0	0·0–16·5	9·6	0·0–15·8	10·0	0·0–16·2
Red meat consumption (g/d)	45·0	0·0–94·6	45·0	0·0–80·0	45·0	0·0–90·0
Fish consumption (g/d)	18·0	0·0–35·4	18·7	0·0–36·7	18·3	0·0–36·2
Sugared drinks consumption (ml/d)‖	85·2*	0·0–167·5	62·1	0·0–126·9	73·3	0·0–131·6
Screen time (h/d)¶	2·0	1·3–3·0	2·0	1·3–3·0	2·0	1·3–3·0
Time spent on physical activity (min/d)‡‡	67·1*	32·1–117·7	45·4	17·1–85·7	55·7	25·0–100·7
Sleep time (h/d)§§	9·0	8·5–9·3	9·0	8·5–9·3	9·0	8·5–9·3
	n	%	*n*	%	*n*	%
Adequate breakfast consumption						
Salty and sweet snacks and other	86	8·3	92	8·8	178	8·6
0 portions of fruits, cereals or dairy products	209	20·3	232	22·3	441	21·3
1 portion of fruits, cereals or dairy products	482	46·7	491	47·1	973	46·9
2 portions of fruits, cereals or dairy products	218	21·1	187	17·9	405	19·5
3 or more portions of fruits, cereals or dairy products	37	3·6	40	3·8	77	3·7
Meal frequency						
2 meals/d	5	0·5	9	0·9	14	0·7
3 meals/d	83	8·0	71	6·8	154	7·4
4 meals/d	266	25·8	293	28·1	559	27·0
5 meals/d	450	43·6	481	46·2	931	44·9
6 meals/d	228	22·1	188	18·0	416	20·1

P25, 25th percentile; P75, 75th percentile.
*Median values were significantly different from those of girls (non-parametric Mann–Whitney test): $P<0·05$.
‡Consumption of milk, yoghurt and cheese.
§Consumption of wholegrain bread and cereal.
‖Consumption of regular soft drinks and sugared fruit juices.
¶Time spent watching television/DVD/videos and/or using games consoles/computer for fun.
‡Time spent on moderate-to-vigorous physical activity.
§§Nightly sleep time on weekdays.

$P=0·002$) after multiple adjustments were made in Model 3. Regarding BIA fat mass indices, percentage fat mass was negatively associated with lifestyle components 1 and 4 ($\beta=-0·048$, $P=0·029$ and $\beta=-0·083$, $P<0·001$ respectively) and percentage trunk fat mass was negatively associated with lifestyle component 4 ($\beta=-0·085$, $P=0·002$) after adjusting for potential confounders in Model 3.

Table 4 Single (Model 1) and multiple (Models 2 and 3) linear regression analysis examining the association of lifestyle components with anthropometric and total, trunk and visceral fat mass indices in schoolchildren aged 9–13 years (*n* 2073) participating in the Healthy Growth Study

	Model 1		Model 2		Model 3	
Component	β	*P* value	β	*P* value	β	*P* value
BMI (kg/m^2)						
Lifestyle component 1	−0·06	0·010	−0·06	0·005	−0·06	0·007
Lifestyle component 2	−0·01	0·722	−0·01	0·702	0·01	0·737
Lifestyle component 3	0·08	<0·001	0·05	0·040	0·04	0·057
Lifestyle component 4	−0·06	0·005	−0·06	0·010	−0·05	0·024
Lifestyle component 5	−0·03	0·248	−0·02	0·444	−0·01	0·627
Waist circumference (cm)						
Lifestyle component 1	−0·06	0·008	−0·06	0·003	−0·06	0·007
Lifestyle component 2	−0·03	0·139	−0·04	0·103	−0·02	0·310
Lifestyle component 3	0·08	<0·001	0·04	0·043	0·04	0·075
Lifestyle component 4	−0·06	0·012	−0·07	0·003	−0·06	0·012
Lifestyle component 5	0·002	0·922	0·001	0·953	0·01	0·703
Sum of skinfold thicknesses (mm)						
Lifestyle component 1	−0·09	<0·001	−0·09	<0·001	−0·08	<0·001
Lifestyle component 2	−0·08	<0·001	−0·08	<0·001	−0·07	0·002
Lifestyle component 3	0·04	0·080	0·02	0·299	0·02	0·353
Lifestyle component 4	−0·06	0·004	−0·05	0·034	−0·04	0·071
Lifestyle component 5	−0·03	0·182	−0·02	0·274	−0·02	0·338
Fat mass (%)						
Lifestyle component 1	−0·05	0·023	−0·05	0·028	−0·05	0·029
Lifestyle component 2	−0·04	0·084	−0·04	0·112	−0·02	0·307
Lifestyle component 3	0·03	0·249	0·003	0·881	0·003	0·894
Lifestyle component 4	−0·11	<0·001	−0·09	<0·001	−0·08	<0·001
Lifestyle component 5	−0·04	0·113	−0·02	0·314	−0·02	0·299
Trunk fat (%)‡						
Lifestyle component 1	−0·06	0·034	−0·05	0·060	−0·04	0·152
Lifestyle component 2	−0·004	0·899	0·01	0·721	0·02	0·528
Lifestyle component 3	0·08	0·005	0·06	0·024	0·05	0·063
Lifestyle component 4	−0·16	<0·001	−0·09	0·001	−0·09	0·002
Lifestyle component 5	−0·13	<0·001	−0·08	0·002	−0·03	0·084
Visceral trunk fat rating‡						
Lifestyle component 1	−0·04	0·151	−0·06	0·028	−0·04	0·058
Lifestyle component 2	0·01	0·769	−0·001	0·984	0·01	0·713
Lifestyle component 3	0·08	0·008	0·06	0·037	0·04	0·130
Lifestyle component 4	0·01	0·839	−0·04	0·146	−0·03	0·280
Lifestyle component 5	−0·04	0·213	−0·06	0·034	−0·05	0·095

β, standardized beta coefficient.
Model 1, unadjusted; Model 2, adjusted for Tanner stage and sex, Model 3, adjusted for sex, Tanner stage, parental BMI, socio-economic status index and birth weight.
Lifestyle component 1, higher dairy consumption and more adequate breakfast; lifestyle component 2, higher consumption of high-fibre food (i.e. fruits, vegetables and wholegrain food); lifestyle component 3, more screen time, shorter sleep duration and higher consumption of sugared beverages; lifestyle component 4, more time spent on moderate-to-vigorous physical activity and more frequent meals; lifestyle component 5, higher red meat consumption and lower fish consumption.
‡Trunk and visceral fat mass data were available for 1211 children.

Logistic regression analyses summarized in Table 5 showed that children whose lifestyle conformed most closely to patterns of lifestyle component 1 (i.e. children with dairy and breakfast consumption patterns within the fourth quartile) were 39·4 %, 45·2 % and 32·2 % less likely to be overweight/obese and in the highest quartile of sum of skinfold thicknesses and percentage fat mass, respectively, than children whose lifestyle was different from this pattern (i.e. children within the first quartile), after controlling for several potential confounders. Furthermore, children in the fourth quartile of lifestyle component 2 (i.e. children with the highest consumption of high-fibre foods) were 27·4 % less likely to be in the highest quartile of sum of skinfold thicknesses than children in the first quartile of lifestyle component 2. Finally, children in the fourth quartile of lifestyle component 4 (i.e. children who spent more time on physical activity and had more frequent meals) were 38·0 %, 26·3 % and 29·5 % less likely to be overweight, centrally obese and in the highest quartile of percentage fat mass than their peers in the first quartile of lifestyle component 4.

Discussion

The present study showed considerably high rates of overweight (29·9 %) and obesity (11·2 %) in a representative sample of Greek children which are two- to threefold higher compared with the prevalences reported for children in central and northern European countries[24].

Table 5 Logistic regression analyses examining the association of quartiles of lifestyle components (independent variable) with anthropometric and total, trunk and visceral fat mass indices (dependent variables) in schoolchildren aged 9–13 years (*n* 2073) participating in the Healthy Growth Study

	Quartile of lifestyle component 1 (higher dairy consumption and more adequate breakfast)						
	First	Second		Third		Fourth	
	(ref.)	OR	95 % CI	OR	95 % CI	OR	95 % CI
Overweight/obesity	1·00	0·82	0·63, 1·07	0·94	0·73, 1·23	0·61	0·46, 0·79
Central obesity	1·00	0·85	0·60, 1·22	0·76	0·53, 1·09	0·92	0·65, 1·31
Sum of skinfold thicknesses, fourth quartile	1·00	0·74	0·55, 0·98	0·66	0·49, 0·88	0·55	0·41, 0·74
Percentage fat mass, fourth quartile	1·00	0·88	0·66, 1·18	0·86	0·64, 1·15	0·68	0·50, 0·92

	Quartile of lifestyle component 2 (higher consumption of high-fibre food)						
	First	Second		Third		Fourth	
	(ref.)	OR	95 % CI	OR	95 % CI	OR	95 % CI
Sum of skinfold thicknesses, fourth quartile	1·00	1·07	0·80, 1·42	0·71	0·53, 0·96	0·73	0·54, 0·98

	Quartile of lifestyle component 4 (more time spent on physical activity and more frequent meals)						
	First	Second		Third		Fourth	
	(ref.)	OR	95 % CI	OR	95 % CI	OR	95 % CI
Overweight/obesity	1·00	0·84	0·65, 1·09	0·65	0·50, 0·84	0·62	0·48, 0·81
Central obesity	1·00	1·04	0·75, 1·46	0·53	0·36, 0·78	0·74	0·52, 0·99
Percentage fat mass, fourth quartile	1·00	0·94	0·71, 1·25	0·55	0·40, 0·75	0·71	0·52, 0·95
Percentage trunk fat, fourth quartile‡	1·00	1·25	0·86, 1·83	0·60	0·40, 1·04	0·80	0·53, 1·22

ref., reference category.
Adjusted for sex, Tanner stage, parental BMI, socio-economic status index and birth weight.
‡Trunk and visceral fat mass data were available for 1211 children.

Most importantly, the current study identified five different lifestyle components, which were associated not only with overweight/obesity in children but also with increased fat mass levels. This is important considering that anthropometric indices of adiposity (i.e. BMI, WC, skinfold thicknesses) are usually subjected to more bias than other techniques that are used to assess body composition, such as BIA[25].

Lifestyle component 1 comprised of higher dairy consumption and more adequate breakfast was negatively associated with BMI, WC, sum of skinfold thicknesses and percentage fat mass. The results of the present study also indicated that the children who tended to conform most closely to the pattern of higher dairy and adequate breakfast consumption (i.e. in the fourth quartile) were less likely to be overweight and to have increased fat mass levels. Dairy consumption is one of the best sources of highly bioavailable dietary Ca, which has been independently associated with reduced body weight[26–28]. More specifically, increased dietary Ca intake inhibits secretion of parathyroid hormone, which in turn lowers Ca influx within the adipocytes[29]. It has been suggested that increased intracellular Ca concentration may stimulate lipogenesis, inhibit lipolysis and reduce thermogenesis, alterations that if sustained for a long period can lead to fat mass accumulation and obesity[30]. In this context a previous study, which evaluated the association between dietary patterns and obesity among women, showed that consumption of low-fat dairy products was related with lower odds of obesity[31]. Still, it is not safe to claim that increasing the consumption of dairy foods results in a reduction in body weight or fat mass levels[32]. Regarding breakfast consumption, a previous study which attempted to identify the possible interactions between lifestyle patterns and BMI revealed that a pattern also comprised of regular breakfast consumption was inversely associated with excess body weight in children[6]. Furthermore, other studies have shown that regular consumption of breakfast that comprises dairy products, cereals and fruits is associated with a lower risk of overweight among children[33–35]. As breakfast consumption is generally considered as a healthy dietary behaviour[36], children who skip breakfast have a greater likelihood of reporting higher energy intake from high-fat, salty and sweet snacks at main meals[37]. Furthermore, breakfast omission seems to favour excessive hunger and rebound overeating[38], which may lead to higher energy intake.

The current study also showed that lifestyle component 2, comprising consumption of high-fibre foods such as fruits, vegetables and wholegrain products, was negatively associated with the sum of skinfold thicknesses, probably due to the higher satiety induced by consumption of these foods[39]. Two other studies performed in Greece and in Great Britain also revealed a negative association between a dietary pattern comprising high-fibre food consumption and excess adiposity in children[6,40]. Other recent research has shown that fibre supplementation in obese individuals can significantly enhance weight loss[41]. Soluble fibre, when fermented in the large intestine, induces production of glucagon-like peptide-1 and peptide YY, two gut hormones that stimulate satiety. Furthermore, increased dietary fibre intake is inversely related to total energy intake, as well as to the diet's metabolizable energy, i.e. gross energy intake minus the energy lost in the faeces, urine and combustible gases[42]. However, further to the physiological mechanisms described above, higher consumption of dairy products, wholegrain cereals, fruits and vegetables could also support the maintenance of lower body weight and fat mass levels since these food groups reflect an overall healthier diet, which is very common among normal-weight individuals.

The findings of the present study further indicated that lifestyle component 4, defined by a combination of more time spent on MVPA and more frequent meals during the day, was negatively associated with BMI, WC, and percentage total body and trunk fat mass. There is plenty of evidence that both physical activity and frequent meals *per se* are negatively correlated with overweight and obesity in childhood[43–46]. A possible explanation for this negative association is that these behaviours are associated with an increased energy expenditure. Regarding physical activity, energy is required for the mechanical work associated with muscle contraction[47]. However, in a previous study when physical activity was clustered in lifestyle patterns no association was found with BMI[6]. As far as meals are concerned, more frequent meal consumption seems to increase diet-induced thermogenesis, thus leading to higher energy expenditure[48]. The importance of consuming frequent meals for maintaining a normal body weight has also been highlighted by other studies, concluding that obese children should be encouraged to eat three main meals and two snacks per day (breakfast, lunch, afternoon meal, dinner, supper)[49].

A number of strengths and limitations can be identified in the present study. Regarding strengths, the Healthy Growth Study was a large-scale epidemiological study conducted using a representative sample of children from four prefectures within the wider region of Greece. Furthermore, the adjustments for specific confounders resulted in the extraction of more accurate results from the linear and logistic regression analyses. However, one of the main limitations of the study is that due to its cross-sectional design it cannot support causal relationships between lifestyle patterns and body composition indices. Another limitation of the study is that the PCA method could not specify how many servings of dairy foods or how many meals one should consume to get the required health benefits. Finally, trunk and visceral fat mass data were available only for 1211 children from the 2073 children initially examined.

The findings of the current study are indicative of strong associations between certain lifestyle patterns and adiposity-related indices in children. More specifically, various anthropometric and bioelectrical impedance

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indices of fat mass were negatively associated with the following three dietary and lifestyle behavioural patterns identified in the present study: (i) increased dairy food and breakfast consumption; (ii) increased consumption of high-fibre foods; and (iii) more time engaged in MVPA concurrently with an increased number of meals consumed per day. These findings could facilitate advice targeting children and their families and the identified lifestyle patterns should be considered as components of future childhood obesity prevention initiatives.

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Healthy Growth Study Group: (i) Harokopio University Research Team/Department of Nutrition and Dietetics: Yannis Manios (Coordinator), George Moschonis (Project Manager), Katerina P. Skenderi, Evangelia Grammatikaki, Odysseas Androutsos, Sofia Tanagra, Alexandra Koumpitski, Paraskevi-Eirini Siatitsa, Anastasia Vandorou, Aikaterini-Efstathia Kyriakou, Vasiliki Dede, Maria Kantilafti, Aliki-Eleni Farmaki, Aikaterini Siopi, Sofia Micheli, Louiza Damianidi, Panagiota Margiola, Despoina Gakni, Vasiliki Iatridi, Christina Mavrogianni, Kelaidi Michailidou, Aggeliki Giannopoulou, Efstathoula Argyri, Konstantina Maragkopoulou, Maria Spyridonos, Eirini Tsikalaki, Panagiotis Kliasios, Anthi Naoumi, Konstantinos Koutsikas, Katerina Kondaki, Epistimi Aggelou, Zoi Krommyda, Charitini Aga, Manolis Birbilis, Ioanna Kosteria, Amalia Zlatintsi, Elpida Voutsadaki, Eleni-Zouboulia Papadopoulou, Zoi Papazi, Maria Papadogiorgakaki, Fanouria Chlouveraki, Maria Lyberi, Nora Karatsikaki-Vlami, Eva Dionysopoulou, Efstratia Daskalou. (ii) Aristotle University of Thessaloniki/Department of Physical Education and Sports Science: Vassilis Mougios, Anatoli Petridou, Konstantinos Papaioannou, Georgios Tsalis, Ananis Karagkiozidis, Konstantinos Bougioukas, Afroditi Sakellaropoulou, Georgia Skouli. (iii) University of Athens/Medical School: George P. Chrousos, Maria Drakopoulou, Evangelia Charmandari, Panagiota Pervanidou.

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PAPER VII

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Accuracy and correlates of visual and verbal instruments assessing maternal perceptions of children's weight status: the Healthy Growth Study

George Moschonis[1], Vasiliki Iatridi[1], Christina Mavrogianni[1], Paraskevi-Eirini Siatitsa[1], Aikaterini E Kyriakou[1], Vasiliki Dede[1], Georgia Skouli[2], Afroditi Sakellaropoulou[3] and Yannis Manios[1,]*

[1]Department of Nutrition & Dietetics, Harokopio University of Athens, 70 El. Venizelou Avenue, 176 71 Kallithea, Athens, Greece: [2]2nd Pediatric Department, Aristotle University of Thessaloniki, Thessaloniki, Greece: [3]Medical Center Zaglivery, Thessaloniki, Greece

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Abstract

Objective: To examine the accuracy of maternal ability to classify their children's weight status correctly using a verbal and a visual classification instrument and to detect significant correlates of maternal misperceptions.
Design: Cross-sectional study.
Setting: Primary schools in four counties from north, west, central and south Greece.
Subjects: A representative sample of 1858 primary-school children aged 9–13 years was examined. Two different instruments to assess maternal perceptions of their children's weight status, i.e. a verbal and a visual one, were used.
Results: Verbal and visual maternal underestimation rates of children's weight status were 15·0 % and 41·3 %, respectively. The frequency of underestimation was much higher among overweight and obese children for both instruments. The highest underestimation rates of 87·9 % and 82·1 % in overweight and obese boys, respectively, were obtained with the visual instrument. Multiple logistic regression modelling revealed that the likelihood of both verbal and visual maternal underestimation of their children's weight status was significantly higher for overweight mothers and for those with a lower educational level. Furthermore, children's male gender and a nanny or someone other than the mother as the child's primary caregiver were found to increase the odds of visual and verbal maternal underestimation of children's weight status, respectively.
Conclusions: The present study showed that the verbal instrument used to assess maternal perceptions of their children's weight status was more accurate compared with the visual one. However, both instruments showed that a considerable number of overweight and obese boys had their weight status underestimated by their mothers. Educating mothers to classify their children's weight status correctly might be a key factor for the implementation of successful childhood obesity prevention initiatives.

Keywords
BMI
Children
Mothers
Underestimation
Obesity

In many developed countries, the prevalence of childhood overweight has more than doubled over the last two decades[1]. Besides the manifestation of clinical signs of morbidity in overweight children, such as hypertension, hyperlipidaemia, etc.[2], childhood overweight often tracks into adulthood[2,3], when it becomes associated with an increased risk of mortality from chronic disease[4]. Changes in dietary and physical activity patterns are the primary causes for the current obesity epidemic and related chronic disease[5]. Specifically, the past few decades have brought marked lifestyle changes resulting in a decline in the time spent in physical activities, as well as in an increase in the consumption of energy-dense foods[5]. However, the aetiology of childhood overweight is probably more complex than the changes induced in lifestyle.

On the basis of recent research, many characteristics and lifestyle patterns that predispose children to overweight are established early in life and are greatly determined by family characteristics, including parents' dietary intake and physical activity patterns, parents' nutritional knowledge, siblings' interaction, etc.[6]. Furthermore, as

*Corresponding author: Email manios@hua.gr

parents are very influential in shaping early dietary and physical activity patterns in their children, their ability to recognize the problem of increased adiposity in their own child is another important determinant of childhood overweight[7]. In general, it is critical for parents to be able to make a distinction between normal and abnormal levels of adiposity to ensure that appropriate preventive and corrective interventions are implemented.

However, several studies have shown that a significant number of parents are unable to correctly perceive their children's weight status when asked to make either a verbal or a visual estimation[8–21], or both[22–24]. This observation implies that public health messages regarding the prevention of childhood obesity may not always reach parents, who often fail to identify the excessive body weight of their children. The factors that are associated with parental misperception of their child's weight status are currently under investigation. For example, some studies have shown that certain family characteristics such as ethnicity[25,26], child's gender[10,18,27] and parental educational status[11,15] may influence parental perception, whereas other studies did not find such associations[9,13].

The objectives of the present study were: (i) to examine the accuracy of maternal ability to classify their children's weight status correctly using a verbal and a visual classification instrument; and (ii) to detect possible demographic, socio-economic, maternal and family correlates of maternal underestimation of their children's weight status.

Subjects and methods

Sampling

The 'Healthy Growth Study' was a large-scale cross-sectional epidemiological study initiated in May 2007 and completed in June 2009. Approval to conduct the study was granted by the Greek Ministry of National Education and by the Ethical Committee of Harokopio University of Athens. The study population comprised schoolchildren aged 9–13 years attending the fifth and sixth grades in primary schools located in municipalities within the wider regions of the counties of Attica, Aitoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multistage and stratified by parental educational level and by the total population of students attending schools within these municipalities. More specifically, the municipalities in the counties under study were divided into three groups on the basis of the average educational level of their adult population (25–65 years of age), which was estimated from data provided by the National Statistical Service of Greece (Census 2001). This procedure yielded two parental educational cut-off points that allowed us to categorize municipalities into three different socio-economic levels (SEL), i.e. higher, medium and lower SEL. Consequently, on the basis of data from the National Statistical Service of Greece, a certain number of municipalities, proportional to the size of their pre-adolescent population (9–14 years of age), were randomly selected from each one of these three SEL groups.

An appropriate number of schools were randomly selected from each one of these municipalities in relation to the population of schoolchildren registered in the fifth and sixth grades in each municipality, on the basis of data obtained from the Greek Child Institute. All seventy-seven primary schools that were invited to participate in the present study responded positively. More specifically, an extended letter explaining the aims of the present study and a consent form for taking full measurements were provided to all parents or guardians having a child in these schools. Parents who agreed to the participation of their children in the study had to sign the consent form and provide their contact details. Signed parental consent forms were collected for 2655 out of 4145 children (response rate: 64·1 %).

Anthropometry and physical examination

Participants from each primary school underwent a physical examination by two trained members of the research team. Anthropometric measurements were obtained from all regions using the same protocol and equipment that was calibrated with standardized weights and rods, according to the calibration procedures described by the manufacturers. The physical examination included basic anthropometric measurements, i.e. weight and height. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770; Hamburg, Germany). Participants were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0·1 cm using a commercial stadiometer (Leicester Height Measure; Invicta Plastics Ltd, Oadby, UK) with participants not wearing shoes, their shoulders in a relaxed position, their arms hanging freely and their head aligned in Frankfort plane. Weight and height were converted to BMI using Quetelet's equation (weight (kg)/height^2 (m^2)). The international age- and sex-specific cut-off points proposed by Cole *et al.*[28,29] for children and adolescents aged 2–18 years were used to categorize participants as 'underweight', 'normal weight', 'overweight' or 'obese'. Regarding physical examination, one well-trained and experienced female paediatrician in each prefecture determined pubertal maturation (Tanner stage) after a thorough visual inspection of breast development in girls and genital development in boys[30].

Demographic, socio-economic data, maternal and family characteristics

Data on the socio-economic and demographic backgrounds of families having at least one child participating in the study were collected from the primary caregiver (most preferably from the mother) during scheduled face-to-face interviews at school. With regard to those primary caregivers who were unable to attend the meetings (approximately 5 % of the total sample), data were collected via telephonic interviews. All interviews were conducted by a research team of ten members who were rigorously trained to minimize the interviewer's effect by using a standardized questionnaire. More specifically, the data collected by primary caregivers included: demographic characteristics such as parent's age and nationality; socio-economic characteristics such as educational level (years of education) and mean annual family income over the last 3 years (€/year); lifestyle characteristics such as smoking habits; and anthropometric characteristics such as mother's height and weight. Mother's age was then classified into three different tertiles. Mother's weight and height were converted to BMI using Quetelet's equation. The International Obesity Task Force cut-off points for adults were used for the categorization of mother's weight status as: 'underweight' ($BMI < 18{\cdot}5\,kg/m^2$), 'normal weight' ($18{\cdot}5 \leq BMI < 25{\cdot}0\,kg/m^2$), 'overweight' ($25{\cdot}0 \leq BMI < 30{\cdot}0\,kg/m^2$) and 'obese' ($BMI \geq 30{\cdot}0\,kg/m^2$). Finally, children's age was calculated on the basis of their date of birth as recorded in the children's paediatric medical records.

Primary caregivers' perceptions of their children's weight status

Maternal perceptions of their children's weight status and body image were assessed using a verbal and a visual classification instrument, respectively. Regarding verbal classification of a child's weight status, the child's primary caregivers were asked to provide their answers to the following two questions: 'According to your perception, what is the current weight of your child?'; and 'According to your perception, what is the current height of your child?' Children's weight and height, as perceived by parents, were also converted to BMI by using Quetelet's equation and thereafter their weight status was classified into different weight categories (underweight, normal weight, overweight and obese) using Cole's cut-off points[28,29]. Regarding visual classification of the children's body shape, this was estimated using the 'Children's Body Image Scale' as proposed by Truby and Paxton[31]. In brief, two sets of age- and gender-specific photographs were available. Each photograph corresponded to a 10-year-old boy or girl with a BMI on or around each of the seven conventional 1979 National Center for Health Statistics (NCHS) percentiles (i.e. 3rd, 10th, 25th, 50th, 75th, 90th and 97th)[32]. More specifically, in each set, one photograph corresponded to an underweight child (3rd percentile), four photographs to children within the normal BMI-for-age percentile range (10th–75th percentiles), one photograph to an overweight child (90th percentile) and the last photograph to an obese child (97th percentile). After presenting this set of photographs in random order, mothers were asked to choose the photograph that most resembled their child's body shape.

Statistical analysis

All variables presented were categorical and expressed as percentages. Associations between categorical variables were examined using the χ^2 or Fisher's exact test whenever appropriate. Cross-classification analysis was further carried out to identify the proportion of participants classified correctly or incorrectly by the verbal and visual classification methods. The two-sample Z-test was also used to compare percentages of correct or incorrect verbal and visual classifications of children's actual weight status. Univariate logistic regression analyses were conducted to test the crude bivariate associations of several demographic, socio-economic and family status variables per se with mothers' verbal and visual underestimation of their children's weight status. Furthermore, multiple logistic regression analysis was conducted to identify which of the demographic, socio-economic, maternal and family status variables were independently associated with maternal underestimation of children's weight status. From all these analyses OR and 95 % CI were computed. All reported P values were based on two-sided tests. The level of statistical significance was set at $P < 0{\cdot}05$. The Statistical Package for the Social Sciences statistical software package version 13·0 (SPSS Inc., Chicago, IL, USA) was used to conduct all statistical analyses.

Results

Complete socio-economic, demographic, maternal, family status, verbal and visual classification data were collected for 1858 children (51·1 % girls and 48·9 % boys) from the 2665 children whose parents had signed the consent forms. This was mainly because of the addition of the visual assessment tool in the parental questionnaire during the course of the study when approximately 800 children and their parents had already been measured. Nevertheless, the study sample of 1858 children examined in the present study was adequate to provide enough statistical power. Approximately 45 % of the participating children were normal weight, 31 % were overweight and 11 % were obese. Regarding the characteristics of mothers, 39·5 % had 9–12 years of education whereas 40·4 % had >12 years. The majority of participating mothers were of Greek nationality (83·6 %) and were married (89·5 %). Significant associations were observed between children's weight status and certain maternal characteristics. More specifically, childhood obesity was found to be positively associated with maternal obesity and smoking status, with a greater proportion of mothers who were obese or smokers having an obese child ($P < 0{\cdot}001$, respectively). On the other hand, maternal educational levels were negatively associated with childhood obesity, since the percentage of obese children was significantly lower for mothers with a higher level of education than for mothers with a medium

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Table 1 Demographic, socio-economic, maternal and family descriptive characteristics of the study population presented by weight groups

	Total (n 1858)	Children's actual weight status: Underweight (n 56)	Normal weight (n 1019)	Overweight (n 574)	Obese (n 209)	
	%	%	%	%	%	P†
Child's age (years)						
9–11	41·8	2·6	54·8	30·9	11·7	0·005
11–13	58·2	3·3	54·9	30·9	10·9	
Child's gender						
Female	51·1	3·6	57·2	30·1	9·1	0·005
Male	48·9	2·4	52·4	31·7	13·5	
Child's primary caregiver						
Mother	53·3	3·2	56·0	29·6	11·2	0·298
Grandparents	40·7	2·6	53·7	31·4	12·3	
Nanny or someone else	6·0	4·6	51·4	37·6	6·4	
Mother's age category						
Lower (<38 years old)	39·8	2·9	54·4	30·2	12·5	0·541
Medium (38–42 years old)	34·5	2·3	55·1	31·6	11·0	
Higher (>42 years old)	26·6	4·1	55·4	30·8	9·7	
Mother's BMI (kg/m^2)						
Underweight	2·6	14·3	63·3	20·4	2·0	<0·001
Normal weight	57·9	3·4	60·2	29·2	7·3	
Overweight	26·9	2·2	50·5	31·5	15·8	
Obese	12·6	0·9	38·2	39·5	21·5	
Mother's educational level (years of education)						
<9	20·2	3·7	49·6	31·2	15·5	0·007
9–12	39·5	3·1	54·4	30·2	12·3	
>12	40·4	2·5	57·9	31·5	8·1	
Mother's nationality						
Greek	83·6	3·1	54·1	31·5	11·3	0·462
Non-Greek	16·4	2·6	58·9	27·6	10·9	
Mother's smoking habits						
Smoker	38·2	2·5	50·4	32·1	14·9	<0·001
Non-smoker	61·8	3·3	57·6	30·1	9·0	
Mother's marital status						
Married	89·5	3·1	55·0	30·7	11·2	0·943
Not married	1·2	4·3	52·2	34·8	8·7	
Divorced	7·5	1·4	56·4	30·7	11·4	
Widowed	1·7	3·1	43·8	37·5	15·6	
SEL of residential area						
Lower	24·8	3·3	57·2	27·6	12·0	0·187
Medium	33·7	3·7	51·9	31·8	12·6	
Higher	41·6	2·3	55·8	32·1	9·7	
Family income (€/year)						
<12 000	22·0	2·9	56·7	27·9	12·5	0·766
12 000–30 000	50·6	3·1	53·7	31·8	11·4	
>30 000	27·4	2·9	55·4	31·6	10·0	

SEL, socio-economic level.
†Associations between weight categories and all other categorical variables included in the table were tested using Pearson's χ^2 or Fisher's exact test whenever appropriate.

or lower educational level ($P = 0·007$). Further descriptive characteristics of the children and their mothers are summarized in Table 1.

Table 2 illustrates the percentages of maternal verbal and visual classification of their children's weight status by percentages of their children's actual weight status. More specifically, correct classification of children's actual weight status was significantly higher for overweight girls (67·7 % *v.* 22·7 %, $P < 0·001$) and boys (72·4 % *v.* 9·7 %, $P < 0·001$), as well as for obese boys (60·9 % *v.* 17·9 %, $P < 0·001$), when mothers estimated their children's weight status using the verbal rather than the visual classification instrument. Furthermore, the percentages of obese girls (4·1 % *v.* 20·9 %, $P < 0·01$) and boys (1·8 % *v.* 48 %, $P < 0·001$) who were misclassified by their mothers as normal weight were significantly lower when mothers tried to perceive their children's weight status using the verbal rather than the visual classification instrument. Overall, a significant percentage of mothers tended to misclassify their overweight or obese child, especially when classifying them visually.

Table 3 summarizes the crude OR and 95 % CI for maternal verbal underestimation of their children's weight status. More specifically, mothers with 9–12 and <9 years of education were 1·47 (95 % CI 1·07, 2·00) and 1·72 (95 % CI 1·20, 2·47) times more likely to verbally underestimate

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Table 2 Maternal verbal and visual classification of children's weight status by children's actual weight status

Primary caregivers' classification of their children's weight status	Children's actual weight status							
	Underweight (*n* 56)		Normal weight (*n* 1019)		Overweight (*n* 574)		Obese (*n* 209)	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
	%	%	%	%	%	%	%	%
Verbal								
Underweight	**61·3**	**42·9**	4·1***	4·8*	0·0	0·8	0·0	0·0
Normal weight	35·5	57·1	**89·9**	**84·8***	29·1***	21·2*	4·1**	1·8*
Overweight	3·2	0·0	5·8***	9·7*	**67·7*** **	**72·4***	41·9	37·3
Obese	0·0	0·0	0·2	0·7	3·1	5·1	**54·1**	**60·9***
Visual								
Underweight	**47·1**	**50·0**	11·2***	25·4*	0·3	0·7	1·2	0·0
Normal weight	52·9	50·0	**87·3**	**72·9***	67·1***	87·2*	20·9**	48·0*
Overweight	0·0	0·0	1·1***	1·3*	**22·7*** **	**9·7***	30·2	34·1
Obese	0·0	0·0	0·4	0·4	9·8	2·4	**47·7**	**17·9***

Percentages in bold indicate correct weight status classification.
**P* < 0·001 for differences between verbal and visual classification of girls' weight status, based on the two-sample *Z*-test.
***P* < 0·01 and ****P* < 0·001 for differences between verbal and visual classification of boys' weight status, based on the two-sample *Z*-test.

Table 3 Logistic regression analyses for the association between maternal verbal underestimation of children's weight status with demographic, socio-economic, maternal and family characteristics of the study population

Independent variable	Verbal underestimation of the child's weight category			
	Crude OR	95 % CI	Adjusted OR	95 % CI
Mother's educational level (years of education)				
>12	Ref.		Ref.	
9–12	**1·47**	**1·07, 2·00**	**1·52**	**1·10, 2·11**
<9	**1·72**	**1·20, 2·47**	**1·89**	**1·30, 2·77**
Mother's weight status				
Under/normal weight	Ref.		Ref.	
Overweight	**1·44**	**1·06, 1·95**	**1·48**	**1·08, 2·02**
Obese	1·30	0·85, 1·95	1·26	0·83, 1·93
Child's primary caregiver				
Mother	Ref.		Ref.	
Grandparents	0·97	0·73, 1·29	1·05	0·78, 1·41
Nanny or someone else	**1·62**	**1·02, 2·72**	**1·70**	**1·00, 2·89**
SEL of residential area				
Lower	Ref.		Ref.	
Medium	**1·38**	**1·03, 1·97**	1·41	0·98, 2·03
Higher	1·08	0·75, 1·54	1·18	0·81, 1·70
Mother's age category				
Lower (<38 years old)	Ref.		Ref.	
Medium (38–42 years old)	0·82	0·60, 1·12	0·87	0·62, 1·21
Higher (>42 years old)	**0·73**	**0·52, 0·98**	0·77	0·54, 1·11
Child's gender				
Male	Ref.			
Female	1·04	0·8, 1·37		
Mother's smoking habit				
Non-smoker	Ref.			
Smoker	1·10	0·84, 1·46		
Maternal marital status				
Married	Ref.			
Not married	0·33	0·44, 2·50		
Divorced	1·10	0·66, 1·80		
Widowed	0·50	0·11, 2·10		
Family income† (€/year)				
<12 000	Ref.			
12 000–30 000	0·94	0·67, 1·33		
>30 000	0·85	0·57, 1·25		

SEL, socio-economic level; Ref., reference category.
Values in bold indicate statistically significant OR.
*Adjusted for the number of people in the household.

their children's weight category compared with mothers with >12 years of education. Furthermore, overweight mothers were 1·44 (95 % CI 1·06, 1·95) times more likely to verbally underestimate their children's weight status compared with under/normal-weight mothers. The likelihood of maternal verbal underestimation of their children's

Table 4 Logistic regression analyses for the association of maternal visual underestimation of children's weight status with demographic, socio-economic, maternal and family characteristics of the study population

	Visual underestimation of the child's weight category			
Independent variable	Crude OR	95 % CI	Adjusted OR	95 % CI
Mother's educational level (years of education)				
>12	Ref.		Ref.	
9–12	0·91	0·74, 1·12	0·90	0·72, 1·13
<9	**0·72**	**0·55, 0·93**	**0·80**	**0·60, 0·99**
Mother's weight status				
Under/normal weight	Ref.		Ref.	
Overweight	**1·38**	**1·11, 1·71**	**1·48**	**1·18, 1·85**
Obese	**1·82**	**1·37, 2·42**	**1·95**	**1·45, 2·62**
Child's gender				
Male	Ref.		Ref.	
Female	**0·42**	**0·34, 0·50**	**0·40**	**0·33, 0·49**
SEL of residential area				
Lower	Ref.		Ref.	
Medium	**1·32**	**1·03, 1·70**	1·28	0·99, 1·66
Higher	1·25	0·98, 1·58	1·15	0·90, 1·48
Family income† (€/year)				
<12 000	Ref.		Ref.	
12 000–30 000	1·14	0·90, 1·45	1·01	0·78, 1·31
>30 000	**1·30**	**1·00, 1·70**	1·09	0·80, 1·49
Mother's nationality				
Greek	Ref.		Ref.	
Non-Greek	**0·78**	**0·61, 0·96**	0·86	0·65, 1·14
Child's primary caregiver				
Mother	Ref.			
Grandparents	1·08	0·89, 1·31		
Nanny or someone else	1·29	0·86, 1·92		
Mother's age category				
Lower (<38 years old)	Ref.			
Medium (38–42 years old)	1·07	0·86, 1·33		
Higher (>42 years old)	1·21	0·96, 1·52		
Mother's smoking habit				
Non-smoker	Ref.			
Smoker	1·09	0·90, 1·31		
Maternal marital status				
Married	Ref.			
Not married	1·09	0·47, 2·50		
Divorced	1·22	0·86, 1·73		
Widowed	0·74	0·35, 1·55		
Tanner stage	1·04	0·94, 1·15		

SEL, socio-economic level; Ref., reference category.
Values in bold indicate statistically significant OR.
†Adjusted for the number of people in the household.

weight status was also higher when the children's primary caregiver was a nanny or someone other than the mother (OR = 1·62; 95 % CI 1·02, 2·72) and lower for older (>42 years old) than for younger (<38 years old) mothers (OR = 0·73; 95 % CI 0·53, 0·98). Table 3 also presents the adjusted OR and 95 % CI derived from the multivariate logistic regression analysis, including in the same model all variables that were found to be significantly related to maternal verbal underestimation of their children's weight category at a univariate level. On the basis of the results derived from this analysis, lower (OR = 1·89; 95 % CI 1·30, 2·77) and medium maternal educational levels (OR = 1·52; 95 % CI 1·10, 2·11), maternal overweight (OR = 1·48; 95 % CI 1·08, 2·02) and nanny or someone else as the child's primary caregiver (OR = 1·70; 95 % CI 1·00, 2·89) remained significantly associated with maternal verbal underestimation of their children's weight status.

Table 4 summarizes the crude and adjusted OR and 95 % CI for maternal visual underestimation of their children's weight status. More specifically, mothers with <9 years of education, mothers with female children and those of non-Greek nationality were 0·72 (95 % CI 0·55, 0·93), 0·42 (95 % CI 0·35, 0·50) and 0·78 (95 % CI 0·61, 0·96) times less likely to visually underestimate their children's weight status compared with mothers with >12 years of education, mothers with male children and those of Greek nationality, respectively. On the other hand, overweight (OR = 1·38; 95 % CI 1·11, 1·71) and obese (OR = 1·82; 95 % CI 1·37, 2·42) mothers were more likely to visually underestimate their children's weight status in comparison with under/normal-weight mothers. Moreover, mothers living in areas of medium SEL (OR = 1·32; 95 % CI 1·03, 1·70) and mothers with an annual family income of >€ 30 000 (OR = 1·30; 95 % CI 1·00, 1·70) were also more likely to visually underestimate their children's weight status compared with mothers living in areas of lower SEL and mothers with an annual family income of <€ 12 000, respectively. Table 4 also presents the adjusted OR and 95 % CI derived from the multivariate logistic regression analysis, including in the same model all variables that were found to be significantly related to maternal verbal underestimation of their children's weight category at a univariate level. On the basis of the results derived from the present analysis, the factors that remained statistically significantly associated with maternal visual underestimation of their children's weight status were maternal lower educational level (OR = 0·80; 95 % CI 0·60, 0·99), maternal overweight (OR = 1·48; 95 % CI 1·18, 1·85) and obesity (OR = 1·95; 95 % CI 1·45, 2·62) and female gender of children (OR = 0·40; 95 % CI 0·33, 0·49).

Discussion

The present study used two different instruments to assess maternal perceptions of their children's weight status, i.e. a verbal and a visual one. The verbal instrument proved to be more accurate than the visual one in classifying children's actual weight status correctly. More specifically, 15·0 % of mothers verbally underestimated their children's weight status, mainly by under-reporting their children's actual weight and much less by over-reporting their children's height. On the other hand, 41·3 % of mothers visually underestimated their children's weight status when they were asked to choose one photograph representing body images of boys and girls in different BMI-for-age percentiles that most resembled their children's body shape (the aforementioned percentages are not shown in the tables). These underestimation rates were found to be even higher for overweight and obese children compared with normal-weight children, reaching 87·9 % and 82·1 %, respectively, in overweight and obese boys, whose weight status was visually assessed by their mothers. These findings were in agreement with those reported by other studies that examined parental perceptions of their children's weight status[8–13,15,16,20–22]. Nevertheless, the verbal instruments used in these studies to assess parental perceptions were not entirely comparable to those used in the present study. Most of the other studies used a verbal classification scale to assess parental perceptions of their children's weight status, mostly with the following question or with one very similar to it: 'How would you describe your child's weight at the moment?', and with one of the answers: underweight, normal weight or about the right weight, overweight and obese[8–13,15,16]. Furthermore, sex-specific sketches or silhouettes corresponding to children of different weight- or BMI-for-age percentiles were used as visual classification scales by two other studies[20,21], whereas both verbal and visual instruments were used in only one previous study[22].

In only one other study were caregivers asked to report their children's actual weight and height[33]. In that study, the accuracy of the verbal classification of children's weight status was comparable to that reported by the present study with respect to underweight, normal-weight and obese children, whereas a lower accuracy was observed for overweight children. Two other studies that used verbal and visual classification instruments, similar to those used in the present study, have failed to report higher accuracy of the verbal classification instrument, whereas others have provided some mixed findings. More specifically, according to the study by Eckstein *et al.*[24], the visual classification instrument (sketches) assessed children's weight status more accurately compared with the verbal classification instrument (word description). Furthermore, the study by Chaimovitz *et al.*[23], which was designed to assess the accuracy of parents' and paediatricians' perceptions of children's weight status, showed that parents displayed higher accuracy using the verbal classification instrument (word description), whereas doctors displayed higher accuracy with the visual classification instrument (sketches).

According to Maynard *et al.*[16], the observation from several studies that mothers tend to underestimate their children's excessive body weight may reflect their reluctance to admit that their children are overweight or their lack of understanding of what 'overweight' means. Thus, recording and understanding maternal perceptions about their children's weight status, as well as the determinants of these perceptions, would be a key step in forming effective links between health professionals and parents[29] that could help overweight prevention programmes be more effective by focusing on the main concern of parents, which is maintenance of 'good health' in their children rather than 'good weight'[34].

In the present study several factors were found to be independently (i.e. at a multivariate level) associated with the likelihood of maternal verbal or visual underestimation of their children's weight status (Tables 3 and 4). Maternal educational level was found to be significantly associated with both verbal and visual maternal underestimation of children's weight status, although in a reverse direction. More specifically, the findings of the present study revealed that mothers with a lower educational level were more likely to make a verbal underestimation but less likely to make a visual underestimation of their children's weight status. The negative association observed between verbal underestimation and maternal educational level is in line with the findings from other similar studies[8,15], whereas a possible interpretation for this association could be the fact that higher educational level may imply a better knowledge of normal body weight and height in children[15]. In addition, more educated mothers are probably more likely to be aware of the physical, social and emotional consequences of obesity. Nevertheless, no other study has reported an increased likelihood of maternal visual underestimation of their children's weight status, making an interpretation of this finding rather difficult.

Furthermore, overweight and obese mothers were significantly more likely to both verbally and visually underestimate their children's weight status. These findings were in agreement with those reported by other investigators who examined parental perceptions of their children's weight status[14,25,26], probably also indicating a reluctance of overweight or obese mothers to understand or admit that their children are overweight[8]. Alternatively, overweight mothers may also believe that young children may grow out of being overweight or that increased fatness signifies health[35].

Another factor that was found to be significantly associated with an increased likelihood of maternal verbal underestimation of children's weight status was when the child's primary caregiver was a person other than the mother or grandparents (i.e. an aunt, older siblings, etc). None of the previous relevant studies have ever examined this variable as a potential correlate of parental perceptions. As many working mothers these days usually leave another person in charge of their children's upbringing (i.e. a nanny, grandparents, an aunt, older siblings, etc.), the limited time that these mothers spend with their children may restrict their ability to identify their children's actual weight or height correctly. Finally, the results of the present study support the idea that parents are more likely to visually underestimate the weight status of boys than of girls[14,16,36]. Although this may relate to sex differences in body composition, it seems more likely to reflect social values as well. Mothers may be more sensitive to weight and body image issues in girls, whereas bigger boys may be seen as having a physical advantage.

The present study has certain potential limitations. First, only 12·6% of mothers were found to be obese. This could be attributed to deliberate under-reporting of weight, which is a common weakness when using self-reported data. Furthermore, the use of photographs that were based on the 1979 NCHS growth charts[31] to visually classify children's weight status could represent another possible limitation of the present study, since childhood obesity has more than doubled during the past three decades[37]. Although there was no other valid visual instrument that could be used alternatively in the present study, this limitation could provide one possible explanation for the higher maternal underestimation rates derived from the use of the visual compared with the verbal classification instrument. This could also be attributed to the fact that the photographs used in the present study represented pre-pubescent children, whereas the sample also included children at a higher stage of sexual maturation. Nevertheless, no significant association was found between the Tanner stage and the accuracy of maternal perceptions of children's weight status with the use of photographs.

To conclude, the verbal instrument used in the present study to assess maternal perceptions of their children's actual weight status was found to be more accurate compared with the visual one. Considerably higher errors of underestimation occurred for overweight and obese children, whereas overweight mothers and those with a lower educational level were more likely to underestimate their children's weight status. Furthermore, children's male gender and a nanny or a person other than the mother as the child's primary caregiver were found to increase the odds of both visual and verbal maternal underestimation of children's weight status, respectively. These findings have important public health implications since – given that maternal perception might be an indirect determinant of their children's body weight – any interventional programme to induce weight loss is unlikely to be successful without parental involvement. Moreover, even though perceptions are strongly influenced by culture, the fact that we live in an era of globalization makes the findings of the present study relevant to public health experts who deal with childhood obesity in almost all developed countries worldwide. The current findings might be even more relevant to minority groups and populations of lower socio-economic level living in developed countries, for whom insufficient awareness on health issues makes the risk of body weight underestimation even greater. Thus, the attempt to combat childhood obesity in clinical and public health settings might be more successful if future research also focuses on detecting mothers who are more likely to misperceive their children's weight status. Educating these mothers to classify their children's weight status correctly might be a key factor for the implementation of successful childhood obesity prevention initiatives.

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PAPER VIII

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Pediatric Diabetes

Original Article

Female sex, small size at birth, and low family income increase the likelihood of insulin resistance in late childhood: the healthy growth study

Manios Y, Moschonis G, Papandreou C, Siatitsa P-E, Iatridi V, Lidoriki I, Lionis C, Chrousos GP, ''Healthy Growth Study'' Group. Female sex, small size at birth, and low family income increase the likelihood of insulin resistance in late childhood: the healthy growth study.
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Objective: To identify among a wide range of perinatal indices, as well as certain family sociodemographic and parental characteristics, those independently associated with insulin resistance (IR) in late childhood.
Methods: A representative sample of 2195 Greek schoolchildren, aged 9–13 yr, was examined, and based on the biochemical indices collected IR was estimated using the homeostasis model assessment (HOMA-IR $<$ 3.16). Perinatal data were recorded from children's medical records, retrospectively, while family sociodemographics and parental anthropometrics were reported by parents.
Results: The overall prevalence of IR was 28.4%, with a higher prevalence observed for girls compared with boys ($p < 0.05$). Examination of univariate associations, *per se*, showed that maternal current and pre-pregnancy overweight/obesity, maternal smoking at early pregnancy, children's small birth weight, and rapid growth at infancy as well as female sex and non-Caucasian race increased the likelihood of IR. In contrast, folate supplementation during pregnancy, as well as higher paternal education and annual family income decreased the likelihood of IR in children. Inclusion of all above variables at a multivariable regression model highlighted female sex [odds ratios (OR): 1.67, 95% confidence intervals (CI): 1.30–2.13], small birth weight (OR: 1.41, 95% CI: 1.03–2.01), and higher annual family income (OR: 0.71, 95% CI: 0.53–0.95 for 12 000–30 000 € and OR: 0.68, 95% CI: 0.48–0.96 for $>$30 000 €) as the only significant correlates of IR after also controlling for children's body mass index (BMI) and Tanner stage.
Conclusions: The current study highlighted small birth weight and female sex as the only perinatal factors independently associated with the occurrence of IR in late childhood, when examined at a multivariable level with a wide range of perinatal indices as well as certain family sociodemographic and parental characteristics.

Yannis Manios[a], George Moschonis[a], Christopher Papandreou[a], Paraskevi-Eirini Siatitsa[a], Vassiliki Iatridi[a], Irene Lidoriki[a], Christos Lionis[b], George P. Chrousos[c] and on behalf of the ''Healthy Growth Study'' Group

[a]Department of Nutrition & Dietetics, Harokopio University of Athens, Athens, Greece; [b]Clinic of Social and Family Medicine, School of Medicine, University of Crete, Heraklion, Crete, Greece; and [c]First Department of Pediatrics, Athens University Medical School, Aghia Sophia"Children's Hospital, Athens, Greece

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Corresponding author:
Yannis Manios,
Department of Nutrition and Dietetics,
Harokopio University,
70, El.Venizelou Ave, 176 71
Kallithea,
Athens, Greece.
Tel: +30 210 9549156;
fax: +30 210 9514759;
e-mail: manios@hua.gr

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Insulin resistance (IR) represents a frequent clinical condition among children and adolescents (1). It reflects the impaired response of tissues to insulin-mediated cellular glucose uptake (2, 3). Health care professionals should be alarmed when clinical signs of IR are detected in children, mainly due to the well-established relations between IR and other related cardiometabolic disorders, such as type 2 diabetes, hypertension, and dyslipidemia, that lead to an increased risk for cardiovascular disease (4). Although the end-points for cardiometabolic risk are not seen in childhood, IR usually tracks from childhood to adulthood, indicating that the precursors of cardiovascular disease are present early in life (5). Therefore, identification of early life risk factors that predispose children to IR could be an important piece of information for researchers, clinicians, and public health policymakers for establishing appropriate initiatives that will facilitate early prevention.

In the context of early prevention of IR identification of the most important perinatal risk factors is essential. Such factors are considered to cause certain physiological and metabolic adaptations of a seemingly permanent nature that can be detrimental to lifelong health. Among these factors, maternal smoking during pregnancy, maternal gestational diabetes (6), small size of infants at birth, infants' feeding with formula and catch-up growth during infancy have been reported as the most salient perinatal risk factors for the later occurrence of IR (7–13). As far as catch-up growth is concerned, this is related to IR via the following mechanism: children born small for gestational age when exposed to an energy-rich environment at early infancy are more susceptible to accumulate abdominal visceral fat mass, an adaptation that is caused by energy deprivation and that is originally intended to safeguard fetal survival (14).

Similar to childhood obesity (15) occurrence of IR in children also seems to follow a socioeconomic and demographic distribution. However, the available evidence on the relationship between IR in children and family sociodemographics is scarce. Furthermore, the literature on the association between perinatal risk factors and IR in children, taking into account the possible role of family sociodemographics and parental characteristics on this association is even scarcer. Hence and further to perinatal risk factors, also recording and understanding family sociodemographic and parental characteristics related to IR can facilitate the design of efficient public health initiatives counteracting IR in childhood.

To our knowledge the current study is a first attempt to identify, among a wide range of perinatal factors, those independently associated with IR in late childhood, also taking into account certain family sociodemographic and parental characteristics.

Participants and Methods

Sampling

The 'Healthy Growth Study' was a cross-sectional epidemiologic study initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of the Harokopio University of Athens. The study population comprised schoolchildren attending the fifth and sixth grades of primary schools located in municipalities within the wider regions of Attica, Aitoloakarnania, Thessaloniki, and Iraklio. The sampling of schools was random, multistage and stratified by parents' educational level and total population of students attending schools within municipalities of these counties, as described in more detail elsewhere (16). The study population was representative of the 9- to 13-yr-old school children living in the four counties included in the study. Nonetheless, these counties are scattered throughout the Greek territory, covering the northern (i.e., Thessaloniki), central (i.e., Attica), western (i.e., Aitoloakarnania), and southern (i.e., Iraklio-Crete) parts of Greece. This, combined with the random, multistage and stratified sampling procedures followed to recruit the sample are indicative of the representativeness of the study population. The sampling procedure yielded 77 primary schools, representative of the total number of schools in the counties under study, which responded positively when they were invited to participate in the study. An extended letter explaining the aims of the study and a consent form for taking full measurements were provided to all parents or guardians having a child in these schools. Parents who agreed to the participation of their children in the study had to sign the consent form and provide their contact details. Signed parental consent forms were collected for 2656 of 4145 children (response rate 64.1 %).

Family sociodemographic, maternal, and perinatal data obtained by mothers and birth certificates

Sociodemographic data, maternal weight, and perinatal data were either reported by the parents or taken from the children's birth certificates and medical records that the parents were instructed to bring along during scheduled interviews conducted at school site. If parents were unable to attend the meeting (approximately 5% of the total sample) data were collected via telephone interviews. All interviews were conducted with the use of a standardized questionnaire by members of the research team who had been rigorously trained to minimize the interviewer's effect. Information on sociodemographic and perinatal factors were collected. More specifically, information included: (i) maternal years of education that were stratified into: less than 9 yr, which is the duration of compulsory education in Greece that leads to a junior high school degree; 9–12 yr of education, which corresponds to having a high school degree; and more than 12 yr of education that corresponds to having a college or a university degree and/or a master and/or a PhD diploma; (ii) child's race; and (iii) region of residence.

Mothers were asked to recall and report the following perinatal information: (i) age of maternal menarche; (ii) parental weight and height from which body mass index (BMI) was calculated and used to categorize each parent into normal weight, overweight, and obese on the basis of International Obesity TaskForce (IOTF) cut-off points (17); (iii) maternal weight before pregnancy and weight gained during pregnancy based on the classification recommended by the Institute of Medicine (IOM) (18); (iv) type of conception, i.e., natural conception or *in vitro* fertilization; (v) smoking habits during pregnancy; (vi) use of nutritional supplements during pregnancy; (vii) medical history of gestational diabetes mellitus and high blood pressure; (viii) type of delivery (normal vs. cesarean); (ix) parity; (x) children's feeding practices from birth to 6 months of age, i.e., exclusive breastfeeding vs. use of formula or mixed feeding, age at which formula was introduced, and weaning age; and (xi) children's growth rate during the first 6 months of life. Birth weight and gestational age were recorded from each child's birth certificate and medical record and were used for the classification of children into small for gestational age (SGA, <10th percentile), appropriate for gestational age (AGA, 10th–89th percentile), and large for gestational age (LGA, ≥90th percentile).

Anthropometry and physical examination

Each anthropometric measure was taken twice by two trained members of the research team. A standard protocol and equipment were used in all schools. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany). Children were weighed without shoes and in the minimum possible clothing. Height was measured to the nearest 0.1 cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd., Oadby, Leicestershire, UK) with the pupil standing still, barefoot, keeping shoulders in a relaxed position, arms hanging freely, and head aligned in the Frankfort plane. BMI was calculated from weight and height using Quetelet's equation [weight (kg)/height^2 (m$^{2)}$]. Pubertal stage of all participants was assessed by a female pediatrician in each prefecture. Children were classified according to the five Tanner stages after visual inspection of breast development in girls and genital development in boys (19).

Biochemical analyses

Early morning blood samples were taken after a 12-h fast. Both parents and children were provided with written reminders from the previous day to ensure compliance with children's fasting. Plasma glucose was determined using commercially available enzymatic colorimetric assays (Roche Diagnostics SA, Vassilia, Switzerland). Serum insulin was determined by a Chemiluminescence immunoassay (Kyowa Medex Ltd, Minami-Ishiki, Japan, for Siemens Diagnostics USA). IR was measured through homeostasis model assessment (HOMA-IR) (20). This index was calculated using fasting glucose (FG) and fasting insulin (IF), as follows: HOMA-IR = (IF (μunits/mL) × FG (mmol/L)/22.5. HOMA-IR > 3.16 (21)] was used as a cut-off point to define insulin-resistant schoolchildren. HOMA-IR has been validated as a surrogate measure of IR in non-diabetic children, with studies showing correlations as high as 0.91 with the hyperinsulinemic–euglycemic clamp, a frequently sampled intravenous glucose tolerance test (22).

Statistical analysis

All variables used in the current analysis were categorical, except for maternal menarche age, which is continuous. To test the effect of the factors under investigation on the occurrence of IR, univariate logistic regression analyses were performed. Furthermore, multivariable logistic regression analysis was performed including all variables that were significantly associated with IR at a univariate level, also controlling for children's BMI and Tanner stage. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) were derived from the univariate and the multivariable regression models. The Statistical Package for Social Sciences (SPSS), version 17.0, was used for all data analyses. The level of statistical significance was set at $p < 0.05$.

Results

The sample consisted of 2195 children attending the fifth and sixth grades of primary school with full perinatal, family sociodemographic, and biochemical data. Table 1 presents the main descriptive characteristics of the study population. The mean age of the study participants was 11.2 ± 0.67 yr and the overall prevalence of IR was 28.4%. Regarding sex differences, girls were found to have significantly lower mean BMI and FG levels but higher mean levels of height, circulating insulin, and HOMA-IR compared with boys ($p < 0.05$). Furthermore, girls had a much higher prevalence of IR than boys (34.2 vs. 22.7%, $p < 0.05$), while more girls than boys were at the higher Tanner stages of 3–5 ($p < 0.05$).

The univariate logistic regression analysis in Table 2 presents the associations between IR and certain parental and family socioeconomic and demographic characteristics. Further to the increased odds for IR observed in girls compared with boys (OR:1.77; 95% CI: 1.46–2.13), non-Caucasian children and children whose mothers were obese were 2.15 (95% CI: 1.19–3.88) and 2.02 (95% CI: 1.52–2.67) times

Table 1. Descriptive characteristics of the study participants presented by gender

	Boys (n = 1097) n (%)	Girls (n = 1098) n (%)	Total (100%) (n = 2195) n (%)
Insulin resistance (HOMA-IR < 3.16)	249 (22.7)	375 (34.2)*	624 (28.4)
Tanner stage			
Stage 1	480 (43.8)	227 (20.7)*	707 (32.2)
Stage 2	483 (44.0)	432 (39.3)*	915 (41.7)
Stage 3	112(10.2)	294 (26.9)*	406 (18.5)
Stage 4	21 (1.9)	117 (10.6)*	138 (6.3)
Stage 5	0 (0.0)	28 (2.6)*	28 (1.3)
	Mean ± SD	Mean ± SD	Mean ± SD
Age (yr)	11.2 ± 0.66	11.2 ± 0.68	11.2 ± 0.67
Weight (kg)	45.5 ± 11.1	45.2 ± 10.7	45.4 ± 10.9
Height (cm)	148.3 ± 7.4	149.3 ± 8.1†	148.8 ± 7.8
BMI (kg/m^2)	20.5 ± 3.9	20.1 ± 3.7†	20.3 ± 3.8
Glucose (mg/dL)	93.5 ± 10.3	91.2 ± 9.1†	92.4 ± 9.8
Insulin (μU/mL)	10.8 ± 8.2	13.1 ± 7.3†	11.9 ± 7.8
HOMA-IR	2.5 ± 2.0	2.9 ± 1.7†	2.7 ± 1.9

* Significantly different from boys (chi-squared test).
† Significantly different from boys (Student's t-test).

more likely of having IR compared with Caucasian children and children with normal-weight mothers, respectively. On the contrary, children whose fathers had a higher educational level of 12–16 and >16 yr of education were 0.77 (95% CI: 0.59–0.99) and 0.66 (95% CI: 0.47–0.92) less likely, respectively, of having IR, than children whose father was less educated (i.e., having <9 yr of education). Similarly, children whose families had an annual income of 12 000–30 000 € and >30 000 € had decreased ORs for IR [0.75 (95% CI: 0.59–0.95) and 0.63 (95% CI: 0.48–0.83)], respectively.

Table 3 presents the perinatal characteristics of the study population and their univariate associations with IR. Increased likelihood for IR was observed for children whose mothers were overweight (OR: 1.33; 95% CI: 1.02–1.72) or obese (OR: 2.44; 95% CI: 1.60–3.73) before pregnancy and were smoking during the first trimester of their pregnancy (OR: 1.97; 95% CI: 1.08–3.61). Similar positive associations were observed for children who were born SGA (OR: 1.48; 95% CI: 1.13–1.95) and had a rapid weight gain during their first 6 months of life (OR: 1.46; 95% CI: 1.20–1.79). On the contrary, the likelihood of IR was lower for children whose mothers had a later menarche (OR: 0.92; 95% CI: 0.86–0.98), were underweight before pregnancy (OR: 0.62; 95% CI: 0.41–0.95), and used folic acid supplements during the second (OR: 0.73; 95% CI: 0.58–0.93) and third trimester (OR: 0.72; 95% CI: 0.56–0.91) of pregnancy.

Table 4 summarizes the results from the multivariable logistic regression analysis that included all perinatal, parental, and sociodemographic variables that were significantly associated with IR at a univariate level, also controlling for children's BMI and Tanner Stage. Regarding socioeconomic and demographic correlates female sex increased the likelihood of IR by 1.67 (95% CI: 1.30–2.13) compared with males, while higher annual family income of 12 000–30 000 € and >30 000 € were associated with a lower likelihood for IR in children (OR: 0.71; 95% CI: 0.53–0.95 and OR: 0.68; 95% CI: 0.48–0.96, respectively) compared with the lower annual family income of <12 000 €. Regarding perinatal predictors of IR, children born SGA and those born LGA were 1.41 (95% CI: 1.03–2.01) and 0.54 (95% CI: 0.34–0.87) more and less likely, respectively, of being insulin resistant.

Discussion

This study revealed a relatively high prevalence of IR (28.4%) among 9- to 13-yr-old schoolchildren (Table 1). Previous epidemiologic studies conducted in the USA (23) and Italy (24) showed that weight status had a strong impact on the prevalence of IR in adolescents. More specifically, in the first study, the prevalence of IR was 3.1, 15, and 52.1% in normal weight, overweight, and obese adolescents, respectively (using HOMA-IR > 4.39 as the threshold), while in the second study IR was present in 3 and 40.8% of normal weight and obese subjects, respectively (using HOMA-IR > 2.5 as the threshold). All aforementioned findings are indicative of a direct positive association between children's BMI and HOMA-IR levels, already confirmed by earlier studies (25). In this context, the considerably high prevalence of overweight and obesity (29.7 and 11.3%, respectively) recently reported for the current population (26) could provide the basis for the high prevalence of IR observed in this study. However, as there is no widely acceptable definition for IR, comparisons of prevalence data reported in the current and by other studies are not feasible (27).

The association between several parental, socioeconomic, and demographic indices with the prevalence of IR in children was tested in this study by conducting multiple univariate logistic regression analyses (Table 2). The univariate analyses revealed several significant correlates of IR in children, with female sex, non-Caucasian race, and maternal overweight increasing the likelihood of IR by 1.77, 2.15, 1.29 times respectively. On the other hand, higher paternal educational level (i.e., >16 yr) and higher annual family income (i.e., > 30 000 €/yr) decreased the likelihood of IR by 0.66 and 0.63 times respectively. Although these associations have also been reported in earlier studies (28), in our study, most of these associations lost their

Table 2. Crude odds ratios (95% confidence intervals) for the association of parental, demographic, and socioeconomic characteristics with insulin resistance

		Odds ratio (95% confidence interval)	
	Cases (% of total)	Insulin resistance (dependent variable)	p-value
Race			
Caucasians	2149 (97.9)	1.00	
Non-Caucasians	46 (2.1)	**2.15 (1.19–3.88)**	0.011
Urbanity			
Metropolitan	1472 (67.1)	1.00	
Semi-urban	326 (14.9)	0.83 (0.64–1.06)	0.145
Rural	397 (18.1)	1.13 (0.90–1.42)	0.277
Father's BMI			
Normal-weight	565 (25.8)	1.00	
Overweight	1182 (53.9)	1.13 (0.90–1.42)	0.281
Obese	448 (20.4)	1.29 (0.97–1.70)	0.075
Mother's BMI			
Normal-weight	1321 (60.2)	1.00	
Overweight	615 (28.0)	**1.29 (1.05–1.61)**	0.018
Obese	259 (11.8)	**2.02 (1.52–2.67)**	<0.001
Father's age			
<42 yr	828 (37.7)	1.00	
42–46 yr	713 (32.5)	0.97 (0.78–1.21)	0.795
>46 yr	654 (29.8)	0.95 (0.75–1.19)	0.641
Mother's age			
<38 yr	856 (39.0)	1.00	0.885
38–42 yr	740 (33.7)	1.02 (0.82–1.27)	0.453
>42 yr	599 (27.3)	1.09 (0.87–1.38)	
Family status			
Two-parent families	1971 (89.8)	1.00	
Single-parent families	224 (10.2)	1.27 (0.94–1.71)	0.123
Nationality			
Greek	1864 (84.9)	1.00	
Non-Greek	331 (15.1)	0.84 (0.66–1.09)	0.191
Paternal education			
<9 yr	560 (25.5)	1.00	
9–12 yr	832 (37.9)	0.80 (0.63–1.01)	0.057
12–16 yr	544 (24.8)	**0.77 (0.59–0.99)**	0.045
>16 yr	259 (11.8)	**0.66 (0.47–0.92)**	0.015
Maternal education			
<9 yr	454 (20.7)	1.00	
9–12 yr	867 (39.5)	0.89 (0.69–1.14)	0.363
12–16 yr	687 (31.3)	0.79 (0.61–1.03)	0.086
>16 yr	187 (8.5)	0.73 (0.49–1.07)	0.106
Family income (€/yr)			
<12 000	494 (22.5)	1.00	
12 000–30 000	1104 (50.3)	**0.75 (0.59–0.95)**	0.018
>30 000	597 (27.2)	**0.63 (0.48–0.83)**	0.001
Mother's current employment status			
Unemployed	720 (32.8)	1.00	
Employed	1475 (67.2)	0.94 (0.77–1.14)	0.531
Household size (m^2/family member)			
<20	834 (38.0)	1.00	0.192
20–25	577 (26.3)	0.85 (0.66– 1.09)	
25–30	406 (18.5)	1.13 (0.87–1.48)	0.362
>30	378 (17.2)	1.06 (0.81–1.40)	0.657
School's socioeconomic level			
Low	571 (26.0)	1.00	
Medium	742 (33.8)	0.82 (0.65–1.05)	0.118
High	882 (40.2)	0.90 (0.71–1.13)	0.360

Figures in bold indicate statistically significant odds ratios.

Table 3. Crude odds ratios (95% confidence intervals) for the association of perinatal factors with insulin resistance

		Odds ratio (95% confidence interval)	
	Cases (% of total)	Insulin resistance (dependent variable)	p-value
Gender			
Boy	1097 (50.0)	1.00	
Girl	1098 (50.0)	**1.77 (1.46–2.13)**	<0.001
Maternal menarche age (yr)	2195 (100)	**0.92 (0.86–0.98)**	0.016
Type of conception			
Physiologic	2135 (97.3)	1.00	
In vitro fertilization (IVF)	60 (2.7)	0.91 (0.51–1.63)	0.759
Mothers' pre-pregnancy weight status			
Normal-weight	1643 (74.4)	1.00	
Underweight	153 (7.0)	**0.62 (0.41–0.95)**	0.027
Overweight	316 (14.4)	**1.33 (1.02–1.72)**	0.032
Obese	92 (4.2)	**2.44 (1.60–3.73)**	<0.001
*Gestational weight gain**			
Within IOM recommendation	717 (32.7)	1.00	
Below IOM recommendation	768 (35.0)	0.88 (0.70–1.10)	0.264
Above IOM recommendation	710 (32.3)	0.99 (0.79–1.24)	0.910
Maternal smoking during pregnancy			
Not smoking	1847 (84.1)	1.00	
Smoking first trimester	44 (2.0)	**1.97 (1.08–3.61)**	0.028
Smoking second and third trimester	58 (2.6)	0.75 (0.40–1.40)	0.366
Smoking first and second trimester	18 (0.8)	0.99 (0.35–2.81)	0.996
Smoking all trimesters	228 (10.4)	1.22 (0.91–1.64)	0.996
High blood pressure during pregnancy			
No	2071 (94.4)	1.00	
Yes	72 (3.3)	1.45 (0.89–2.37)	0.138
Not known	52 (2.4)	1.25 (0.69–2.24)	0.464
Diabetes mellitus during pregnancy			
No	2082 (94.9)	1.00	
Yes	56 (2.6)	1.21 (0.69–2.14)	0.507
Not known	57 (2.6)	1.49 (0.86–2.58)	0.151
Use of folic acid: first pregnancy trimester			
No	1845 (84.1)	1.00	
Yes	350 (15.9)	0.88 (0.68–1.14)	0.333
Use of folic acid: second pregnancy trimester			
No	1716 (78.2)	1.00	
Yes	479 (21.8)	**0.73 (0.58–0.93)**	0.011
Use of folic acid: third pregnancy trimester			
No	1747 (79.6)	1.00	
Yes	448 (20.4)	**0.72 (0.56–0.91)**	0.006
Gestational age (wk)			
<37	413 (18.8)	1.00	
≥37	1782 (81.2)	0.80 (0.635–1.00)	0.059
Parity			
Uniparous	1075 (49.0)	1.00	0.677
Multiparous	1120 (51.0)	0.96 (0.80–1.16)	
Birth weight for gestational age			
Appropriate (10th–89th percentile)	1769 (80.6)	1.00	
Small (<10th percentile)	263 (12.0)	**1.48 (1.13–1.95)**	0.004
Large (>90th percentile)	163 (7.4)	0.68 (0.46–1.01)	0.054
Type of delivery			
Normal	1554 (70.8)	1.00	
Cesarean	641 (29.2)	1.04 (0.85–1.28)	0.694
Weight gain in the first 6 months			
Average (−1 to +1 z-score change)	1248 (56.9)	1.00	
Poor (< −1 z-score change)	231 (10.5)	0.86 (0.62–1.20)	0.381
Rapid (> +1 z-score change)	716 (32.6)	**1.46 (1.20–1.79)**	<0.001
Breast feeding			
Not exclusive	2010 (91.6)	1.00	
Exclusive	185 (8.4)	0.82 (0.58–1.16)	0.262
Time of solid food initiation			
≤4 months	378 (17.2)	1.00	0.937
5–6 months	1466 (66.8)	1.01 (0.79–1.30)	0.976
>6 months	351 (16.0)	1.00 (0.72–1.37)	

Figures in bold indicate statistically significant odds ratios.
* Based on recommendations by the Institute of Medicine (18).

Table 4. Adjusted* odds ratios (95% confidence intervals) for the association of certain parental anthropometric, demographic, socioeconomic, and perinatal factors with insulin resistance

	Odds ratio (95% confidence interval)	
	Insulin resistance	p-value
Maternal menarche age (yr)	1.01 (0.93–1.09)	0.864
Race		
Caucasians	1.00	
Non-Caucasians	1.68 (0.81–3.46)	0.161
Mother's BMI		
Normal weight	1.00	
Overweight	0.96 (0.73–1.26)	0.767
Obese	0.93 (0.60–1.44)	0.750
Paternal education		
<9 yr	1.00	0.088
9–12 yr	0.76 (0.52–1.06)	0.964
12–16 yr	0.99 (0.70–1.41)	0.329
>16 yr	0.78 (0.48–1.28)	
Maternal education		
<9 yr	1.00	0.224
9–12 yr	1.22 (0.89–1.68)	0.449
12–16 yr	1.15 (0.80–1.65)	0.638
>16 yr	1.14 (0.65–2.01)	
Family income (€/yr)		
<12 000	1.00	0.020
12 000–30 000	**0.71 (0.53–0.95)**	0.029
>30 000	**0.68 (0.48–0.96)**	
Gender		
Boy	1.00	
Girl	**1.67 (1.30–2.13)**	<0.001
Mothers' pre-pregnancy weight status		
Normal-weight	1.00	
Underweight	0.79 (0.49–1.23)	0.360
Overweight	0.99 (0.69–1.43)	0.965
Obese	1.66 (0.90–3.04)	0.103
Maternal smoking during pregnancy		
Not Smoking	1.00	
Smoking first trimester	1.65 (0.77–3.53)	0.195
Smoking second and third trimester	0.57 (0.27–1.20)	0.136
Smoking first and second trimester	1.15 (0.35–3.76)	0.821
Smoking all trimesters	0.88 (0.61–1.26)	0.474
Use of folic acid: second pregnancy trimester		
No	1.00	
Yes	0.86 (0.52–1.41)	0.550
Use of folic acid: third pregnancy trimester		
No	1.00	
Yes	0.92 (0.55–1.53)	0.748
Birth weight for gestational age		
Appropriate (10th–89th percentile)	1.00	
Small (<10th percentile)	**1.41 (1.03–2.01)**	0.048
Large (>90th percentile)	**0.54 (0.34–0.87)**	0.012
Weight gain in the first 6 months		
Average (−1 to +1 z-score change)	1.00	
Poor (< −1 z-score change)	0.97 (0.65–1.44)	0.869
Rapid (> +1 z-score change)	1.08 (0.83–1.40)	0.563

Figures in bold indicate statistically significant odds ratios.
* Adjusted for children's BMI and Tanner stage.

statistical significance when they were tested at a multivariable level. Female sex and higher (12 000–30 000 € and >30 000 €) annual family income were the two main sociodemographic indices that were independently associated with IR at a multivariable level, as the former increased the odds of IR by 1.67 times whereas the latter decreased the odds of IR by 0.71 and 0.68 times respectively (Table 4).

While the increased odds for IR in girls compared with boys could be attributed to their earlier pubertal development and the subsequent hormonal changes, still adjustments made for Tanner stage did not weaken the statistical significance of this association (29). The sex difference in IR could not be explained by differences in individual anthropometric variables, as compared with girls, boys had significantly greater BMI. Sex-linked genes may account for the intrinsic sex difference observed and such genes may have an important impact on the development of IR (30). Regarding the association reported in the current study between lower annual family income and IR in children, there is general consensus in social and health sciences of an increased risk for obesity and related metabolic disorders, including IR, in children of lower socioeconomic status in several developed countries (31). Lower socioeconomic status (SES) of the family most commonly defined as lower family income or parental educational level has been reported to be positively related to adverse health behaviors followed by children, including higher consumption of high energy and glycemic index foods and beverages, such as fats, sweets, sugar-sweetened beverages etc. (32–34). Furthermore, mothers of lower SES could be less aware of healthy practices during their child's early development, thus increasing the odds for smoking and excess weight gain during pregnancy, formula- instead of breast-feeding of their baby, etc.

The univariate analyses revealed several statistically significant associations between IR in children and certain perinatal risk factors (Table 3). However, at a multivariable level and further to female sex, small birth weight for gestational age was the only independent perinatal risk factor of IR, increasing the likelihood of IR by 1.41 times (Table 4). This association has been repeatedly confirmed by other previous prospective and cross-sectional studies with retrospectively collected data as the present one (13, 35, 36). On the other hand, a negative association was observed in the current study between large birth weight and IR in children, as children born LGA were 0.46 times less likely to develop IR in their late childhood. Taking the above into account, there are several studies reporting a 'U'-shaped relationship between birth weight and childhood overweight, thus suggesting a more complex association between fetal size at birth and metabolic disorders in later life (37). Regarding the loss of the statistical significance of the associations between other perinatal factors and IR in children, this could be attributed to the mediating effect of BMI and Tanner stage (38). In this context, early exposures of the fetus and the infant to several pre- and postnatal factors have been reported to produce a number of physiological and metabolic epigenetic adaptations of a seemingly permanent nature in the child that can adversely affect satiety and hunger cues and consequently increase the odds of overweight/obesity (39, 40). Because biological maturation and puberty come earlier in overweight and obese children (41), the hormonal changes induced during the transitional life stage of preadolescence could be another important factor partially mediating the direct associations between perinatal factors and IR in late childhood.

The findings of the present study should be interpreted in the context of its limitations and strengths. Its cross-sectional design represents its main limitation, as it cannot provide cause-and-effect relations. The interpretation of the current results should be limited to descriptive purposes and further prospective studies and/or clinical trials, whenever appropriate, need to be conducted in order to understand the causality of relations between the aforementioned factors and IR. Moreover, parental anthropometric data (i.e., weight and height) were self-reported, while children's perinatal data were collected retrospectively by asking parents and using pediatric medical records. Regarding strengths, the Healthy Growth Study was a large-scale, epidemiological study covering the central, northern, southern, and western parts of the Greek territory, thus providing sufficient representativeness.

In conclusion, this study identified small birth weight and female sex as the only perinatal factors independently associated with the occurrence of IR in late childhood, when examined at a multivariable level with a wide range of perinatal indices, as well as certain family sociodemographic and parental characteristics. Considering that fetal size at birth is known to reflect the product of the nutritional and hormonal milieu in which the fetus develops, controlling the trajectory of fetal growth via optimization of maternal nutrition and health status during pregnancy seems fundamental (42). As this study also highlighted low family income as the only sociodemographic factor associated with IR in late childhood, early prevention initiatives should mainly target lower SES families, where the problem is and thus the need for intervention is bigger.

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PAPER IX

Revision of the Healthy Lifestyle-Diet Index and association of the revised index with obesity and iron deficiency in schoolchildren: The Healthy Growth study

Yannis Manios[1,*], George Moschonis[1], Euthimia Politidou[1], Anthi Naoumi[1], Georgia Kourlaba[2], Dimitris Peppas[1], Christos Lionis[3] and George P. Chrousos[4], on behalf of the "Healthy Growth Study" group.

[1]Department of Nutrition & Dietetics, Harokopio University of Athens, Greece.

[2]Department of Health Services Management, National School of Public Health, Athens, Greece.

[3]Clinic of Social and Family Medicine, School of Medicine, Heraklion, University of Crete, Crete, Greece;

[4]First Department of Pediatrics, Athens University Medical School, Aghia Sophia"Children's Hospital, Athens, Greece;

[*]**Corresponding author:**

Dr Yannis Manios, Assistant Professor,

Harokopio University of Athens,

Department of Nutrition and Dietetics,

70, El.Venizelou Ave,

17671 Kallithea,

Athens-Greece.

Tel: +30 210 9549156.

Fax: +30 210 9514759.

E-mail: manios@hua.gr

Abstract

Purpose: To revise the Healthy Diet-Lifestyle Index (HDL-index) in order to more accurately assess the degree of adherence to existing dietary and lifestyle guidelines for school-aged children and to examine the association of the revised HDL-index (R-HDL-index) with obesity and iron deficiency (ID).

Methods: A representative sample of 2660 primary schoolchildren (9-13 years old) participating in the "Healthy Growth Study" was examined. Twelve components related to dietary and lifestyle patterns were used in order to develop the R-HDL-index. Scores from 0 to 4 were assigned to all components. The R-HDL-index total score ranged between 0 and 48.

Results: The overall mean score of the R-HDL-index was 17.1±4.5. Higher R-HDL-index scores were associated with lower proportion of children being obese. More specifically, logistic regression analysis showed that for every 1/48 unit increase in the R-HDL-index score the likelihood of childhood obesity decreased by 6%. However there were no significant associations between the R-HDL-index and ID.

Conclusions: The R-HDL-index may be a useful tool for public health policy makers and other health professionals in order to assess diet quality and lifestyle patterns of primary schoolchildren. Furthermore higher scores of this index are associated with an increased likelihood for childhood obesity but not with ID.

Keywords: Primary schoolchildren, Diet, Lifestyle patterns, Obesity, Iron deficiency

Introduction

Increasing obesity prevalence is of major global health concern [1], while at the same time iron deficiency (ID) is still considered the most common nutrient deficiency worldwide [2]. Unhealthy dietary and other lifestyle patterns have been reported as significant risk factors for both childhood obesity and ID [3]. More specifically, the positive energy balance, brought by increased consumption of energy-dense, high-fat and low-fiber foods in combination with low physical activity levels, is the main risk factor of childhood obesity. In this context higher consumption of foods with an increased content of nutrients that inhibit intestinal absorption of dietary non-heme iron (e.g. phytates in fruits and vegetables, calcium in dairies, polyphenols in tea and coffee) combined with lower consumption of foods rich in nutrients that enhance dietary non-heme iron bioavailability (e.g vitamin C in citrus fruits) has strongly been implicated in the aetiology of ID in children [4]. Furthermore, the chronic inflammatory state induced by obesity per se has been identified as an independent risk factor of ID [5].

Considering the above, the development of a practical tool that will provide a quantitative measure of such patterns, also applicable in assessing the risk for both obesity and ID seems very important from a public health perspective. The development of an index appropriate for this purpose could be one such approach. To the best of our knowledge, the only composite indices that aim to evaluate the overall diet quality and are applicable to schoolchildren and adolescents are the Youth Healthy Eating Index, the Mediterranean Diet Quality Index and E-KINDEX [6-9]. However, some of these indices are not easily applicable because they require the estimation of specific nutrients' intake as their components. Moreover, lifestyle characteristics have not been included as index components in none of the aforementioned existing indices that are applicable to this particular age group. The authors of the present study have previously developed the Healthy Lifestyle-Diet Index (HLD-index) to evaluate the degree of adherence to existing dietary and lifestyle guidelines for school-aged children from the USDA's My Pyramid (www.mypyramid.gov) and reported significant associations with insulin resistance [10].

The aim of this work was to revise the HDL-index in order to more accurately assess the degree of children's adherence to existing age-appropriate dietary and lifestyle guidelines based on USDA's Choose my Plate (www.choosemyplate.gov) and to examine the association of the revised HDL-index (R-HDL-index) with obesity and ID.

Methods

Sampling

The 'Healthy Growth Study' was a cross-sectional epidemiological study initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens. The study population comprised schoolchildren attending the 5th and 6th grades of primary schools located in municipalities within the wider regions of Attica, Aitoloakarnania, Thessaloniki and Heraklion. The sampling procedure is fully described in a previous study [11]. In brief, the sampling of schools was random, multistage and stratified by parental educational level and by the total population of students attending schools within these municipalities. An extended letter explaining the aims of the present study and a consent form for conducting full measurements were provided to all parents or guardians having a child in these schools. Signed parental consent forms were collected for 2660 out of 4145 children (response rate 64.2 %).

Anthropometry

Weight was measured without shoes in light clothes using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany) to the nearest 0.1 kg. Height was measured to the nearest 0.1cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Oadby, UK) with the participants not wearing shoes, their shoulders in a relaxed position, their arms hanging freely and their head in Frankfort horizontal plane. Two trained members of the research team carried out these measurements.

Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). The anthropometric categorization of children was made according to International Obesity Taskforce (IOTF) [12,13] and children were divided into four groups: underweight, normal, overweight and obese.

Dietary intake

Dietary intake data were obtained for two consecutive weekdays and one weekend day using 24-h recalls. Food intake data were analyzed using the Nutritionist V diet analysis software (version 2.1, 1999, First Databank, San Bruno, CA, USA), which was extensively amended to include traditional Greek foods and recipes, as described in Food Composition Tables and Composition of Greek Cooked Food and Dishes [14]. The food-grouping scheme was designed for all foods or entries (core and recipe) appearing in Nutritionist V. Forty-seven food groups were initially established, based on similar source characteristics and nutrient content. Composite food items, such as recipes, were de-composed and were assigned to food groups according to primary ingredients. A similar methodology for the extraction of food groups has been previously reported in studies with not only smaller sample size, but also only one 24-h recall available [15]. Examples of foods included in the food groups have been documented previously [16].

Other characteristics that were recorded

Children's TV/video viewing and computer games playing time was assessed by children's report with regard to their TV/video viewing time and time playing computer games during a usual weekday and a usual weekend. The mean daily TV/video viewing and computer games playing time was calculated using the following equation: daily TV/video viewing and computer games playing hours =((weekday TV/ video viewing and computer games playing hours x 5) + weekend TV/video viewing and computer games playing hours)/7. Physical activity during leisure time was assessed using a standardized activity interview, based on a questionnaire completed by the participants in the presence of a member of the research team. Further details regarding the reliability and validity of the questionnaire are provided elsewhere [17]. Respondents reported the time spent on various physical activities on two weekdays and one weekend day, most preferably Sunday. Reported activities were grouped by a member of the research team into moderate-to-vigorous physical activities (MVPA) (intensity higher than 4 metabolic equivalents), including activities such as brisk walking, bicycling, gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, jumping rope and general participation in active outdoors games. Given the age group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity.

Biochemical analysis

Blood samples were obtained for biochemical and haematological screening tests between 08.30 and 10.30 hours after a 12 h overnight fast. Reminders were distributed the previous day to both parents and children in order to ensure compliance with fasting. Professional staff performed venipuncture, using two types of test tubes, one of which contained EDTA, to obtain a maximum of 10 ml blood. The remaining blood was collected in plain test tubes for the preparation of serum, which was divided into aliquots and stored at -80^{0}C. When blood collection was completed in Aitoloakarnania, Thessaloniki and Heraklion, all serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where biochemical analyses and central storage of back-up samples at 80^{0}C took place. Serum iron and total iron-binding capacity (TIBC) levels were determined by colorimetric assays (Roche Diagnostics SA, Basel, Switzerland). Transferrin saturation (TS) was calculated by dividing serum iron levels by TIBC and multiplying by 100. Iron deficiency (ID) was defined using the following age- and sex-specific thresholds proposed by UNICEF and the WHO [18]: ID was defined as TS $<$ 16 %. The Mentzer Index (MCV (fl)/RBC (M/ml)) [19] was also calculated for all pupils participating in the present study to differentiate beta-thalassemia from ID. On the basis of this index, children with thalassemia minor (eighteen cases) were excluded from further analysis.

Component selection for diet index development

The revised HLD-index (R-HLD-index) resulted from the modification of the HDL-index [10], in an attempt to increase its accuracy in the estimation of the degree of children's adherence to available dietary and lifestyle guidelines. For the development of the R-HLD-index, 12 components were used. The first 10 components measure the frequency consumption of fruits, vegetables, grains, milk/dairy products, meat and meat products, fish/seafood, legumes, eggs, soft drinks and sweets. The other two components reflect the physical activity status of children through measuring the time children spend watching television (TV) or playing computer games (sedentary life) and the time children spend on moderate to vigorous physical activity (MVPA). These components were selected based on dietary recommendations from USDA's ChooseMyPlate (www.choosemyplate.gov) and the recommendations of American Academy of Pediatrics with regard to the TV viewing time [20,21].

Scoring system for the development of R-HLD-index

A five-point scoring system (0–4) was used to assign the appropriate score to each index component. The highest score was ascribed to that dietary intake or participation in moderate to vigorous activity or watching TV being closed to the recommended guidelines, while the lowest score was assigned to the above dietary-lifestyle behaviours being far from the recommendations [www.choosemyplate.gov, 20,21]. Proportionally, the intermediate scores were derived based on the recommendations. Regarding fruit intake, score 4 was assigned to a frequency of consumption ranging from 1 to 3 servings per day containing <50% juice and as for vegetables to a consumption frequency from 2-3 servings per day. With regards to grains, score 4 was assigned to the consumption of 4-6 servings per day, with more than half being whole grains. As concerns milk/dairy consumption, score 4 was assigned to 2-4 servings per day with >50% of them being fat-free or low-fat dairy products. In terms of legumes and eggs preferable a consumption of 3-5 and 2-3 servings per week, respectively, received the highest score of 4. Moreover, with regard to meat component, score 4 was assigned to a consumption frequency of 2-3 servings per week. The other components were scored in the same way as in the original HLD-index [10]. More details for the scores assigned to each component are presented in Table 1.

The total score of the R-HLD-index was obtained by summing the scores assigned to each index component. The values of this index range between 0 and 48. Higher values of the R-HLD-index indicate greater adherence to dietary–lifestyle recommendations or otherwise greater adherence to a ‘healthy’ dietary–lifestyle pattern. Then, primary schoolchildren were divided into three groups using the tertiles of R-HLD-index as follows: those considered as having (a) an ‘unhealthy diet–lifestyle pattern’ (1st tertile); (b) a ‘moderate healthy diet–lifestyle pattern’ (2nd tertile); and (c) a ‘healthy diet–lifestyle pattern’ (3rd tertile).

Statistical analysis

Continuous variables are presented as mean ± standard deviation and categorical variables are summarized as relative frequencies (%). Associations between categorical variables were tested by using the x^2 test and the two-sample z-test for proportions whenever appropriate. The associations between the continuous and binary variables (sex) were evaluated through Student’s t-test or Mann-Whitney test when continuous variables were normally distributed or skewed, respectively. Comparisons between continuous variables (food groups’ consumption) and the tertiles of the R-HLD-index were performed using the one-way analysis of variance, after testing for equality of variances or the Kruskal-Wallis test, as appropriate. Bonferroni correction was used to account for increase in type I error owing to multiple comparisons.

Multiple logistic regression models were used to assess the association between the HLD-index score (either as a quantitative variable or as a categorical variable with three categories based on its tertiles), obesity and iron deficiency.

Participants' gender, age, Tanner stage, total energy intake, as well as body mass index category were used as potential confounders for the association between R-HLD-index score and iron deficiency, whereas age, sex, Tanner stage, total energy intake, birth weight, mother's body mass index category and moderate to vigorous physical activity were used as potential confounders for the association between R-HLD-index score and obesity. The results are presented as odds ratios and 95% confidence interval. The Statistical Package for Social Sciences (SPSS) Version 17.0 was used for data analysis. The level of statistical significance was set at $p<0.05$.

Results

The weight and iron status prevalence data of the study participants are presented in Table 2. In total, 2660 children were recruited from 10 to 12 years of age. Overall, the observed prevalence was 3.1 % for underweight, 55.1 % for normal-weight, 30.3 % for overweight and 11.5 % for obesity. Table 2 also summarizes the differences in the prevalence of underweight, normal weight, overweight and obesity among boys and girls. Specifically, the prevalence of obesity was found to be significantly higher in boys than girls (13.6% *v.* 9.4%, p=0.001). It was also found that 15.7% of children under study had iron deficiency with no significant difference observed between sexes (i.e. 16.9% in boys and 15.7% in girls).

The prevalence of iron deficiency by weight group is shown in Table 3. The prevalence of ID was found to be higher in obese children compared to their overweight and under/normal-weight peers (p=0.004).

Table 4 illustrates descriptive characteristics of the R-HLD-index among all schoolchildren of the Healthy Growth Study per sex and weight group, separately. The overall mean ± standard deviation of the R-HLD-index score was 17.12 + 4.5 and this score was normally distributed. No statistically significant difference was seen in the R-HLD-index score between boys and girls (p=0.572) as well as between children with normal iron status and iron deficiency (p=0.462). However, there were significant differences in the score among normal, overweight and obese children (p=0.015) with obese children having significantly lower mean score compared to under/normal-weight children.

The mean ± standard deviation of the portions consumed from several food groups and of the time spent on TV viewing and MVPA by the tertiles of the R-HLD-index are depicted in Table 5. The mean consumption of fruits, vegetables, fishes and seafood, dairy (full-fat, low-fat, skimmed), whole grains, legumes and the mean daily time spent on MVPA per week was significantly higher among children in the 3rd tertile of the index compared with the rest of children (P<0.001). On the other hand consumption of sweets and soft drinks as well as time spent on TV viewing was significantly lower for children in the higher R-HLD-index tertile (P<0.001).

Logistic regression analysis showed that for every 1/48 unit increase in the R-HDL-index score the likelihood of childhood obesity decreased by 6%. Moreover, the likelihood of being obese was 44% lower among children with higher R-HDL-index score (3rd tertile) compared with children with lower score (i.e. 1st tertile) (Table 6). No significant associations were observed between R-HDL-index and ID.

Discussion

The current study tried to revise an index that was initially constructed for assessing the degree of children's adherence to existing dietary and lifestyle guidelines (i.e. the HLD-index) and to assess the association of the revised index (i.e. R-HLD-index) with obesity and ID. The main differentiations derived from the revision of the HLD-index involved changes in the scoring of certain components, as well as the addition of new components i.e. the consumption frequency of legumes and eggs. These changes were required because dietary recommendations for kids, based on which the original HLD-index had been developed [10], were updated, recently (www.choosemyplate.gov). Changes to the scoring algorithm of index components were conducted to the following components: fruit, vegetables, grains, milk/dairy and meat /meat products. Regarding fruits consumption, fruit juice was also included in the scoring with children consuming more frequently fruits but less frequently fruit juice receiving higher scores than children consuming more fruit juice. This change was mainly due to the positive associations observed between fruit juice consumption and childhood obesity by several studies conducted with children [22]. Another difference between the two indices was noticed in the scoring of vegetables consumption with 2-3 servings per day representing the ideal consumption according to the new index as opposed to >4 servings per day set in the previous index. The reason for this change was to follow the updated guidelines in which the daily recommendation for vegetables consumption is set to 1.5 cup. Vegetables contain among others fiber, known for its health benefits [23]. However, there is some concern that a high intake of fiber may impair the absorption of minerals, like iron [24].

Moreover, a simplification of the scoring system used for the grains component was performed in the revised index taking into account that the recent recommendations suggest that at least half of all the grains eaten should be whole grain (www.dietaryguidelines.gov). Similarly, in milk/dairy component the percent of low- or no fat milk was included to adjust for the quality of dairy products consumed. Finally, eggs and legumes were separated from meat/meat product component as were incorporated in the original index constituting independent components in the new one. The egg is a nutrient-dense food with favourable health effects as well as legumes [25,26].

In the current study the revised index was applied to a sample of 2660 primary schoolchildren while its accuracy was examined in relation to children's food consumption, physical and sedentary activity patterns. The R-HLD-index is a fairly accurate instrument to assess healthy diet and lifestyle patterns in primary schoolchildren, considering that higher scores of this index are strongly associated with higher intakes of foods that are necessary to support optimum growth and preserve children's health. Furthermore, higher scores of the R-HLD-index were also related to more hours participating in MVPA and also to lower intakes of unhealthy foods and fewer hours spent on sedentary activities, i.e. TV viewing.

In accordance with the overall prevalence reported for other Greek cohorts [27,28], the present study showed relatively high rates of overweight (30.3%) and obesity (11.5%) in primary schoolchildren. A strong inverse association of the R-HLD-Index with weight status was also noticed, since under/normal-weight children had a higher score compared to their obese counterparts. Consequently, the revised index could be a candidate instrument for assessing the likelihood of obesity in children. Our findings are in agreement with that reported from a previous work conducted with Cypriot children showing that a higher score of the E-KINDEX was related to 80% lower probability of being overweight/obese [9]. However, another study conducted with Greek children and using the Healthy Eating Index to assess diet quality, revealed no significant differences between normal-weight and obese children [29].

However, the R-HLD-index did not correlate strongly with ID. The purpose of the revised index was to extend its utility by assessing the risk for both obesity and ID. The previous HLD-index was negatively associated with insulin resistance which in turn has been found to correlate well with obesity [30]. Thus, the association between the R-HLD-index and obesity was somehow expected. Many recent studies show that the obese children are usually malnourished and this could increase the risk for nutritional deficiencies like ID [31]. It would be important to mention here that the aetiology of ID is multifaceted [32], hence it is not dependent on diet alone. Some recent findings from the Healthy Growth Study showed that ID was more common among obese children indicating an independent association of ID with obesity [5]. Moreover, the components of the R-HLD-index possibly cannot have a generic application to assess the risk for all clinical conditions. These indices assess the degree that individuals are conformed to the recommendations, without being designed to predict the occurrence or the outcome of the diseases. For this reason, although these indices are generally associated with morbidity or the risk of diseases development, these associations are not strong enough [33].

There are several methodological limitations that warrant brief discussion. Firstly, the present study is cross-sectional and thus cannot examine cause-effect relationships. Although a valid structured questionnaire was used for physical activity assessment, a more reliable method, like accelerometry would provide more valid information. Similarly children's TV viewing time was self-reported and their validity of their report was not examined.

In conclusion, the proposed R-HDL-index is a simple diet-lifestyle score for assessing adherence to existing dietary and lifestyle guidelines available for school-aged children and a valuable tool to assess the risk of obesity to primary schoolchildren. It could be used from public health policy makers and other health-care professionals to identify subgroups in the population following poor diet-lifestyle patterns and being at increased probability for obesity so as to develop effective preventive measures. Still utilization of the R-HLD-index in prospective studies conducted with different populations is the only way to evaluate its predictive validity against several health outcomes.

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Conflict of interest The authors declare that they have no conflict of interest.

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Table 1 The scoring system for the revised HLD-index (R-HLD-index)

Index components	*Frequency*	*Score*
Fruit	Never or 1-6 servings per week containing <50% juice	0
	1-6 serving per week containing <50% juice or >3 servings per day >50% juice	1
	>3 servings per day containing <50% juice	2
	1-3 servings per day containing >50% juice	3
	1-3 servings per day containing <50% juice	4
Vegetables	Never	0
	1-6 servings per week	1
	1-2 servings per day or >4 servings per day	2
	3-4 servings per day	3
	2-3 servings per day	4
Fish and seafood	Never or rarely	0
	≥4 servings per week	1
	1-2 servings per week	2
	3-4 servings per week	3
	2-3 servings per week	4
Sweets	≥1 serving per day	0
	4-6 servings per week	1
	2-4 servings per week	2
	1-2 servings per week	3
	Never or rarely	4
Soft drinks	≥1serving per day	0
	4-6 servings per week	1
	2-4 servings per week	2
	1-2 servings per week	3
	Never or rarely	4
Grains	< 1 serving per day or 1-2 servings per day and <50% whole grains	0
	1-2 servings per day and 50% whole grains or >6 servings and <50% whole grains	1
	2-4 servings per day and <50% whole grains or >6 servings per day and >50% whole grains	2
	2-4 servings per day and >50% whole grains or 4-6 servings per day and <50% whole grains	3
	4-6 servings per day and > 50% whole grains	4
Milk/dairy	<1 serving per day or 1-2 servings per day and >50% low- or no-fat	0
	1-2 servings per day and >50% low- or no-fat, >4 servings per day and <50% low- or no-fat	1
	<4 servings per day and >50% low- or no-fat	2
	2-4 servings per day and <50% low- or no-fat	3
	2-4 servings per day and >50% low- or no-fat	4
Legumes	<1 serving per week	0
	>7 servings per week	1
	1-3 servings per week	2
	5-7 servings per week	3
	3-5 servings per week	4
Eggs	<1 serving per week	0
	>7 servings per week	1
	3-4 servings per week	2
	1-2 servings per week	3
	2-3 servings per week	4
Meat and meat products	<1 serving per week	0
	>4 servings per week	1

	1-2 servings per week	2
	3-4 servings per week	3
	2-3 servings per week	4
TV viewing	>4 h/day	0
	3-4 h/day	1
	2-3 h/day	2
	1-2 h/day	3
	<1 h/day	4
MVPA	0-15 min/day	0
	15-30 min/day	1
	30-45 min/day	2
	45-60 min/day	3
	>60 min/day	4

R-HDL-index, Revised Healthy Lifestyle-Diet index; MVPA, moderate to vigorous physical activity; TV, television.

The total score of R-HDL-Index was obtained summing the scores assigned to each component and this score range between 0 and 48.

Fruits: All fresh, frozen, canned and dried fruits and all fresh or packaged fruit juices with no added sugar or fat. The serving is equal to a medium fruit or ½ cup of fruit juice.
Vegetables: All vegetables with no added sugar or fats. The serving is equal to ½ cup.
Total grains: All whole-grain products and refined grain products and grains, such as breads, rice, pasta, cereals, crackers and oatmeal. The serving is equal to 1 slice of bread, ½ cup of cereals, pasta and rice.
Total dairy products: All milks, yogurts and cheeses. The serving is equal to 1 cup of milk or yogurt and 1.5 oz. of cheese.
Total meat: beef, pork, lamb, goat, meatballs, poultry, rabbits and legumes. The serving is equal to 2 oz.
Fish and seafood: All fishes and octopus, squid, cattlefish, shrimps and crabs. The serving is equal to 2 oz.
Sweets: All chocolates and cakes, biscuits, cookies, ice cream, butter cookies, traditional sweets, doughnuts, waffles, candies, lollipops, fruits in syrup and jelly beans.
The serving is equal to 1 oz.

Table 2 Descriptive characteristics of the study population including obesity and iron deficiency prevalence.

	Boys (%)	Girls (%)	P-value†	Total (%)
Underweight	2.5	3.8	0.001	3.1
Normal weight	52.9	57.3		55.1
Overweight	31.0	29.5		30.3
Obese	13.6	9.4		11.5
Normal iron status	83.1	85.4	0.219	84.3
Iron deficiency	16.9	15.7		15.7

†*P*-values derived from the x^2 test

P<0.05 derived from 2-sample z-test for proportions.

Table 3 Prevalence of iron deficiency based on weight status.

	Normal iron status (%)	Iron deficiency (%)	P-value†
Under/normal-weight	86.0	14.0	0.004
Overweight	82.9	17.1	
Obese	74.1	25.9	
Total	84.3	15.7	

†*P*-values derived from the x^2 test

Table 4 Descriptive characteristics of the R-HLD-index per sex, iron status and weight group

	Sex				Iron status			Weight status			
Descriptive characteristics	Total	Boys	Girls	P-value†	Normal iron status	Iron deficiency	P-value†	Under/normal-weight	Overweight	Obese	P-value‡
Mean	17.12	17.18	17.06	0.572	17.04	17.28	0.462	17.28*	16.96	16.07*	0.015
Standard deviation	4.50	4.44	4.56		4.4	4.4		4.48	4.55	4.39	

†P-values derived from Student's T-test

‡ P-value derived from one-way ANOVA, using the Bonferroni rule for post-hoc multiple comparisons.
*P<0.05 for the comparison between under/normal-weight and obese children

Table 5 Comparisons of food groups consumption, TV viewing and MVPA by the tertiles of the R-HLD-index.

	Tertiles of R-HDL-Index			
	1st (0-16 points)	2nd (17-20 points)	3rd (20-48 points)	P-value
Fruits	1.7±1.9	2.5±1.9[a]	2.7±1.9[a,b]	<0.001
Fruit juices	35.4±42.0	50.6±41.2[a]	47.8±38.8[a,b]	<0.001
Vegetables	0.75±1.0	1.0±1[a]	1.4±1.1[a,b]	<0.001
Soft drinks	3.2±3.5	1.4±2.6[a]	0.5±1.4[a,b]	<0.001
Sweets	19.5±13.3	14.4±12.5[a]	9.5±12.1[a,b]	<0.001
Fish/seafood	0.4±2.1	0.59±2.1[a]	0.9±2.6[a,b]	0.001
Milk/dairy	2.0±1.4	2.6±1.2[a]	2.9±1.0[a,b]	<0.001
Low- or no-fat Milk/dairy	0.1±0.5	0.2±0.6[a]	0.3±0.6[a,b]	<0.001
Grains	4.9+2.7	5.5±2.5[a]	5.3±1.9[a,b]	<0.001
Whole grains	0.1±0.4	0.2±0.7[a]	0.4±0.8[a,b]	<0.001
Legumes	1.4±5.0	2.1±5.9[a]	2.6±5.8[a,b]	<0.001
Meat	6.7±6.8	7.0±6.5	6.1±5.7	0.076
Eggs	1.4±4.3	1.7±4.3	1.9±4.1	0.201
MVPA (h/week)	51.2±57.4	71.7±64.8[a]	85.2±54.9[a,b]	<0.001
TV viewing (h/day)	2.8±1.5	2.2±1.3[a]	1.7±1.05[a,b]	<0.001

R-HDL-Index, Revised Healthy Lifestyle-Diet Index; MVPA, moderate to vigorous physical activity; TV, television.

[a]: p<0.05 for the comparison with the 1st tertile of R-HLD-Index after Bonferroni correction for multiple comparisons.

[b]: p<0.05 for the comparison with the 2nd tertile of R-HLD-Index after Bonferroni correction for multiple comparisons.

Table 6 Associations between the R-HLD-index (independent variable), iron deficiency and obesity (dependent variables).

	Dependent variable: Iron deficiency[1]		Dependent variable: obesity[2]	
Independent variable	OR (95% CI)	P-value	OR (95% CI)	P-value
R-HDL-Index	1.020 (0.987-1.055)	0.233	0.940 (0.895-0.988)	0.014
Tertiles of R-HDL-Index				
1st tertile	Reference		Reference	
2nd tertile	1.263 (0.892-1.789)	0.356	0.648 (0.403-1.042)	0.073
3rd tertile	1.095 (0.760-1.578)	0.625	0.576 (0.330-0.973)	0.039

CI, confidence interval; R-HDL-Index, Revised Healthy Lifestyle-Diet Index; OR, odds ratio.

[1]Adjusted for age, sex, Tanner stage, total energy intake, body mass index category.

[2]Adjusted for age, sex, Tanner stage, total energy intake, birth weight, mother's body mass index category and moderate to vigorous physical activity.

Revision of the Healthy Lifestyle-Diet Index and association of the revised index with obesity and iron deficiency in schoolchildren: The Healthy Growth study

Yannis Manios[1,*], George Moschonis[1], Euthimia Politidou[1], Anthi Naoumi[1], Georgia Kourlaba[2], Dimitris Peppas[1], Christos Lionis[3] and George P. Chrousos[4], on behalf of the "Healthy Growth Study" group.

[1]Department of Nutrition & Dietetics, Harokopio University of Athens, Greece.

[2]Department of Health Services Management, National School of Public Health, Athens, Greece.

[3]Clinic of Social and Family Medicine, School of Medicine, Heraklion, University of Crete, Crete, Greece;

[4]First Department of Pediatrics, Athens University Medical School, Aghia Sophia"Children's Hospital, Athens, Greece;

[*]**Corresponding author:**

Dr Yannis Manios, Assistant Professor,

Harokopio University of Athens,

Department of Nutrition and Dietetics,

70, El.Venizelou Ave,

17671 Kallithea,

Athens-Greece.

Tel: +30 210 9549156.

Fax: +30 210 9514759.

E-mail: manios@hua.gr

Abstract

Purpose: To revise the Healthy Diet-Lifestyle Index (HDL-index) in order to more accurately assess the degree of adherence to existing dietary and lifestyle guidelines for school-aged children and to examine the association of the revised HDL-index (R-HDL-index) with obesity and iron deficiency (ID).

Methods: A representative sample of 2660 primary schoolchildren (9-13 years old) participating in the "Healthy Growth Study" was examined. Twelve components related to dietary and lifestyle patterns were used in order to develop the R-HDL-index. Scores from 0 to 4 were assigned to all components. The R-HDL-index total score ranged between 0 and 48.

Results: The overall mean score of the R-HDL-index was 17.1±4.5. Higher R-HDL-index scores were associated with lower proportion of children being obese. More specifically, logistic regression analysis showed that for every 1/48 unit increase in the R-HDL-index score the likelihood of childhood obesity decreased by 6%. However there were no significant associations between the R-HDL-index and ID.

Conclusions: The R-HDL-index may be a useful tool for public health policy makers and other health professionals in order to assess diet quality and lifestyle patterns of primary schoolchildren. Furthermore higher scores of this index are associated with an increased likelihood for childhood obesity but not with ID.

Keywords: Primary schoolchildren, Diet, Lifestyle patterns, Obesity, Iron deficiency

Introduction

Increasing obesity prevalence is of major global health concern [1], while at the same time iron deficiency (ID) is still considered the most common nutrient deficiency worldwide [2]. Unhealthy dietary and other lifestyle patterns have been reported as significant risk factors for both childhood obesity and ID [3]. More specifically, the positive energy balance, brought by increased consumption of energy-dense, high-fat and low-fiber foods in combination with low physical activity levels, is the main risk factor of childhood obesity. In this context higher consumption of foods with an increased content of nutrients that inhibit intestinal absorption of dietary non-heme iron (e.g. phytates in fruits and vegetables, calcium in dairies, polyphenols in tea and coffee) combined with lower consumption of foods rich in nutrients that enhance dietary non-heme iron bioavailability (e.g vitamin C in citrus fruits) has strongly been implicated in the aetiology of ID in children [4]. Furthermore, the chronic inflammatory state induced by obesity per se has been identified as an independent risk factor of ID [5].

Considering the above, the development of a practical tool that will provide a quantitative measure of such patterns, also applicable in assessing the risk for both obesity and ID seems very important from a public health perspective. The development of an index appropriate for this purpose could be one such approach. To the best of our knowledge, the only composite indices that aim to evaluate the overall diet quality and are applicable to schoolchildren and adolescents are the Youth Healthy Eating Index, the Mediterranean Diet Quality Index and E-KINDEX [6-9]. However, some of these indices are not easily applicable because they require the estimation of specific nutrients' intake as their components. Moreover, lifestyle characteristics have not been included as index components in none of the aforementioned existing indices that are applicable to this particular age group. The authors of the present study have previously developed the Healthy Lifestyle-Diet Index (HLD-index) to evaluate the degree of adherence to existing dietary and lifestyle guidelines for school-aged children from the USDA's My Pyramid (www.mypyramid.gov) and reported significant associations with insulin resistance [10].

The aim of this work was to revise the HDL-index in order to more accurately assess the degree of children's adherence to existing age-appropriate dietary and lifestyle guidelines based on USDA's Choose my Plate (www.choosemyplate.gov) and to examine the association of the revised HDL-index (R-HDL-index) with obesity and ID.

Methods

Sampling

The 'Healthy Growth Study' was a cross-sectional epidemiological study initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens. The study population comprised schoolchildren attending the 5^{th} and 6^{th} grades of primary schools located in municipalities within the wider regions of Attica, Aitoloakarnania, Thessaloniki and Heraklion. The sampling procedure is fully described in a previous study [11]. In brief, the sampling of schools was random, multistage and stratified by parental educational level and by the total population of students attending schools within these municipalities. An extended letter explaining the aims of the present study and a consent form for conducting full measurements were provided to all parents or guardians having a child in these schools. Signed parental consent forms were collected for 2660 out of 4145 children (response rate 64.2 %).

Anthropometry

Weight was measured without shoes in light clothes using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany) to the nearest 0.1 kg. Height was measured to the nearest 0.1cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Oadby, UK) with the participants not wearing shoes, their shoulders in a relaxed position, their arms hanging freely and their head in Frankfort horizontal plane. Two trained members of the research team carried out these measurements.

Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). The anthropometric categorization of children was made according to International Obesity Taskforce (IOTF) [12,13] and children were divided into four groups: underweight, normal, overweight and obese.

Dietary intake

Dietary intake data were obtained for two consecutive weekdays and one weekend day using 24-h recalls. Food intake data were analyzed using the Nutritionist V diet analysis software (version 2.1, 1999, First Databank, San Bruno, CA, USA), which was extensively amended to include traditional Greek foods and recipes, as described in Food Composition Tables and Composition of Greek Cooked Food and Dishes [14]. The food-grouping scheme was designed for all foods or entries (core and recipe) appearing in Nutritionist V. Forty-seven food groups were initially established, based on similar source characteristics and nutrient content. Composite food items, such as recipes, were de-composed and were assigned to food groups according to primary ingredients. A similar methodology for the extraction of food groups has been previously reported in studies with not only smaller sample size, but also only one 24-h recall available [15]. Examples of foods included in the food groups have been documented previously [16].

Other characteristics that were recorded

Children's TV/video viewing and computer games playing time was assessed by children's report with regard to their TV/video viewing time and time playing computer games during a usual weekday and a usual weekend. The mean daily TV/video viewing and computer games playing time was calculated using the following equation: daily TV/video viewing and computer games playing hours =((weekday TV/ video viewing and computer games playing hours x 5) + weekend TV/video viewing and computer games playing hours)/7. Physical activity during leisure time was assessed using a standardized activity interview, based on a questionnaire completed by the participants in the presence of a member of the research team. Further details regarding the reliability and validity of the questionnaire are provided elsewhere [17]. Respondents reported the time spent on various physical activities on two weekdays and one weekend day, most preferably Sunday. Reported activities were grouped by a member of the research team into moderate-to-vigorous physical activities (MVPA) (intensity higher than 4 metabolic equivalents), including activities such as brisk walking, bicycling, gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, jumping rope and general participation in active outdoors games. Given the age group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity.

Biochemical analysis

Blood samples were obtained for biochemical and haematological screening tests between 08.30 and 10.30 hours after a 12 h overnight fast. Reminders were distributed the previous day to both parents and children in order to ensure compliance with fasting. Professional staff performed venipuncture, using two types of test tubes, one of which contained EDTA, to obtain a maximum of 10 ml blood. The remaining blood was collected in plain test tubes for the preparation of serum, which was divided into aliquots and stored at -80^{0}C. When blood collection was completed in Aitoloakarnania, Thessaloniki and Heraklion, all serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where biochemical analyses and central storage of back-up samples at 80^{0}C took place. Serum iron and total iron-binding capacity (TIBC) levels were determined by colorimetric assays (Roche Diagnostics SA, Basel, Switzerland). Transferrin saturation (TS) was calculated by dividing serum iron levels by TIBC and multiplying by 100. Iron deficiency (ID) was defined using the following age- and sex-specific thresholds proposed by UNICEF and the WHO [18]: ID was defined as TS < 16 %. The Mentzer Index (MCV (fl)/RBC (M/ml)) [19] was also calculated for all pupils participating in the present study to differentiate beta-thalassemia from ID. On the basis of this index, children with thalassemia minor (eighteen cases) were excluded from further analysis.

Component selection for diet index development

The revised HLD-index (R-HLD-index) resulted from the modification of the HDL-index [10], in an attempt to increase its accuracy in the estimation of the degree of children's adherence to available dietary and lifestyle guidelines. For the development of the R-HLD-index, 12 components were used. The first 10 components measure the frequency consumption of fruits, vegetables, grains, milk/dairy products, meat and meat products, fish/seafood, legumes, eggs, soft drinks and sweets. The other two components reflect the physical activity status of children through measuring the time children spend watching television (TV) or playing computer games (sedentary life) and the time children spend on moderate to vigorous physical activity (MVPA). These components were selected based on dietary recommendations from USDA's ChooseMyPlate (www.choosemyplate.gov) and the recommendations of American Academy of Pediatrics with regard to the TV viewing time [20,21].

Scoring system for the development of R-HLD-index

A five-point scoring system (0–4) was used to assign the appropriate score to each index component. The highest score was ascribed to that dietary intake or participation in moderate to vigorous activity or watching TV being closed to the recommended guidelines, while the lowest score was assigned to the above dietary-lifestyle behaviours being far from the recommendations [www.choosemyplate.gov, 20,21]. Proportionally, the intermediate scores were derived based on the recommendations. Regarding fruit intake, score 4 was assigned to a frequency of consumption ranging from 1 to 3 servings per day containing <50% juice and as for vegetables to a consumption frequency from 2-3 servings per day. With regards to grains, score 4 was assigned to the consumption of 4-6 servings per day, with more than half being whole grains. As concerns milk/dairy consumption, score 4 was assigned to 2-4 servings per day with >50% of them being fat-free or low-fat dairy products. In terms of legumes and eggs preferable a consumption of 3-5 and 2-3 servings per week, respectively, received the highest score of 4. Moreover, with regard to meat component, score 4 was assigned to a consumption frequency of 2-3 servings per week. The other components were scored in the same way as in the original HLD-index [10]. More details for the scores assigned to each component are presented in Table 1.

The total score of the R-HLD-index was obtained by summing the scores assigned to each index component. The values of this index range between 0 and 48. Higher values of the R-HLD-index indicate greater adherence to dietary–lifestyle recommendations or otherwise greater adherence to a 'healthy' dietary–lifestyle pattern. Then, primary schoolchildren were divided into three groups using the tertiles of R-HLD-index as follows: those considered as having (a) an 'unhealthy diet–lifestyle pattern' (1st tertile); (b) a 'moderate healthy diet–lifestyle pattern' (2nd tertile); and (c) a 'healthy diet–lifestyle pattern' (3rd tertile).

Statistical analysis

Continuous variables are presented as mean ± standard deviation and categorical variables are summarized as relative frequencies (%). Associations between categorical variables were tested by using the x^2 test and the two-sample z-test for proportions whenever appropriate. The associations between the continuous and binary variables (sex) were evaluated through Student's t-test or Mann-Whitney test when continuous variables were normally distributed or skewed, respectively. Comparisons between continuous variables (food groups' consumption) and the tertiles of the R-HLD-index were performed using the one-way analysis of variance, after testing for equality of variances or the Kruskal-Wallis test, as appropriate. Bonferroni correction was used to account for increase in type I error owing to multiple comparisons.

Multiple logistic regression models were used to assess the association between the HLD-index score (either as a quantitative variable or as a categorical variable with three categories based on its tertiles), obesity and iron deficiency.

Participants' gender, age, Tanner stage, total energy intake, as well as body mass index category were used as potential confounders for the association between R-HLD-index score and iron deficiency, whereas age, sex, Tanner stage, total energy intake, birth weight, mother's body mass index category and moderate to vigorous physical activity were used as potential confounders for the association between R-HLD-index score and obesity. The results are presented as odds ratios and 95% confidence interval. The Statistical Package for Social Sciences (SPSS) Version 17.0 was used for data analysis. The level of statistical significance was set at $p<0.05$.

Results

The weight and iron status prevalence data of the study participants are presented in Table 2. In total, 2660 children were recruited from 10 to 12 years of age. Overall, the observed prevalence was 3.1 % for underweight, 55.1 % for normal-weight, 30.3 % for overweight and 11.5 % for obesity. Table 2 also summarizes the differences in the prevalence of underweight, normal weight, overweight and obesity among boys and girls. Specifically, the prevalence of obesity was found to be significantly higher in boys than girls (13.6% *v.* 9.4%, p=0.001). It was also found that 15.7% of children under study had iron deficiency with no significant difference observed between sexes (i.e. 16.9% in boys and 15.7% in girls).

The prevalence of iron deficiency by weight group is shown in Table 3. The prevalence of ID was found to be higher in obese children compared to their overweight and under/normal-weight peers (p=0.004).

Table 4 illustrates descriptive characteristics of the R-HLD-index among all schoolchildren of the Healthy Growth Study per sex and weight group, separately. The overall mean $\pm$ standard deviation of the R-HLD-index score was 17.12 + 4.5 and this score was normally distributed. No statistically significant difference was seen in the R-HLD-index score between boys and girls ($p=0.572$) as well as between children with normal iron status and iron deficiency ($p=0.462$). However, there were significant differences in the score among normal, overweight and obese children ($p=0.015$) with obese children having significantly lower mean score compared to under/normal-weight children.

The mean $\pm$ standard deviation of the portions consumed from several food groups and of the time spent on TV viewing and MVPA by the tertiles of the R-HLD-index are depicted in Table 5. The mean consumption of fruits, vegetables, fishes and seafood, dairy (full-fat, low-fat, skimmed), whole grains, legumes and the mean daily time spent on MVPA per week was significantly higher among children in the 3rd tertile of the index compared with the rest of children ($P<0.001$). On the other hand consumption of sweets and soft drinks as well as time spent on TV viewing was significantly lower for children in the higher R-HLD-index tertile ($P<0.001$).

Logistic regression analysis showed that for every 1/48 unit increase in the R-HDL-index score the likelihood of childhood obesity decreased by 6%. Moreover, the likelihood of being obese was 44% lower among children with higher R-HDL-index score (3rd tertile) compared with children with lower score (i.e. 1st tertile) (Table 6). No significant associations were observed between R-HDL-index and ID.

Discussion

The current study tried to revise an index that was initially constructed for assessing the degree of children's adherence to existing dietary and lifestyle guidelines (i.e. the HLD-index) and to assess the association of the revised index (i.e. R-HLD-index) with obesity and ID. The main differentiations derived from the revision of the HLD-index involved changes in the scoring of certain components, as well as the addition of new components i.e. the consumption frequency of legumes and eggs. These changes were required because dietary recommendations for kids, based on which the original HLD-index had been developed [10], were updated, recently (www.choosemyplate.gov). Changes to the scoring algorithm of index components were conducted to the following components: fruit, vegetables, grains, milk/dairy and meat /meat products. Regarding fruits consumption, fruit juice was also included in the scoring with children consuming more frequently fruits but less frequently fruit juice receiving higher scores than children consuming more fruit juice. This change was mainly due to the positive associations observed between fruit juice consumption and childhood obesity by several studies conducted with children [22]. Another difference between the two indices was noticed in the scoring of vegetables consumption with 2-3 servings per day representing the ideal consumption according to the new index as opposed to >4 servings per day set in the previous index. The reason for this change was to follow the updated guidelines in which the daily recommendation for vegetables consumption is set to 1.5 cup. Vegetables contain among others fiber, known for its health benefits [23]. However, there is some concern that a high intake of fiber may impair the absorption of minerals, like iron [24].

Moreover, a simplification of the scoring system used for the grains component was performed in the revised index taking into account that the recent recommendations suggest that at least half of all the grains eaten should be whole grain (www.dietaryguidelines.gov). Similarly, in milk/dairy component the percent of low- or no fat milk was included to adjust for the quality of dairy products consumed. Finally, eggs and legumes were separated from meat/meat product component as were incorporated in the original index constituting independent components in the new one. The egg is a nutrient-dense food with favourable health effects as well as legumes [25,26].

In the current study the revised index was applied to a sample of 2660 primary schoolchildren while its accuracy was examined in relation to children's food consumption, physical and sedentary activity patterns. The R-HLD-index is a fairly accurate instrument to assess healthy diet and lifestyle patterns in primary schoolchildren, considering that higher scores of this index are strongly associated with higher intakes of foods that are necessary to support optimum growth and preserve children's health. Furthermore, higher scores of the R-HLD-index were also related to more hours participating in MVPA and also to lower intakes of unhealthy foods and fewer hours spent on sedentary activities, i.e. TV viewing.

In accordance with the overall prevalence reported for other Greek cohorts [27,28], the present study showed relatively high rates of overweight (30.3%) and obesity (11.5%) in primary schoolchildren. A strong inverse association of the R-HLD-Index with weight status was also noticed, since under/normal-weight children had a higher score compared to their obese counterparts. Consequently, the revised index could be a candidate instrument for assessing the likelihood of obesity in children. Our findings are in agreement with that reported from a previous work conducted with Cypriot children showing that a higher score of the E-KINDEX was related to 80% lower probability of being overweight/obese [9]. However, another study conducted with Greek children and using the Healthy Eating Index to assess diet quality, revealed no significant differences between normal-weight and obese children [29].

However, the R-HLD-index did not correlate strongly with ID. The purpose of the revised index was to extend its utility by assessing the risk for both obesity and ID. The previous HLD-index was negatively associated with insulin resistance which in turn has been found to correlate well with obesity [30]. Thus, the association between the R-HLD-index and obesity was somehow expected. Many recent studies show that the obese children are usually malnourished and this could increase the risk for nutritional deficiencies like ID [31]. It would be important to mention here that the aetiology of ID is multifaceted [32], hence it is not dependent on diet alone. Some recent findings from the Healthy Growth Study showed that ID was more common among obese children indicating an independent association of ID with obesity [5]. Moreover, the components of the R-HLD-index possibly cannot have a generic application to assess the risk for all clinical conditions. These indices assess the degree that individuals are conformed to the recommendations, without being designed to predict the occurrence or the outcome of the diseases. For this reason, although these indices are generally associated with morbidity or the risk of diseases development, these associations are not strong enough [33].

There are several methodological limitations that warrant brief discussion. Firstly, the present study is cross-sectional and thus cannot examine cause-effect relationships. Although a valid structured questionnaire was used for physical activity assessment, a more reliable method, like accelerometry would provide more valid information. Similarly children’s TV viewing time was self-reported and their validity of their report was not examined.

In conclusion, the proposed R-HDL-index is a simple diet-lifestyle score for assessing adherence to existing dietary and lifestyle guidelines available for school-aged children and a valuable tool to assess the risk of obesity to primary schoolchildren. It could be used from public health policy makers and other health-care professionals to identify subgroups in the population following poor diet-lifestyle patterns and being at increased probability for obesity so as to develop effective preventive measures. Still utilization of the R-HLD-index in prospective studies conducted with different populations is the only way to evaluate its predictive validity against several health outcomes.

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Conflict of interest The authors declare that they have no conflict of interest.

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Table 1 The scoring system for the revised HLD-index (R-HLD-index)

Index components	*Frequency*	*Score*
Fruit	Never or 1-6 servings per week containing <50% juice	0
	1-6 serving per week containing <50% juice or >3 servings per day >50% juice	1
	>3 servings per day containing <50% juice	2
	1-3 servings per day containing >50% juice	3
	1-3 servings per day containing <50% juice	4
Vegetables	Never	0
	1-6 servings per week	1
	1-2 servings per day or >4 servings per day	2
	3-4 servings per day	3
	2-3 servings per day	4
Fish and seafood	Never or rarely	0
	≥4 servings per week	1
	1-2 servings per week	2
	3-4 servings per week	3
	2-3 servings per week	4
Sweets	≥1 serving per day	0
	4-6 servings per week	1
	2-4 servings per week	2
	1-2 servings per week	3
	Never or rarely	4
Soft drinks	≥1serving per day	0
	4-6 servings per week	1
	2-4 servings per week	2
	1-2 servings per week	3
	Never or rarely	4
Grains	< 1 serving per day or 1-2 servings per day and <50% whole grains	0
	1-2 servings per day and 50% whole grains or >6 servings and <50% whole grains	1
	2-4 servings per day and <50% whole grains or >6 servings per day and >50% whole grains	2
	2-4 servings per day and >50% whole grains or 4-6 servings per day and <50% whole grains	3
	4-6 servings per day and > 50% whole grains	4
Milk/dairy	<1 serving per day or 1-2 servings per day and >50% low- or no-fat	0
	1-2 servings per day and >50% low- or no-fat, >4 servings per day and <50% low- or no-fat	1
	<4 servings per day and >50% low- or no-fat	2
	2-4 servings per day and <50% low- or no-fat	3
	2-4 servings per day and >50% low- or no-fat	4
Legumes	<1 serving per week	0
	>7 servings per week	1
	1-3 servings per week	2
	5-7 servings per week	3
	3-5 servings per week	4
Eggs	<1 serving per week	0
	>7 servings per week	1
	3-4 servings per week	2
	1-2 servings per week	3
	2-3 servings per week	4
Meat and meat products	<1 serving per week	0
	>4 servings per week	1

	1-2 servings per week	2
	3-4 servings per week	3
	2-3 servings per week	4
TV viewing	>4 h/day	0
	3-4 h/day	1
	2-3 h/day	2
	1-2 h/day	3
	<1 h/day	4
MVPA	0-15 min/day	0
	15-30 min/day	1
	30-45 min/day	2
	45-60 min/day	3
	>60 min/day	4

R-HDL-index, Revised Healthy Lifestyle-Diet index; MVPA, moderate to vigorous physical activity; TV, television.

The total score of R-HDL-Index was obtained summing the scores assigned to each component and this score range between 0 and 48.

Fruits: All fresh, frozen, canned and dried fruits and all fresh or packaged fruit juices with no added sugar or fat. The serving is equal to a medium fruit or ½ cup of fruit juice.
Vegetables: All vegetables with no added sugar or fats. The serving is equal to ½ cup.
Total grains: All whole-grain products and refined grain products and grains, such as breads, rice, pasta, cereals, crackers and oatmeal. The serving is equal to 1 slice of bread, ½ cup of cereals, pasta and rice.
Total dairy products: All milks, yogurts and cheeses. The serving is equal to 1 cup of milk or yogurt and 1.5 oz. of cheese.
Total meat: beef, pork, lamb, goat, meatballs, poultry, rabbits and legumes. The serving is equal to 2 oz.
Fish and seafood: All fishes and octopus, squid, cattlefish, shrimps and crabs. The serving is equal to 2 oz.
Sweets: All chocolates and cakes, biscuits, cookies, ice cream, butter cookies, traditional sweets, doughnuts, waffles, candies, lollipops, fruits in syrup and jelly beans.
The serving is equal to 1 oz.

Table 2 Descriptive characteristics of the study population including obesity and iron deficiency prevalence.

	Boys (%)	Girls (%)	P-value†	Total (%)
Underweight	2.5	3.8	0.001	3.1
Normal weight	52.9	57.3		55.1
Overweight	31.0	29.5		30.3
Obese	13.6	9.4		11.5
Normal iron status	83.1	85.4	0.219	84.3
Iron deficiency	16.9	15.7		15.7

†*P*-values derived from the x^2 test

P<0.05 derived from 2-sample z-test for proportions.

Table 3 Prevalence of iron deficiency based on weight status.

	Normal iron status (%)	Iron deficiency (%)	P-value†
Under/normal-weight	86.0	14.0	0.004
Overweight	82.9	17.1	
Obese	74.1	25.9	
Total	84.3	15.7	

†*P*-values derived from the x^2 test

Table 4 Descriptive characteristics of the R-HLD-index per sex, iron status and weight group

	Sex				Iron status			Weight status			
Descriptive characteristics	Total	Boys	Girls	P-value†	Normal iron status	Iron deficiency	P-value†	Under/normal-weight	Overweight	Obese	P-value‡
Mean	17.12	17.18	17.06	0.572	17.04	17.28	0.462	17.28*	16.96	16.07*	0.015
Standard deviation	4.50	4.44	4.56		4.4	4.4		4.48	4.55	4.39	

†P-values derived from Student's T-test

‡ P-value derived from one-way ANOVA, using the Bonferroni rule for post-hoc multiple comparisons.
*P<0.05 for the comparison between under/normal-weight and obese children

Table 5 Comparisons of food groups consumption, TV viewing and MVPA by the tertiles of the R-HLD-index.

	Tertiles of R-HDL-Index			
	1st (0-16 points)	2nd (17-20 points)	3rd (20-48 points)	P-value
Fruits	1.7±1.9	2.5±1.9[a]	2.7±1.9[a,b]	<0.001
Fruit juices	35.4±42.0	50.6±41.2[a]	47.8±38.8[a,b]	<0.001
Vegetables	0.75±1.0	1.0±1[a]	1.4±1.1[a,b]	<0.001
Soft drinks	3.2±3.5	1.4±2.6[a]	0.5±1.4[a,b]	<0.001
Sweets	19.5±13.3	14.4±12.5[a]	9.5±12.1[a,b]	<0.001
Fish/seafood	0.4±2.1	0.59±2.1[a]	0.9±2.6[a,b]	0.001
Milk/dairy	2.0±1.4	2.6±1.2[a]	2.9±1.0[a,b]	<0.001
Low- or no-fat Milk/dairy	0.1±0.5	0.2±0.6[a]	0.3±0.6[a,b]	<0.001
Grains	4.9+2.7	5.5±2.5[a]	5.3±1.9[a,b]	<0.001
Whole grains	0.1±0.4	0.2±0.7[a]	0.4±0.8[a,b]	<0.001
Legumes	1.4±5.0	2.1±5.9[a]	2.6±5.8[a,b]	<0.001
Meat	6.7±6.8	7.0±6.5	6.1±5.7	0.076
Eggs	1.4±4.3	1.7±4.3	1.9±4.1	0.201
MVPA (h/week)	51.2±57.4	71.7±64.8[a]	85.2±54.9[a,b]	<0.001
TV viewing (h/day)	2.8±1.5	2.2±1.3[a]	1.7±1.05[a,b]	<0.001

R-HDL-Index, Revised Healthy Lifestyle-Diet Index; MVPA, moderate to vigorous physical activity; TV, television.

[a]: p<0.05 for the comparison with the 1st tertile of R-HLD-Index after Bonferroni correction for multiple comparisons.

[b]: p<0.05 for the comparison with the 2nd tertile of R-HLD-Index after Bonferroni correction for multiple comparisons.

Table 6 Associations between the R-HLD-index (independent variable), iron deficiency and obesity (dependent variables).

	Dependent variable: Iron deficiency[1]		Dependent variable: obesity[2]	
Independent variable	OR (95% CI)	P-value	OR (95% CI)	P-value
R-HDL-Index	1.020 (0.987-1.055)	0.233	0.940 (0.895-0.988)	0.014
Tertiles of R-HDL-Index				
1st tertile	Reference		Reference	
2nd tertile	1.263 (0.892-1.789)	0.356	0.648 (0.403-1.042)	0.073
3rd tertile	1.095 (0.760-1.578)	0.625	0.576 (0.330-0.973)	0.039

CI, confidence interval; R-HDL-Index, Revised Healthy Lifestyle-Diet Index; OR, odds ratio.

[1]Adjusted for age, sex, Tanner stage, total energy intake, body mass index category.

[2]Adjusted for age, sex, Tanner stage, total energy intake, birth weight, mother's body mass index category and moderate to vigorous physical activity.

George B. Moschonis is a Nutritionist-Dietitian, with a M.Sc. in "Clinical Nutrition". During the last 8 years he is working as a Research Fellow in several EU-funded and national research projects in the Department of Nutrition and Dietetics at Harokopio University, Athens.