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PhD Dissertation

Micronutrient intakes in a representative sample of Greek children and adolescents: the Hellenic National Nutrition and Health Survey

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΤΡΟΦΙΜΩΝ ΚΑΙ ΔΙΑΤΡΟΦΗΣ ΤΟΥ ΑΝΘΡΩΠΟΥ

Διδακτορική Διατριβή

Διατροφική πρόσληψη μικροθρεπτικών συστατικών σε αντιπροσωπευτικό δείγμα παιδιών και εφήβων στην Ελλάδα: Πανελλαδική Μελέτη Διατροφής και Υγείας

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Επιβλέπων Καθηγητής: Αντώνης Ζαμπέλας

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

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Στους αγαπημένους μου γονείς,

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ΕΥΧΑΡΙΣΤΙΕΣ

Ολοκληρώνοντας αυτό το ταξίδι της γνώσης και της εκπαίδευσης θα ήθελα να ευχαριστήσω όλους τους ανθρώπους που με βοήθησαν σε αυτή την προσπάθεια.

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Θα ήθελα επίσης να ευχαριστήσω τα άλλα δύο μέλη της τριμελούς επιτροπής, την καθηγήτρια Μαρία Καψοκεφάλου και τον καθηγητή Δημοσθένη Παναγιωτάκο, που δέχτηκαν να επιβλέψουν την παρούσα διατριβή. Οφείλω ιδιαίτερες ευχαριστίες στη Δρ. Εμμανουέλλα Μαγριπλή για την καθοδήγηση της σχετικά με τις στατιστικές αναλύσεις και την ερμηνεία των αποτελεσμάτων.

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Ευχαριστώ τέλος από καρδιάς την οικογένεια μου, τους γονείς μου και τους αδερφούς μου Νίκο και Μάριο, για την υπομονή που έδειξαν, τη συναισθηματική ενίσχυση και τη συνεχή συμπαράσταση όλο αυτό το διάστημα.

Περίληψη

Γενική Επισκόπηση: Η παιδική και εφηβική ηλικία αποτελούν κρίσιμα στάδια της ζωής κατά τη διάρκεια των οποίων οι απαιτήσεις σε βιταμίνες και μέταλλα αυξάνονται σημαντικά προκειμένου να διασφαλιστεί η βέλτιστη ανάπτυξη και να προληφθούν οι αρνητικές επιπτώσεις στην υγεία. Επιπρόσθετα, αναπτύσσονται οι διατροφικές συνήθειες κατά την παιδική και εφηβική ηλικία, και τείνουν να παραμένουν στα μεταγενέστερα στάδια της ζωής. Αυτές οι πληροφορίες τονίζουν τη σημασία να παρακολουθηθεί η διατροφική κατάσταση των παιδιών και των εφήβων. Προκειμένου να σχεδιαστούν αποτελεσματικές διατροφικές παρεμβάσεις, είναι σημαντικό να γνωρίζουμε ποια τρόφιμα συνεισφέρουν στην πρόσληψη των μικροθρεπτικών συστατικών. Επιπλέον, παρόλο που η παράλειψη του πρωινού συσχετίζεται με φτωχή ποιότητα διατροφής μεταξύ παιδιών και εφήβων, δεν έχουν διερευνηθεί οι συσχετίσεις για άλλα διατροφικά μοντέλα (όπως μοντέλα γευμάτων και σνακ). Η κατανόηση του τρόπου με τον οποίο συσχετίζονται τα διατροφικά μοντέλα με την ποιότητα διατροφής είναι χρήσιμη για να ενημερωθούν οι επιστήμονες υγείας.

Στόχοι: Οι στόχοι της παρούσας διδακτορικής διατριβής ήταν να: (α) παραχθούν επικαιροποιημένα δεδομένα για τη συνήθη πρόσληψη 20 μικροθρεπτικών σε αντιπροσωπευτικό δείγμα Ελλήνων παιδιών και εφήβων; (β) αξιολογηθεί η επάρκεια διατροφικής πρόσληψης στο πληθυσμό; (γ) υπάρξει εικόνα για τις βασικές πηγές τροφίμων στα μικροθρεπτικά συστατικά; και (δ) εξεταστεί πως διαφορετικά μοντέλα γευμάτων και σνακ συσχετίζονται με τη πρόσληψη μικροθρεπτικών και την ποιότητα διατροφής.

Μεθοδολογία: Αυτή η συγχρονική ανάλυση περιλάμβανε 598 παιδιά και εφήβους ηλικίας 1-19 ετών που συμμετείχαν στην Πανελλαδική Μελέτη Διατροφής και Υγείας. Η πρόσληψη βιταμινών και μετάλλων, και τα μοντέλα γευμάτων και σνακ προέκυψαν από τις δύο ανακλήσεις 24-ώρου. Οι υπολογισμοί έγιναν χρησιμοποιώντας τη μεθοδολογία του National Research Council method για να ληφθεί υπόψη η ενδό- και μεταξύ-των ατόμων-διακύμανση. Ο επιπολασμός της ανεπάρκειας των μικροθρεπτικών στο δείγμα

υπολογίστηκε χρησιμοποιώντας τη Μέση Απαίτηση (EAR). Υπολογίστηκε η συνεισφορά 38 ομάδων τροφίμων στη πρόσληψη θρεπτικών συστατικών για να προσδιοριστούν οι πηγές τροφίμων σε μικροθρεπτικά. Ο Μέσος Όρος Επάρκειας (MAR) χρησιμοποιήθηκε ως μέθοδος αξιολόγησης της συνολικής ποιότητας διατροφής. Διεξάχθηκε πολλαπλή γραμμική παλινδρόμηση, προσαρμοσμένη στους συμπαράγοντες, για να εξετασθούν οι συσχετίσεις μεταξύ των διατροφικών μοντέλων με τις προσαρμοσμένες-σε ενέργεια προσλήψεις θρεπτικών και με το MAR.

Αποτελέσματα: Ένα σημαντικό ποσοστό παιδιών και εφήβων είχαν μειωμένες προσλήψεις σε μικροθρεπτικά συστατικά. Η συνήθης πρόσληψη των βιταμινών D, K και του καλίου ήταν χαμηλή σε σχεδόν όλα τα άτομα. Η βιταμίνη A, το φυλικό οξύ, το ασβέστιο και το μαγνήσιο ήταν επίσης χαμηλή σε σημαντικό ποσοστό, ειδικά στα κορίτσια ηλικίας 14-18 ετών. Το παντοθενικό οξύ επισημάνθηκε ως συστατικό ενδιαφέροντος καθώς μόλις ένα στα 10 αγόρια ηλικίας 9-13 και κορίτσια ηλικίας 14-19 είχαν πρόσληψη που ξεπερνούσε το EAR. Τα δεδομένα έδειξαν πως οι ομάδες τροφίμων που κατείχαν υψηλή θέση στη συνεισφορά της ενέργειας δεν ήταν απαραίτητα σημαντικές πηγές μικροθρεπτικών. Αναφορικά με τα μοντέλα γευμάτων και σνακ, εντοπίστηκαν τέσσερα πιο συχνά αναφερόμενα διατροφικά σχήματα που περιλάμβαναν πρωινό (Π), μεσημεριανό (Μ), δείπνο (Δ) και 2 σνακ (Σ) (22.8%); Π, Μ, Δ και 1 Σ (17.6%); Π, Μ, Δ και 3 Σ (11.2%); Και Π, Μ και Δ (7.9%). Με βάση αυτά τα μοντέλα, η καθημερινή πρόσληψη όλων των κυρίως γευμάτων επισημάνθηκε. Στα παιδιά και εφήβους ηλικίας 4-19 ετών, η αύξηση συχνότητας των σνακ σχετίστηκε θετικά με τις προσλήψεις των βιταμινών A, D, K, ριβοφλαβίνης, παντοθενικού και φυλικού οξέος, μαγνησίου, και χαλκού. Μία αντίστροφη συσχέτιση καταγράφηκε για τις βιταμίνες E, B_6, ασβέστιο και σίδηρο. Μεταξύ των παιδιών ηλικίας 1-3, στατιστικά σημαντική συσχέτιση με τον αριθμό των σνακ παρατηρήθηκε μόνο στο χαλκό, με την ομάδα "Π-Μ-Δ-2Σ" να παρουσιάζει τη μέγιστη πρόσληψη. Όσον αφορά στην συνολική ποιότητα διατροφής,

μεταξύ όλων των συμμετεχόντων δεν υπήρχε στατιστικά σημαντική συσχέτιση του MAR με τον τύπο του μοντέλου γευμάτων και σνακ, και κατ’ επέκταση με τη συχνότητα των σνακ.

Συμπεράσματα: Τα αποτελέσματα δείχνουν πως θα πρέπει να βελτιωθεί η επάρκεια σε μικροθρεπτικά συστατικά της διατροφής παιδιών και εφήβων στην Ελλάδα. Δεδομένης της υψηλής συχνότητας κατανάλωσης σνακ από τα παιδιά και τους εφήβους, η αντικατάσταση των παρόντων τροφίμων που καταναλώνονται ως σνακ με πλούσιες-σε-θρεπτικά συστατικά επιλογές θα μπορούσε να οδηγήσει σε βελτίωση της θρεπτικής ποιότητας της διατροφής. Αυτά τα ευρήματα θα μπορούσαν να παράσχουν καθοδήγηση στους επιστήμονες υγείας προκείμενου να βοηθήσουν τα νεαρά άτομα στην Ελλάδα να βελτιώσουν τις επιλογές τους σε τρόφιμα.

Επιστημονικός τομέας: Επιδημιολογία της Διατροφής

Λέξεις Κλειδιά: μικροθρεπτικά συστατικά, συνήθης πρόσληψη, γεύματα και σνακ, παιδιά, έφηβοι

Abstract

Background: Childhood and adolescence are crucial periods of life during which the requirements for vitamins and minerals increase substantial in order to ensure optimal growth and prevent negative implications to health. Moreover, dietary habits are developed in childhood and adolescence and tend to remain through later stages of life. This information highlights the importance of monitoring nutritional status in childhood and adolescence. To help design effective dietary interventions, it is important to know what foods contribute to micronutrient intakes. Additionally, skipping breakfast is associated with poorer diet quality among children and adolescents, but evidence of associations for other eating patterns (e.g., meal and snack patterns) is scarce. An understanding of how eating patterns are associated with diet quality is needed to inform public health policy makers.

Objectives: The purposes of this thesis were to: (a) provide updated estimates of the usual intake of 20 micronutrients in a representative sample of Greek children and adolescents; (b) evaluate nutrient intake adequacy in the population; (c) get insight into the main food sources of nutrients; and (d) examine how different meal and snack patterns are associated with micronutrient intakes and diet quality.

Methods: This cross-sectional analysis included 598 children and adolescents aged 1-19 years from the Hellenic National Nutrition and Health Survey. Two 24-h recalls were used to estimate vitamin and mineral intakes, and to identify meal and snack patterns. Estimates were calculated using the National Research Council method to account for within- and between- person variation. The prevalence of nutrients' inadequacy among sample was estimated with the use of Estimated Average Requirement (EAR). The contribution of 38 food groups to nutrient intake was estimated to identify micronutrients food sources. Mean Adequacy Ratio (MAR) was used as an overall measure of diet quality. Multiple linear regression adjusted for covariates was conducted to examine associations between eating patterns, energy-adjusted nutrient intakes and MAR.

Results: A substantial percentage of children and adolescents had insufficient intakes of numerous micronutrients. Usual intake of vitamins D, K and potassium was inadequate in practically all individuals. Vitamin A, folate, calcium, magnesium were also insufficient in a considerable percentage, especially in girls aged 14-18 years. Pantothenic acid was highlighted as nutrient of interest since only one out of ten boys 9-13 y and girls 14-19 y had intake above the EAR. Data demonstrated that food groups highly ranked in energy contribution were not necessarily important sources of micronutrients. Regarding meal and snack patterns, four most frequently reported eating schemes were identified including breakfast (B), lunch (L), dinner (D) and 2 snacks (S) (22.8%); B, L, D and 1S (17.6%); B, L, D and 3S (11.2%); and B, L and D (7.9%). Based on these schemes, the daily consumption of all main meals from the majority of the sample was highlighted. In children and adolescents aged 4-19 years, increasing snack frequency was positively associated with intakes of vitamins A, D, K, riboflavin, pantothenic acid, folate, magnesium, and copper. An inverse association was recorded for vitamins E, B_6, calcium and iron. Among children aged 1-3, only copper was significantly associated with number of snacks, with the group of “B-L-D-2S” presenting the highest intake. As for the overall diet quality, among all participants there was no significant association of MAR with the type of meal and snack pattern, and thus the snack frequency.

Conclusions: Results suggest that micronutrient density of children and adolescents’ diet should be improved in Greece. Given the high incidence of snacking behavior among children and adolescents, modifying current snack foods with nutrient-rich choices could lead to an improvement of their diet’s nutritional quality. These findings could provide guidance to public health policy makers in order to help young people optimize their food choices in Greece.

Scientific area: Nutritional Epidemiology

Keywords: nutrients, usual intake, meals and snacks, children, adolescents

Table of Contents

Abbreviations

ACP	Acyl Carrier Protein
AI	Adequate Intake
BMI	Body Mass Index
CVD	Cardiovascular Disease
CoA	Coenzyme A
DASH	Dietary Approach to Stop Hypertension
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
EO	Eating Occasion
FAD	Flavin Adenine Dinucleotide
FMN	Flavin Mononucleotide
FNB-IOM	Food and Nutrition Board of the American Institute of Medicine
HEI	Healthy Eating Index
HNNHS	Hellenic National Nutrition and Health Survey
IOM	Institute of Medicine
MAR	Mean Adequacy Ratio
MK	Menaquinone
MGP	Matrix Gla-protein
NCD	Non-Communicable Disease
NTD	Neural Tube Defection
PL	Pyridoxal
PLP	Pyridoxal 5’-phosphate
PM	Pyridoxamin

PMP	Pyridoxamin 5’-phosphate
PN	Pyridoxine
PNP	Pyridoxine 5’-phosphate
RDA	Recommended Dietary Allowance
UL	Tolerable Upper Intake
WHO	World Health Organization

1. Introduction – Thesis overview

1.1 Malnutrition

Nutrition is a key pillar of human life, health and development, throughout the entire lifespan (1). As it has long been recognized, from the earliest stages of fetal development, at birth and throughout infancy, childhood, adolescence and adulthood, proper food and good nutrition are essential for survival, physical growth, mental development, performance and productivity, health and well-being. Yet, today's world is characterized by the coexistence of agricultural bounty and widespread malnutrition (2).

Although the terms malnutrition and undernutrition are often used indiscriminately, without sufficient attention to their true meanings, malnutrition denotes both undernutrition (insufficient nutrition) and overnutrition (too many nutrients) (3). According to the World Health Organization (WHO), malnutrition is defined as "the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions" (4). A recent report for the Word Committee on Food Security argued that "malnutrition in all its forms – not only hunger, but also micronutrient deficiencies, as well as overweight and obesity- is … a critical challenge not only in the developing but also in the developed countries" (5). Furthermore, many countries, irrespective of their economic development, are now facing a "double burden" of disease: on one hand they struggle with the problems of infectious diseases and undernutrition, and on the other hand they are experiencing a rapid increase in risk factors of noncommunicable diseases such as overweight and obesity (6). It is not uncommon to find undernutrition and overnutrition existing side-by-side within the same country, the same community and even within the same household. Thus, solutions to all forms of malnutrition need to focus on ensuring an adequate supply of food, but equally, on the quality of diets (2).

1.1.1 Forms of malnutrition

The term malnutrition addresses three broad groups of conditions: a) undernutrition, b) overnutrition, and c) diet-related noncommunicable diseases (7). The following section summarizes those forms.

1.1.1.1 Undernutrition

Undernutrition is the outcome of food intake that is continuously insufficient to meet dietary energy requirements, poor absorption and/or poor biological use of nutrients consumed (7). This usually results in loss of body weight. Malnutrition in the form of undernutrition is the most significant risk factor for the burden of disease in developing countries (8), causing approximately 300,000 deaths per year accountable for more than half of the deaths occurring in children in the developing countries (9, 10). Consequences of inadequate nutrition are enormous. Chronic undernutrition is a risk to health and impairs function, including ability to undertake physical activities and work and, possibly, impaired immune function with a predisposition to infection (11).

There are four sub-forms of undernutrition: underweight, stunting, wasting and micronutrient deficiencies (7).

1.1.1.1.1 Underweight

Underweight is defined as low weight-for-age (11), and is usually the result of acute insufficiency of food and nutrient intake . Underweight increases one’s susceptibility to infections, related morbidity, disability and mortality, leading to decreased national productivity and economic growth (12, 13). Ordinarily, all forms of undernutrition can penetrate in an individual who is underweight (7).

1.1.1.1.2 Stunting

Stunting is defined as low height-for-age (below 2 standard deviations compared to the reference population of the same age and sex), and this condition is usually an indication of chronic insufficient food and nutrient intake and frequent infections (2, 7). Stunting is associated with an underdeveloped brain, with long-lasting harmful consequences, including diminished mental ability and learning capacity, poor school performance in childhood, reduced earnings and increased risks of nutrition-related chronic diseases, such as diabetes, hypertension, and obesity in the future (14). Globally, approximately 155 million children under five are stunted, according to 2016 figures (15), with the highest rates being found in eastern Africa and south-central Asia (16).

1.1.1.1.3 Wasting

Wasting is defined as low weight for height, and is an indicator of acute malnutrition which results from recent food deprivation or disease (2, 7). An individual who is moderately wasted has a weight-for-height that is below 2 standard deviations of the reference population, whilst a severely wasted individual’s weight-for-age is below 3 standard deviations. Children suffering from wasting have weakened immunity, are susceptible to long term developmental delays, and face an increased risk of death (17). In 2016, nearly 52 million children under five worldwide were wasted and 17 million were severely wasted, most of the global burden of wasting being found in developing countries. Two clinical phenotypes of severe acute malnutrition are classically recognized: marasmus and kwashiorkor (18). Whereas marasmus is characterized by severe wasting, kwashiorkor presents with a range of clinical signs, including nutritionally induced bilateral pitting edema, loss of hair pigmentation, skin lesions, hypoalbuninemia, and hepatic steatosis.

1.1.1.1.4 Micronutrient deficiencies – Hidden hunger

Micronutrient deficiencies are less visible than protein-energy malnutrition but not less important, and is the most prevalent form of undernutrition in industrialized nations (19). Before the discovery of vitamins, food was mainly viewed as a source of protein and energy (20). This has changed with the recognition that a diet containing an appropriate amount of micronutrients is the cornerstone for preventing diseases such as beriberi, and scurvy. However, putting dietary recommendations into practice continues to be a substantial challenge. Despite the efforts in the last decades to ameliorate micronutrient deficiencies and its consequences in the developing countries, nutrient deficiencies are still affecting the lives and health of billions of people around the whole world. Particularly, in 2012, an estimated 2 billion people worldwide suffered from hidden hunger, which means that people have enough calories, however have inadequate micronutrient intakes. Single micronutrient deficiencies rarely occur alone; multiple micronutrient deficiencies coexist (19). The long-term consequences of this type of undernutrition are not only seen at the individual level but also have deleterious impacts on the economic development and human capital at the country level. Thus, tackling micronutrient deficiencies is a major global health priority.

1.1.1.2 Overnutrition

Overnutrition refers to a chronic condition where intake of food is in excess of dietary energy requirements, resulting in overweight and/or obesity (21). Prevalence of overweight and obesity is defined based on body-mass index (BMI) calculated by mass as measured in kilograms divided by the square of height measured in meters (kg/m^2). For adults (individuals above the age of 18 years), overweight is defined as having a BMI greater than or equal to 25 and lower than 30; obesity is defined as having a BMI greater than or equal to 30. For children and adolescents, classification of overweight and obesity is based on the International Obesity Task Force definition. In 2013, nearly 2 billion out of 5 billion adults worldwide were overweight or obese, and there were 41 million overweight and obese children under age 5 years (22). Once considered a problem for small number of people in affluent societies, obesity has now reached epidemic proportions, and is an important contributor to the global burden of chronic disease and disability. It is associated with several deleterious outcomes such as cardiovascular disease, hypertension, type 2 diabetes, increased risk of metabolic syndrome, depression, increased all-cause mortality, and reduced life expectancy (21, 23). Obesity and its associated comorbidities have also a significant economic impact on national health care systems.

1.1.1.3 Chronic Non-Communicable Diseases

Today, chronic non-communicable diseases (NCDs), mainly cardiovascular diseases, cancers, chronic respiratory diseases and diabetes, constitute one of the greatest threats to public health and economic growth at local, national and global levels (24). In addition to health care costs, NCDs contribute substantially to costs associated with lost productivity. Of the 57 million global deaths in 2008, these four diseases were responsible for 36 million deaths, or 63%, with 80% of NCD deaths occurring in low- and middle-income countries. In Europe, NCDs play an even more substantial role, accounting for nearly 86% of all deaths and 77% of the disease burden, putting increasing strain on health systems, economic development and the well-being of large parts of the population, in particular people aged 50 years and older (25). NCDs, also known as chronic diseases, are the result of a combination of genetic, physiological, environmental and behavioral factors, and they are preventable. By eliminating risk factors such as unhealthy diet, physical inactivity, tobacco use and alcohol consumption, 80% of heart disease, stroke and type 2 diabetes, and over 30% of cancers could be avoided. Unless untreated, the number of deaths by NCDs will increase by 17% on a global scale over the next ten years according to estimates by WHO (26).

1.2 Micronutrients

Micronutrients is the umbrella term used to represent essential vitamins and minerals required in small quantities from the diet to sustain virtually all normal cellular and molecular functions (19). There is a growing interest in the role of the micronutrients in optimizing health, and in in prevention or treatment of disease (27). This stems mainly from the increase in knowledge and understanding of the biochemical functions of these nutrients. This dissertation will focus on twelve vitamins and eight minerals, and the following section provides a short description on their functions, sources and deficiency consequences.

1.2.1 Vitamin A

Vitamin A is a collective name for a group of lipophilic biomolecules, including performed Vitamin A (retinol, retinal or retinoic acid) and provitamin A (β-carotene and other carotenoids) (28). Vitamin A is arguably one of the most multifunctional vitamins in the human body, as it is essential from embryogenesis to adulthood (29). Traditionally, it is known best for being fundamental for good vision as an essential component of rhodopsin, a protein that absorbs light in the retinal receptors, and because it supports the normal differentiation and functioning of the conjunctival membranes and cornea (30-32). Other important functions of vitamin A are regulation of growth and differentiation of cells, growth and development of embryos, apoptosis, bone growth and tooth development, support of reproductive and immune functions and regulation of lipid metabolism and energy homeostasis (29, 31, 33). Furthermore, it is worthy to mention here that vitamin A is an important antioxidant, a property shared with vitamins E and C, the fat soluble and the water soluble vitamins, respectively (30).

Preformed vitamin A (retinoid) can be found almost exclusively in animal products, such as offal and meat, fish oil, butter, milk and eggs (31, 34). Plants are the major source for dietary provitamin A. b-Carotene-rich vegetables and fruits include dark-green leafy (e.g. spinach), sweet potatoes, pumpkin, kale, squash, carrots, mangoes and melons (31, 35). To a lesser extent, carotenoids can also be ingested in eggs, poultry and fish, where typically plant or algal products have been included in the feed of poultry or fish itself (36). Besides the natural contribution from food, vitamin A may be added to foods (e.g. margarines and dairy products) (34).

The primary consequence resulting from vitamin A deficiency is impaired vision, a condition known as “night blindness” (30). Chronic persistent vitamin A deficiency can produce xerophthalmia. Lack of vitamin A deficiency also induces impaired immunity, anemia and adversely affects reproduction (30, 37).

1.2.2 Vitamin D

Vitamin D comes in two forms: vitamin D_3, derived from endogenous synthesis in the skin or alternatively from the diet; and vitamin D_2, derived only from the diet (38). The principal function of vitamin D is to maintain calcium and phosphorus homeostasis in the circulation, together with parathyroid hormone and fibroblast growth factor (39, 40). Additionally, vitamin D is involved in bone formation, resorption, and mineralization, and in maintaining neuromuscular function (40). An additional development has been the recognition that vitamin may have a physiological extra-skeletal role considering the broad distribution of vitamin D receptors in a number of cell types not directly involved in the classical endocrine functions of vitamin D (41). More specifically this micronutrient modulates the transcription of cell cycle proteins, which decrease cell proliferation and increase cell differentiation of a number of specialized cells of the body (41). Other functions include maintenance of the health of immune system and reducing the risk of certain cancers, diabetes, metabolic syndrome and autoimmune diseases (41-43).

The exposure to UVB radiation (wavelengths of 290-320 nm) is the prime natural source of vitamin D for human (44), it is known as the "sunshine vitamin" (45). In addition to latitude and season, the vitamin D synthesis in the skin of humans is affected by several factors, including time spent outdoors, the use of sunscreen, clothing, skin type and age (46). Dietary intake contributes only small amounts of vitamin D overall, with vitamin D found in reasonable amounts in oily fish such as salmon, herring, sardines and tuna, egg, butter, red meat and liver (47-49). The vitamin D within the same food products may also vary, with previous research showing that the vitamin D content of fish may vary enormously depending on whether fish is farmed or wild. For instance, Chen *et al.* (50) analyzed the vitamin D content of both wild and farmed salmon and discovered that wild salmon contained four times the vitamin D content of farmed salmon. Further sources of dietary vitamin D are fortified foods (most often milk, margarine and/ or butter, and breakfast cereals and dietary supplements (39).

Inadequate Vitamin D intake is a serious problem that leads to skeletal malformation and weakness, that manifests itself as rickets in children and osteomalacia in adults (42). Serious and persistent rickets in young women leads to pelvic deformities that impair or prevent natural childbirth, so vitamin D deficiency can directly impact reproductive success. Prolonged vitamin D deficiency may lead to low bone mineral density and may dispose older subjects, particularly post-menopausal women, for osteoporosis, a situation characterized by a reduction in bone mass, reduced bone quality and an increased risk of bone fracture (39, 51). The main sites of fracture involve the wrist, vertebrae and hip. Vitamin D insufficiency is also related to increased susceptibility to infectious diseases, certain cancers, autoimmune diseases and hypertension (39, 42, 52, 53).

1.2.3 Vitamin E

Vitamin E is an essential fat-soluble vitamin that includes a group of eight chemical forms (α-, β-, γ-, and δ-tocopherols and tocotrienols) (54, 55). In most cases, the main source of vitamin E is from the diets rich in γ-tocopherol; however, α-tocopherol is the dominant form in the bloodstream and the most biologically active form of vitamin E. Although most of the data in the past have focused in the antioxidant activities of all vitamin E forms (56), research has recently focused on its non-antioxidant functions. In fact vitamin E is important for the normal morphology of erythrocytes and is thought to be involved in slowing the aging process (57). Furthermore, this vitamin inhibits platelet aggregations, and thus it may play a protective role against the atherosclerotic process and cardiovascular disease (54). Vitamin E seems to have a protective role against arthritis, cataracts, neurological disease and immunological disorders (57, 58). Vitamin E forms have been also associated with cancer prevention, because of their antioxidant activities and their involvement in the regulation of inflammatory response (59, 60). Finally, the role of the different forms of vitamin E in protecting from Alzheimer's disease (55) and in preventing/ treating from Parkinson disease (61) is under investigation.

The main sources of vitamin E are vegetable oils, nuts, and seeds, with consumption of each chemical form varying by dietary source (55). Common food sources of α-tocopherol are almonds, avocados, hazelnuts, peanuts, and sunflower seeds; of β-tocopherol oregano and poppy seeds; of γ-tocopherol pecans, pistachios, sesame seeds, and walnuts; and of δ-tocopherol edamame and rasberries. Tocotrienols prevail in rice bran, barley, oats and palm oil. Other sources of tocotrienols include grape fruit seed oil, hazelnuts, maize, olive oil, Buckthorn berry, rye, flax seed oil, poppy seed oil, and sunflower oil.

Vitamin E deficiency is scare and overt deficiency symptoms have not been found in healthy people who obtain little vitamin E from their diets (62). Premature babies of very low birth weight (<1,500 grams) might be deficient in this vitamin. Due to its fat solubility, vitamin E requires lipid for its absorption in the gastrointestinal tract (63). Consequently, people with fat malabsorption disorders are more likely to become deficient than people without such disorders. Examples of fat malabsorption disorders include genetic defects in the microsomal TG transfer protein or in apoB, cystic fibrosis, gastric bypass, liver disease or pancreatic insufficiency) (62, 63). Patients with abetalipoproteinemia frequently have very low serum vitamin E concentrations, below measurable levels. Clinical features of vitamin E deficiency include a progressive neurologic disorder, spinocerebellar ataxia, which occurs as a result of a dying-back neuropathy in sensory neurons (61, 62). With progressing deficiency there is also muscle deterioration, and this deterioration can include the heart muscle. Furthermore, low levels of plasma α-tocopherol concentrations are associated with increased infection, anemia, stunting of growth and poor outcomes during pregnancy for both the infant and the mother (very low birth weight and high pre-eclampsia risk) (62).

1.2.4 Vitamin K

Vitamin K is a fat-soluble vitamin that exists naturally as vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinone, MK-4 through MK-10) (64). Vitamin K is required as a co-factor for the γ-carboxylation of several vitamin K-dependent proteins, turning inactive uncarboxylated proteins into active carboxylated forms to confer functioning (65). Recent interest in vitamin K has been motivated by evidence of physiological roles beyond that of coagulation (64). Vitamin K appears to counteract excessive bone loss and helps to prevent fractures due to osteopenia and osteoporosis (64, 66, 67). Its beneficial role is thought to be unrelated to increasing bone mineral density but rather increasing bone health. A previous meta-analysis has shown that vitamin K_2 (45 mg/day) significantly reduces hip (77% reduction), vertebral (60% reduction) and all non-vertebral fractures (81% reduction) (68). Moreover, based on the literature review, there is a growing interest on the potential beneficial role of vitamin K on cardiovascular disease (CVD) (65, 69, 70). Vascular calcification is an important cause of cardiovascular morbidity and mortality. Matrix Gla-protein (MGP) is an inhibitor of vascular calcification. Through carboxylation of MGP, vitamin K may help reduce coronary calcification and thereby reduce the risk of CVD.

Vitamin K_1 is present in all photosynthetic plants and is the main dietary form of vitamin K (64, 71). Its primary sources include dark green leafy vegetables (e.g. spinach, lettuce and other salad plants) and Brassica (flowering, head or leafy) (64, 72). Other sources are some seed oils, spreadable vegetable fats and blended fats/ oils (73). Vitamin K_2, which is primarily of bacterial origin, is present in modest amounts in various animal-based and fermented foods (72). More specifically, vitamin K_2 is found in small amounts in liver, poultry products (as poultry feed is a rich source of vitamin K_2), milk, butter, egg yolks and certain types of cheese (66, 72). Furthermore, vitamin K_2 is present in large amounts in fermented soybeans (commonly called *natto*), which is a traditional food in eastern Japan (64). Alternatively people may convert high doses of vitamin K_1 into vitamin K_2 in their bodies (69).

In severe cases vitamin K deficiency is clinically characterized by a bleeding and hemorrhage tendency in relation to the essential role of the vitamin in the activation of coagulation proteins (74). Newborn infants are in increased risk of deficiency because of the low content of vitamin K in breast milk, the low placental transfer of vitamin K, the low levels of clotting factors at birth and of a sterile gut (70). Because vitamin K is required for the carboxylation of osteocalcin in bone, several studies suggest that low levels of vitamin K are related to pathological fractures and osteoporosis (68, 70, 74).

1.2.5 Vitamin C

Vitamin C, also known as ascorbic acid, is an essential water-soluble vitamin which cannot be synthetized by humans due to loss of a key enzyme in the biosynthetic pathway (75). After much intensive studying of this nutrient since its isolation in 1928, many of its biological activities have been highlighted (76). To begin with, vitamin C plays an important role in the biosynthesis of collagen, carnitine, and certain neurotransmitters, and is implicated in protein metabolism (76-78). Collagen represents about one quarter of the total body protein and constitutes the principal protein of skin, bones, teeth, and connective tissues (79). Its synthesis is required for maintaining normal vascular function but also for tumor angiogenesis (77). Vitamin C plays also an important role in the antioxidant defense system of human body (77, 80), and is able to regenerate other antioxidants, including α-tocopherol (vitamin E) (76). In addition to its synthetic and antioxidant activities, vitamin C contributes to immune defense (75) and improves the absorption of non-heme iron, the form of iron present in plant-based foods (77). Ongoing research is focusing on whether vitamin C, by limiting the damaging effects of free radicals through its antioxidant activity, might help prevent the development or treat certain cancers, cardiovascular disease, and other diseases in which oxidative stress plays a causal role (76, 77, 81).

Food with a naturally high vitamin C content are fruits like berries, lychee, papaya, kiwi, and citrus fruits, vegetables like Brussels sprouts, cauliflower, cabbage, sweet pepper, and herbs/ spices such as parsley, sorrel and chives (82). Animal tissues also contain vitamin C in lower amounts, with kidney and liver representing good sources. The vitamin C content of food may be reduced by prolonged storage and by cooking. Because of its water solubility, vitamin C may be lost when the cooking water is discarded.

A deficiency of vitamin C results in the clinical presentation of scurvy, the oldest recognized nutritional deficiency disease (83). Even 8-12 weeks of irregular or inadequate intake of vitamin C can result in clinical symptoms (82, 83). The initial manifestations in adults include fatigue, anemia and aching joints and muscle weakness, while there are important effects on bone tissue in children. As vitamin C deficiency progresses, collagen synthesis becomes impaired and connective tissues become weakened, causing petechiae, ecchymoses, poor wound healing, hyperkeratosis and cork screw hairs. Additional signs of scurvy include depression, swollen, loosen and bleeding gums and tooth loss. In children deficiency of vitamin C may result in a poor formation of the bone osteoid causing disruption in enchondral bone formation (83). Early manifestations mentioned above are followed by leg swelling (mostly marked at the knees and the ankles). Left untreated, scurvy is fatal.

1.2.6 Thiamin

Thiamin, also called vitamin B_1, is one of the water-soluble B vitamins (84). This vitamin plays a fundamental role in energy metabolism and, therefore, in the growth, development and function of cells (84, 85). Other roles that are connected in this vitamin are its involvement in brain development, brain function, maintenance, and interneuronal communication (84-86); it has an essential role in the metabolism of glucose, on which the brain depends as its energy source (85). Thiamin is also involved in nerve tissue repair and nerve signal modulation (84-86). Finally, thiamin has been known to play a role in the immune and the antioxidant defense system.

Thiamin is widely distributed in foods but most contain only concentrations of the vitamin (85, 87). The principal food sources include liver (especially pork liver), wheat germ, whole grains, meat, fish, beans and peas, and nuts. Dairy products, fruits and vegetables are not good sources. Food processing (alkaline pH, high temperatures, exposure to sulphites) contributes to considerable thiamin losses.

Thiamin deficiency is related, in addition to insufficient dietary intake, to alcoholism and drug abuse, and other risk groups include people with HIV, after bariatric surgery, gastrectomy, and with chronic gastrointestinal and liver disorders (87, 88). Thiamin deficiency usually presents with symptoms of peripheral neuritis, cardiac insufficiency and a tendency for edemas (86, 87). In its early phases, it can cause fatigue, loss of appetite and weight loss, irritability, forgetfulness, paresthesia, myalgia, back pain and gastrointestinal disturbances. The classic syndrome resulting from thiamin deficiency in humans is beriberi with mostly neurological and cardio vascular manifestations. People with this condition have impaired sensory, motor, and reflex functions. In rare cases, it causes congestive heart failure that leads to edema in the lower limbs and occasionally to death. Finally, lack of thiamin impairs metabolic function of the brain and can lead to Wernicke's encephalopathy, which is clinically characterized by ocular abnormalities, ataxia, and disturbances of consciousness, and to Korsakoff's syndrome (psychosis) resulting in amnesia, disorientation and often confabulation (86, 87, 89-91). Wernicke-Korsakaoff syndrome can be triggered by excessive chronic alcohol consumption.

1.2.7 Riboflavin

Riboflavin, also called vitamin B_2, is one of the water-soluble B vitamins (92). This vitamin is the integral part of two important coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (92-94). FMN and FAD have central role in the metabolism of carbohydrate, amino acids, lipids, and some vitamins such as pyridoxine, niacin and folate in their synthesis and activation. Riboflavin also supports cellular antioxidant protection. The results of the reviewed studies by Ashoori *et al.* (95) confirm its antioxidant nature and indicate that this vitamin can protect the body against oxidative stress. Oxidative stress is defined as an increased generation of reactive oxygen species or a reduced ability to deactivate them. It is well known that oxidative stress is involved in the development of many chronic diseases such as cancer (96), CVD (97) and diabetes (98). Riboflavin also helps to maintain the integrity of mucous membranes, skin, eyes, and the nervous system (92-94). In addition, this vitamin is involved in erythropoiesis, improves iron absorption, and aids in the mobilization of ferritin from tissues.

Riboflavin is found in a wide variety of animal and plant foods (93, 94). Rapidly growing green leafy vegetables are rich in the vitamin; however, meat and dairy products are the most important contributors of riboflavin dietary intake in developed countries. The other riboflavin-rich foods are eggs and organ meat (kidney and liver). Riboflavin is heat stable, however, UV light exposure or alkali can destroy riboflavin (93, 95).

Although present in a variety of foods, riboflavin deficiency (ariboflavinosis) still occurs in both developing and industrialized countries (92, 95). It is usually due to insufficient dietary intake but also occurs in people with long-standing infections, liver disease, and alcoholism. Clinical signs of riboflavin deficiency include skin disorders, hyperemia (excess blood) and edema of the mouth and throat, sore throat, angular stomatitis, cheilosis, glossitis, hair loss, cataract development and decrease in hemoglobin status (92, 93). A swollen tongue, seborrheic dermatitis, anemia and impaired nerve function can be developed, if riboflavin deficiency is severe and prolonged.

1.2.8 Niacin

Niacin, also called vitamin B_3, nicotinic acid or nicotinamide, is one of the water-soluble B vitamins (99). This vitamin is required for the biosynthesis of the pyridine nucleotides, NAD and NADP, through which niacin has key roles in virtually all aspects of metabolism (100). Over 400 enzymes require NAD and NADP to accept or donate electrons for redox reactions. NAD and NADP appear to support distinct functions. Generally, NAD is important in the degradation (catabolism) of carbohydrates, fats, proteins, and alcohol. NAD also participates in protein modification, cell signaling, calcium mobilization and DNA repair (100-102). NADP functions in biosynthetic (anabolic) reactions, such as in the synthesis of fatty acids, steroids (e.g. cholesterol, bile acids, and steroid hormones), and building blocks of other macromolecules (103).

Niacin occurs in a wide range of foods (104). Good sources include brewers' yeasts, meat, poultry, red fish (e.g., tuna), cereals (especially fortified cereals), nuts and legumes.

Foods rich in protein, such as milk, cheese and eggs, provide some niacin. Coffee is also a source of niacin.

Long-term inadequate intake of niacin can lead to the development of pellagra. The three classic “Ds” of pellagra include dermatitis, diarrhea, and dementia, which occur in unpredictable orders and combinations, depending perhaps on genetics, lifestyle or infectious conditions (100). These are followed ultimately by death if pellagra is left untreated. Pellagrous dermatitis has a characteristic appearance on parts of the body that is triggered by exposure to sunlight, heat, or mild trauma, such as the face, neck, hands, feet, and elbows. Symptoms related to the digestive system include inflammation of the mouth and tongue, vomiting, constipation, abdominal pain, and ultimately diarrhea. Mental symptoms include irritability, headaches, sleeplessness, loss of memory, and emotional instability. In the 21st century, pellagra is now rare in developed countries. Niacin deficiency may still be observed in people with chronic alcoholism, with anorexia nervosa and severe malabsorption disorders, and as a drug-induced condition (105).

1.2.9 Pantothenic acid

Pantothenic acid, also called vitamin B_5, is one of the water-soluble B vitamins (106). Its primary function is to serve as substrate for the synthesis of coenzyme A (CoA), which is used by many cellular enzymes, and acyl carrier protein (ACP) (106-108). Principally, CoA is involved in fatty acid synthesis and degradation, acetyl and acyl transfer, and a multitude of other anabolic and catabolic processes. ACP is involved mainly in fatty acid synthesis.

Pantothenic acid is found in a wide variety of foods of both plant and animal origin (108). Particularly rich sources of this vitamin include chicken, beef, liver and other organ meats, whole grains, potatoes, and tomato products. Royal bee jelly and ovaries of tuna and cod also have high levels of pantothenic acid. Because of its thermal lability and susceptibility to oxidation, significant amounts of the vitamin are lost from highly processed foods, including refined grains and cooked or canned meats and vegetables. Processing and refining whole grains results in its 37-47% loss, while canning of meats, fish and dairy products leads to losses of 20-35%.

Since pantothenic acid is such a ubiquitous component of foods, both animal and vegetable, deficiency of this vitamin in humans is very rare. If present, pantothenic acid deficiency is usually associated with multiple nutrient deficiencies, thus, making it difficult to discern effects specific to a lack of pantothenic acid (108). On the basis of the experience of prisoners of the World War II and experimental studies in humans of diets lacking pantothenic acid in combination with administration of an antagonist of pantothenic acid metabolism, a deficiency is associated with numbness and burning of the hands and feet, headache, fatigue, insomnia and anorexia with gastric disturbances.

1.2.10 Vitamin B_6

Vitamin B_6, is one of the water-soluble B vitamins and it is comprised of a group of six chemically related compounds, pyridoxine (PN), pyridoxal (PL), pyridoxamin (PM), and their respective phosphorylated derivatives pyridoxine 5'-phosphate (PNP), pyridoxal 5'-phosphate (PLP) and pyridoxamin 5'-phosphate (PMP) (109). The primary vitamin B_6 coenzymic form, PLP, serves as a cofactor in over 140 enzymatic reactions mainly in the metabolism of amino acids but also in reactions of carbohydrates and lipids (110). This underlines the wide variety of chemical reactions that PLP-dependent enzymes promote in human body and shows the importance of vitamin B_6. Besides these roles, PLP is also involved in hemoglobin formation through the regulation of homocysteine and the synthesis of cysteine (111), in one-carbon metabolism (112, 113), and in cognitive development through the biosynthesis of neurotransmitters (113). Apart from its function as cofactor for PLP-dependent enzymes, recent research on vitamin B_6 on its role and as anti-inflammatory agent (114-116) as antioxidant (110). It has been shown that vitamin B_6 exhibits antioxidant activity that even exceeds that of vitamins C and E (111).

Vitamin B_6 is widely distributed in foods of animal and plant origin (110, 113). Beef, pork, chicken, fish and nuts are good sources, and organ meats are particularly rich in vitamin B_6. Whole grains are good sources of vitamin B_6, as are bananas and to a lesser extent, potatoes. Although whole-wheat flour is good source of the vitamin, white flour is low due to the removal of vitamin B_6 in the endosperm.

Overall, in a healthy population with access to a balanced diet, vitamin B_6 is uncommon. Vitamin B_6-deficient people experience symptoms such as microcytic anemia, dermatitis, or electroencephalographic abnormalities (113). Sometimes, impaired cell-mediated immunity, depression and confusion have also been reported. In infants, suboptimal vitamin B_6 intake causes irritability and convulsive seizures (117).

1.2.11 **Folate**

Folate, also known as folic acid or vitamin B_9, is one of the water soluble B vitamins (118). Folate functions as coenzyme or cosubstrate in a network of reactions in one-carbon metabolism, including their involvement in the metabolism of key amino acids and the synthesis of nucleic acids (DNA and RNA) (119). As such, the vitamin is a key factor in essential functions of cell metabolism such as DNA replication, repair and methylation, and synthesis of nucleotides, amino acids and some vitamins (120). Folate is also fundamental for the conversion of homocysteine through the action of methionine synthase (118). Additionally, some studies (121-123) have reported a positive association of folate status and birth weight, which is explained by extension of the gestational age or prevention of preterm birth.

Folate occurs in a wide variety of foods of both plant and animal origin; however, there are a few foods that can be considered particularly rich sources. Dark green leafy vegetables, pulses, orange and grapefruit (juice), fortified grain products, peanuts and almonds are the principal sources (119, 120). Meat generally contains low amounts of folate, with the exception of offal such as liver and kidney, which are particularly high in folate.

Chronic severe folate deficiency is associated with decreased DNA synthesis necessary for normal cell division which results in abnormal red cell precursors and hence cells become megaloblastic (megaloblastic anemia) (120). Furthermore, severe folate deficiency leads to hyperhomocysteinemia which is a risk factor for neural tube defects (NTDs) (124). NTDs, an important cause of mortality and morbidity, are congenital malformations of the brain and spinal cord caused by failure of the neural tube to close between 21 and 28 days following conception. Defects range from anaencephaly, through enecephalocoeles to spina bifida, which is more variable in severity and effect. An estimated 4800 pregnancies are affected by NTDs each year in Europe (125). At least 50% of these birth defects can be prevented by improving maternal folate status before conception. In addition to poor birth outcome, folate deficiency and hyperhomocysteinemia have been related to maternal health, particularly the risk for preeclampsia. Finally, there is an ongoing debate on the effects of hyperhomocysteinemia on cardiovascular disease, cognitive function and bone mineral density (118, 120, 126).

1.2.12 Vitamin B_{12}

Vitamin B_{12}, also known as cobalamin, is one of the eight B vitamins and its role in cellular metabolism is closely intertwined with that of folate (127). Vitamin B_{12} has essential roles in human health as it is required for the metabolism of DNA and the formation of red blood cells (128, 129). Other vitamin B_{12}-dependent metabolic reactions play key roles in the synthesis of neurotransmitters, DNA, RNA and protein methylation (130). Vitamin B_{12} is also involved in the metabolism of folate and homocysteine, and in the transformation of lipid acids to energy (126, 130).

Animal tissues accumulate vitamin B_{12}, hence, it is found only in foods of animal origin (128). The richest sources are liver and kidney. Other good sources are beef and lamb, fish, eggs, chicken and dairy foods (126, 128).

The effects of B_{12} deficiency are mainly seen in the blood and nervous system. The hematological effect of B_{12} deficiency is megaloblastic anemia, which results from disruption of DNA synthesis (127). In addition to anemia, there might also be a decrease in the numbers of all blood cells (pancytopenia). The neurologic symptoms of vitamin B12 deficiency include numbness and tingling of the hands and, more commonly, the feet, difficulty walking, memory loss, disorientation, and dementia with or without mood changes (126). Although the progression of neurologic complications is usually gradual, such symptoms may not be reversed with treatment of vitamin B_{12} deficiency, especially if they have been present for a long time. Neurologic complications are not always associated with megaloblastic anemia and are the only clinical symptoms of vitamin B_{12} deficiency in about 25% of cases. As with folate deficiency, low vitamin B_{12} status and B_{12} deficiency during pregnancy and lactation can have consequences for offspring, including NTDs (131). Additionally, suboptimal levels of vitamin B_{12} result in homocysteine accumulation (126), the causes of which on cardiovascular disease, cognitive function and bone mineral density are not still clear (118, 120, 126).

1.2.13 Calcium

Calcium is an integral component of the skeleton; approximately 99% of total body calcium is found in bones and teeth (132). The remaining calcium stores are found in the cells of the soft tissue (0.9%) and in the bloodstream and extracellular fluid (0.1%). It has a structural role, and is needed for tissue rigidity, strength and elasticity (133). Bone is a reservoir for calcium and other inorganic nutrients, and participates in whole-body mineral homeostasis through the process of bone formation and resorption. Calcium deposition into bone is an ongoing process throughout childhood and into adolescence, reaching maximum accretion during the pubertal growth spurt (134, 135). Measures of bone density in adolescent girls indicate that about 37% of total skeletal bone mass is achieved between pubertal stages 2 (mean age 11 years) and 4 (mean age 15 years), with an average daily calcium accretion rate of 300 to 400 mg/day (136). In addition to structural roles, calcium serves a vital role in nerve impulse transmission, muscular contraction, blood coagulation, hormone secretion, and intercellular adhesion (137-139).

Rich food sources of calcium include classically dairy products such as milk, yogurt and cheese (140). Fortified products like tofu or some beverages (e.g. fruit juice), fish with soft bones (e.g. tinned sardines), Chinese cabbage, savoy, chard, spinach and broccoli, legumes and nuts have also important calcium intake, but not all have the same bioavailability (135, 140). Moreover, although grains do not have particularly high amounts of calcium unless they are fortified, they contribute to the dietary intake of calcium because they contain small amounts of the mineral and people tend to consume them frequently (133).

During chronic calcium deficiency the mineral is resorbed from the skeleton to maintain a normal circulating concentrations, thereby compromising bone health (133). Consequently, chronic calcium deficiency is one of several important causes of poor bone mineralization and osteoporosis. Suboptimal calcium intake can also cause rickets in children and adolescents (141, 142), and increases the risk of bone fractures for both children and adults (135, 143). Furthermore, low amounts of calcium reaching the lower bowel can increase vulnerability to colon cancer (144, 145), and kidney stones (146). Failure to maintain extracellular calcium concentrations may increase risk of hypertension (147), pre-eclampsia (148), premenstrual syndrome (149), polycystic ovary syndrome and hyperparathyroidism (150).

1.2.14 Phosphorus

Phosphorus is the most abundant anion in the human body and comprises approximately 1% of total body weight (151). The majority of phosphate is present in bone and teeth (85%), with the remainder distributed between other tissues (14%) and extracellular fluid (1%). Phosphate is in involved in many biologic processes in the human body. Adequate phosphorus intake is critical for proper bone development and mineralization, and for skeletal integrity (151, 152). Additionally, phosphorus is involved in cell membrane structure as phospholipids, in information coding as a necessary component of DNA and RNA, and in for cellular metabolism as an energy source in the form of ATP (153, 154). It also plays a vital role in enzymatic activation, by phosphorylation of catalytic proteins (154) and helps maintain normal acid-base balance (pH), by acting as one of the body's most important buffers (155). Finally, many intracellular signaling processes depend on phosphorus-containing compounds (155).

Phosphorus is found widely distributed in various foods because it exist in virtually all living organisms, with the major sources being protein-rich foods. Milk and its products are the main food sources, followed by meat, fish, poultry, eggs, grain products, legumes and peanuts (151, 156). However, phosphorus is also a component of many food additives that are used in food processing, and is present in processed meats and in beverages such as sodas, juices, and sport drinks (157).

Phosphorus deficiency is expressed as hypophosphatemia (158). This rarely occurs because of inadequate dietary phosphorus intake, and is almost always due to metabolic disorders. Although rare in the general population, the incidence of phosphorus deficiency is high in certain subgroup of patients, such as those with sepsis, chronic alcoholism, major trauma, or chronic obstructive pulmonary disease. The effects of moderate to severe hypophosphatemia may include loss of appetite, anemia, muscle atrophy and weakness, rhabdomyolysis, bone pain, rickets (in children), osteomalacia (in adults), increased susceptibility to infection, conscious disturbance, numbness and tingling of the extremities, difficulty walking, cardiac dysfunction and respiratory failure (152, 157, 159). Severe hypophosphatemia may occasionally be life threatening.

1.2.15 Magnesium

Magnesium is the fourth most abundant mineral in the human body and the second most abundant intracellular divalent cation (160). The bones store approximately 50-60% of the total magnesium content, while muscles and other soft tissues store around 40-50%. Less than 2% of magnesium in the body is available in serum and red blood cells, accounting for the extracellular magnesium in the body. This mineral is an essential cofactor for a diverse metabolic reactions involving more than 300 enzymes within the human body (161). It acts as counter ion for the energy-rich ATP and nucleic acids, regulates transmembrane transport, and has various roles in function and structure of proteins, nucleic acid and mitochondria. Additionally, magnesium plays a key role in the active transport of calcium and potassium ions across cell membranes, a process that is important for nerve impulse conduction, muscular relaxation, vasomotor tone and normal heart rhythm (162, 163). Finally, magnesium contributes to bone mineralization, blood glucose control and several other cellular functions.

Chlorophyll (and thus green vegetables such as spinach) is the major source magnesium (162). Nuts, seeds and unprocessed cereals are also rice in the mineral (161, 162). Legumes, fruit, fish and meat have an intermediate magnesium concentration. Some types of food processing, such as refining grains in ways that remove the nutrient-rich germ and bran, lower magnesium content substantially. Low magnesium concentrations are found in dairy products, except milk. Finally, the magnesium content of tap/ bottled water can make a significant contribution to intake.

Early signs of magnesium deficiency include loss of appetite, lethargy, nausea, vomiting, fatigue and weakness (162). Because of the widespread involvement of the mineral in numerous physiological functions, chronic magnesium deficiency has been associated with a number of chronic diseases. Specifically, low serum magnesium contributes to osteoporosis (164); in fact, magnesium intake was positively associated with bone mass density in elderly (165) and so it was in young adulthood if intake had been correct during pre-adolescent ages (166). Furthermore, magnesium deficiency has been linked to the metabolic syndrome, cardiac arrhythmia, hypertension, insulin resistance and type 2 diabetes mellitus, hypocalcaemia, respiratory diseases and Alzheimer’s disease (161, 162).

1.2.16 Iron

Iron is involved in a broad range of biologically important reactions, as it is a component of innumerable proteins: (a) heme proteins (e.g., hemoglobin, myoglobin, neuroglobin), (b) iron-sulfur proteins (e.g., aconitase), (c) mononuclear iron proteins (e.g., superoxide dismutase), and (d) diiron-carboxylate proteins (e.g., ribonucleotide reductase, ferritin) (167, 168). Among those categories, the last three protein groups are detected at lower levels, but they are functionally important (167). Almost two-thirds of the body iron is incorporated in the hemoglobin present in circulating erythrocytes, 25% is stored in a readily mobilized pool in the liver, and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of cellular enzymes (169). Heme-proteins are transporters of oxygen, carbon dioxide, carbon monoxide, and nitric oxide (e.g. hemoglobin and neuroglobin), stores of oxygen (e.g. myoglobin and neuroglobin), and scavengers of free radicals (169, 170). Iron-sulfur proteins are involved in electron transfer, structural stabilization, transcriptional regulation and catalysis, while the other forms of protein-associated iron are involved in inflammatory responses (169).

Dietary iron is found in heme and non-heme forms (171). The primary sources of heme iron are hemoglobin and myoglobin from consumption of meat, poultry, and fish, whereas non-heme iron is obtained from cereals, pulses, legumes, fruits and vegetables (172). Furthermore, there are many processed products fortified with iron. Heme iron has higher bioavailability that non-heme iron, and other dietary factors have less effect on the absorption of heme iron than non-heme iron (171). The bioavailability of iron is approximately 14% - 18% from mixed diets that include substantial amounts of meat, seafood, and vitamin C (ascorbic acid, which enhances the bioavailability of non-heme iron) and 5% - 12% from vegetarian diets. In addition to vitamin C, meat, poultry, and seafood can improve the uptake of non-heme iron. In contrary, inhibitors of non-heme iron's availability include calcium, phytates (present in whole-grain cereals and legumes) and certain polyphenols (found in plant foods, tea, coffee and wine).

The most widely recognized clinical manifestation of iron deficiency is anemia. Global anemia prevalence in 2010 was 32.9% (*i.e.*, more than 2.2 billion people were affected), with iron deficiency being the most common etiology (173). Clinical features of iron deficiency anemia depend on the severity of anemia and include koilonychias (spoon-shaped finger nails), soft nails, glossitis, cheilitis, mood changes, muscle weakness, and impaired immunity (174). However, iron deficiency can exist with or without anemia. Specifically, major health consequences of iron deficiency with or without anemia include impaired immune mechanisms, impaired cognitive and physical development in children, reduced physical performance and work productivity in adults, cognitive decline in the elderly (172, 174, 175). Iron deficiency during pregnancy is associated with a variety of adverse outcome for both mother and infant, including increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight (172).

1.2.17 Zinc

Zinc is one of the most important trace elements in the body with catalytic, structural and regulatory functions (176). It is present in all body tissues and secretions in relatively high concentrations, with 85% of whole body zinc in muscle and bones, 11% in skin and the liver, and the remaining in all the other tissues, with the highest concentrations in the prostate and parts of the eye (177). Zinc is required for the biological function of over 300 different enzymes. In particular, it is essential and directly involved in catalysis and co-catalysis by the enzymes which control many cell processes (177, 178). These processes include DNA synthesis, normal growth, brain development, behavioral response, reproduction, fetal development, membrane stability, bone formation and wound healing. Zinc serves also to stabilize the structure of proteins involved in DNA replication and reverse transcription. Furthermore, sufficient availability of this element is of particular importance to taste acuity, immune system function, bone mineralization, proper thyroid function, manufacture of insulin, blood clotting, oxidative stress and apoptosis (177, 179). Apoptosis is a major mechanism of programmed cell death, and its dysregulation has negative implications for growth and development, as well as a number chronic diseases (178). In addition, zinc regulates body fluid pH, it promotes the formation of collagen to make hair, skin, nails, and it helps to enhance memory and improves mental development (179).

Zinc is widely distributed in foods. Beef and other red meats, shellfish, and poultry are the major contributors of zinc in the adult omnivorous diet; however, dairy products and many staple vegetables provide amounts of zinc similar to those found in animal tissues (180). Vegetarians obtain a substantial amount of zinc from dairy foods, cereals, grains, legumes, pulses, nuts, and seeds. Green leafy vegetables and fruits are only moderate sources of this element because of their high water content. In addition to the total zinc content of the diet, a range of other dietary components influences positively or negatively zinc absorption from food. Factors that have a positive effect on its absorption include the amount of protein in a meal, individual amino acids, and other low-molescular-weight ions. In contrary, the primary dietary factor recognized to have a major negative effect on the absorption of zinc is phytate (181).

Given its multitude of functions, it is not surprising that a deficit of this element can pose serious and diverse physiological challenges. Clinical signs depend on the severity of the condition, and organ systems known to be affected include epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems (176). Mild zinc deficiency results in impaired growth velocity in children, decreased serum testosterone level and oligospermia in men, immune system impairment, hyperammonemia, hypogeusia, decreased dark adaptation and decreased lean body mass (182). Moderate zinc deficiency results in growth retardation, hypogonadism in male adolescents, rough skin, poor appetite, mental lethargy, delayed wound healing, dysfunction in cell-mediated immunity, and abnormal neurosensory changes. Severe zinc deficiency can cause bullous pustular dermatitis, alopecia, diarrhea, psychological impairment, weight loss, infections, hypogonadism in men, neurosensory disorders, and problematic healing of ulcers.

1.2.18 Copper

Copper, an essential micronutrient in the human body, has no known structural role, nor is there a major copper storage reservoir; rather copper serves as an important catalytic co-factor for several enzymes known as cyproenzymes (183). As such, copper is critical in the human body for the proper function of organs and several metabolic processes such as energy production, hemoglobin synthesis, iron oxidation, activation of neuropeptides, neurotransmitter biosynthesis, cellular respiration, pigment formation, antioxidant defense peptide amidation, and connective tissue formation (184, 185).

Copper content in the diet varies widely because foodstuffs differ in their natural copper content (186). Factors such as season (copper concentration is higher in greener portions), soil quality, geography, water source and use of fertilizers influence the final content in food. The richest dietary sources of copper include organ meat (liver), some seafood (oysters), cocoa products, nuts (particularly cashew) and seeds. In contrary, milk (especially milk from cow) and dairy products are poor sources of copper. Besides food, drinking water can be an additional source of copper.

Copper deficiency is relatively rare in humans, although a number of cases continue to appear in the clinical literature in the last decades (184, 185). Groups that are susceptible to developing copper deficiency include the following: individuals at any age receiving total parenteral nutrition without supplemental copper for extended periods; preterm infants fed milk-based formula without adequate copper; infants recovering from malnutrition or chronic diarrhea, patients undergoing chronic peritoneal dialysis; severe burn patients; ambulatory renal dialysis patients; individuals with malabsorption syndromes; and individuals consuming excessive doses of zinc, antacids, or copper chelators. In addition, Menkes disease is an X-linked neurodegenerative disorder resulting from subnormal copper transport, and its features are similar with those of copper deficiency (187). Low copper status has been associated with bone malformation during development, risk of developing osteoporosis in later life, impaired melanin synthesis, poor immune response and increased frequency of infections, poor cardiovascular health, and alterations in cholesterol metabolism (186). Copper deficiency impairs normal hematopoiesis resulting anemia and leukopenia, neutropenia being the most common (185). Moreover, oxidative stress as a consequence of copper deficiency has been linked with a faster decline in cognitive ability in Alzheimer's disease (186).

1.2.19 Selenium

Selenium, an essential trace element frequently labeled as an "antioxidant", is not an actual antioxidant on its own but it is an integral component of selenoproteins, several of which are involved in protection of cells against oxidative stress (188). Twenty-five selenoproteins have been identified so far in the human genome, whereas only few of them have been functionally characterized. Most selenoproteins participate in antioxidant defense and redox state regulation (188-190). In addition some selenoproteins are involved in thyroid hormone metabolism, immune function, spermatogenesis, selenium homeostasis and transport and skeletal and cardiac muscle metabolism.

On a global scale, there is a wide range of concentrations of selenium in different types of food (191). Those variations are due to differences in the selenium content of the soil on which a plant is grown or an animal is raised, but also to factors that determine the availability of selenium to the food chain, including selenium speciation, soil pH and organic-matter content, and the presence of ions that can complex with selenium. Consequently, selenium levels in foods can vary manifold, not only between countries but also between regions in a country. Selenium intake in humans is principally from the consumption of meat, fish and egg, which contain high levels of the element in relation to other foods (192). Brazil nuts, garlic and brassicas (e.g., cabbage, broccoli, mustard) are also able to effectively accumulate selenium.

The best known diseases associated with prolonged selenium deficiency are Keshan disease and Kashin-Beck disease. Keshan disease is a serious cardiomyopathy that was first described among young women and children in a selenium-deficient region of China (193). The acute form of the disease is characterized by the sudden onset of cardiac insufficiency, while the chronic form results in moderate-to-severe heart enrlagement with varying degrees of cardiac insufficiency. It should be mentioned that the disease cannot be fully prevented by selenium supplementation, which indicates that additional etiological factors, especially an infectious agent, might be involved (193, 194). Kashin-Beck disease is a disabling disorder of the cartilage, bone, and joints that results in stunted growth and deformity that leads to restricted movement and joint enlargement (195). Seen primarily in Siberia, northern Korea and the central regions of China, its etiology is multi-factorial, with selenium deficiency being one of the underlying factors that alone cannot explain its occurrence (195, 196). Moreover, patients receiving selenium-free total parenteral nutrition are at risk and the observed symptoms include skeletal myopathy, muscle weakness, pseudoalbinism and red blood cell macrocytosis (197).

1.2.20 Potassium

Potassium is the most abundant cation in the human body (198). Approximately 98% of the body's total potassium are located intracellularly, the rest 2% is distributed in the extracellular fluid. Due to its location, potassium is the predominant osmotically active element inside cells. It plays a major role in the distribution of water inside and outside cells, assists in the regulation of the acid-base balance, and contributes to establishing a membrane potential which supports electrical activity in nerve fibres and muscle cells (198, 199). It is also involved in cell metabolism by taking part in energy transduction, hormone secretion, and the regulation of protein and glycogen synthesis. In addition, several studies have suggested that a high potassium intake plays a protective role against hypertension, stroke, cardiac dysfunctions, renal damage, hypercalciuria, kidney stones, and osteoporosis (200-204); however, these beneficial effects are not entirely consistent (203, 205, 206).

Potassium is widely distributed along foods. In particular, foods containing high concentrations of potassium include avocado, apricots, cantaloupes, lima beans, oranges, and all meats, poultry, and fish (203, 207). Milk, yogurt and nuts are recognized as being excellent sources of potassium. Apple juice, asparagus beets, carrots, peas, and lettuce are considered to have moderate levels. Those often listed as low in potassium are blueberries, cabbage, beans, mushrooms, pineapples and plums. The Dietary Approach to Stop Hypertension (DASH)-style diet represents a food-based approach to increasing potassium to levels recommended by the Institute of Medicine (IOM).

Potassium deficiency, presented as hypokalemia, can be caused either by decreased intake of potassium or by excessive losses of potassium in the urine or through the gastrointestinal tract (208). The most common causes of hypokalemia include use of diuretic drugs, endocrine diseases, kidney disorders, diarrhea or vomiting, chronic laxative abuse, intestinal obstruction and infections. Mild hypokalemia may be asymptomatic or present with muscle weakness, constipation, fatigue and malaise. Patients with underlying cardiac diseases are prone to arrhythmias. Moderate hypokalemia may result in more severe constipation, an inability to concentrate urine accompanied by polyuria, and a tendency for the development of encephalopathy in patients with concomitant renal disease. Severe hypokalemia can result in muscular paralysis and in poor respiration because of immobilization of the diaphragm and decreased blood pressure.

1.3 Tracking behaviors from childhood into adulthood

In epidemiology, tracking is defined as the stability of a given variable over a period of time (209). Dietary tracking values can therefore be considered to illustrate the maintenance of dietary habits, nutrient intakes or food consumption over time. In the past, several studies have demonstrated some consistency in food choices, and other health-related behaviors from childhood through adolescence and into adulthood. In terms of dietary patterns, Mikkila *et al.* (210) observed tracking of the pattern scores in the Cardiovascular Risk in Young Finns Study. The first cross-sectional study was carried out in 1980, when the subjects were children aged 3-18 years. All those who participated in 1980 were invited in 1986 and in 2001 to the follow-up studies. Tracking was stronger among subjects who were adolescents (15-18 years) at baseline. Of those originally belonging to the uppermost quintile of pattern 1 and 2 scores, 41% and 38% respectively, persisted in the same quintile 21 years later. Lake *et al.* (211, 212) found significant tracking of nutrient intakes from childhood into adulthood. They followed up 202 people at age 11-12 and 32-33 years. They also examined tracking of food groups from the Balance of Good Health and found significant tracking for the three out the five groups. Intakes of fruit and vegetables; bread, cereals and potatoes; and meat, fish and alternatives were all found to track significantly.

In terms of food and nutrient intakes, Welten *et al.* (213) found calcium intakes tracked from adolescence (13 years old) into adulthood (27 years old) with individuals maintaining their relative position within the group over this 15 year period despite an increase in the mean calcium intake with age. In the same study, the likelihood of remaining in the same quartile of intake of dairy foods (milk, milk products and cheese) was relatively high. A relevant stability was observed in intakes of fruits, vegetables, sweets, chocolates and soft drinks among from 14 to 21 years of age (214). As adolescents moved into young adulthood, although fruit and vegetable consumption decreased and intakes of soft drinks increased, the relative ranks remained stable within the population. More recently, the stability of children and adolescents in Sweden was examined (215). Children and adolescents were recruited at 9-15 years old, completed a 24-h dietary recall and were reexamined after six years. Fair tracking was seen between childhood and adolescence for the milk, fil and yogurt food group, and between adolescence and young adulthood for fruit. Slight tracking was observed for most other food groups and fair to slight tracking for all nutrients studied.

To sum up, irrespective of the time period between data collections and the method of data collection used, evidence suggests that there is slight to high tracking for most food groups, nutrients and dietary patterns, indicating some modest stability of diet at individual level. Adequate nutrition during this young vulnerable period of life is therefore important to support general health during adulthood. Thus, assessing and understanding diet and nutritional habits of children and adolescents is important for planning population-based dietary guidance.

1.4 Assessing nutritional status at a population level

There are several indicators used to measure nutritional status. These include body composition, clinical signs of deficiency, physical function, biochemical compounds, metabolic processes or dietary intake (3). The choice of which of these indicators is used is dependent on the question being asked. In clinical settings, it is common to use a combination of qualitative and quantitative descriptions of malnutrition. For example serum retinol level, a biochemical measure, can be used to measure vitamin A deficiency and a clinical feature e.g. xerophthalmia can also be used as a measure of vitamin A deficiency. The most efficient and commonly used methodology to assess nutritional status at a population level is assessing dietary intake (216). Multiple 24-h recalls or food records are used for dietary assessment in large national epidemiological studies because they provide rich detail about the types and amounts of foods and beverages consumed. Furthermore, the use of recall or record methods permits considerable flexibility for data analysis, since data may be analyzed by nutrients, as well as by individual foods, by any food grouping scheme desired or by meals.

Evaluating the nutritional adequacy of the diet is based on the comparison of the usual intake of vitamins and minerals to daily recommended intakes, for which various techniques are employed (216). In the last few decades, several sophisticated methods based on statistical modeling have been evolved that allow for taking into account within-person variation (217), as well as excluding dietary under-reporters (218). As for the daily recommended intakes, the Food and Nutrition Board of the American Institute of Medicine (FNB-IOM) established in 1997 the first Dietary Reference Intakes (DRIs) for vitamins, minerals, and macronutrients (219). The IOM DRIs are used from several studies around the world, as they are regularly updated and frequently used compared to reference values provided by other scientific bodies of organizations (220). **Table 1** summarizes the DRIs concepts. Several countries e.g. the UK (221) or Germany (222) have published their own dietary recommendations.

Table 1. Dietary Reference Intakes concepts

EAR (*Estimated Average Requirement*): how much of a nutrient meets the needs of 50% of the healthy subjects of a specific population's group
RDA (*Recommended Dietary Allowances*): how much of a nutrient meets the needs of 97.5% (mean + 2 SD) of the healthy subjects of a specific population's group
AI (*Adequate Intake*): how much of a nutrient is adequate for a population's group, according to the average intakes of apparently healthy people
UL (*Tolerable Upper Intake L*evel): maximum intake of a nutrient not bound to any adverse effect

An estimate of the prevalence of inadequate intakes of a nutrient by a specific gender or life stage group can be obtained by determining the percentage of the individuals in the group whose usual intakes are less than the EAR (219).

1.5 The epidemiology of micronutrient deficiencies among children and adolescents across Europe

Although the European food environment supplies an overabundance of energy and nutrients, several national dietary surveys reveal inadequate intakes for a number of micronutrients among children and adolescents. It has been suggested that this phenomenon could be explained by changes observed in many industrialized countries regarding food consumption patterns. More specifically, the consumption of nutrient-rich core food groups (e.g., whole grains, vegetables and low-fat dairy products) has been decreased, while at the same time there is an increase toward the consumption of foods with a low micronutrient density (e.g., fast-food, sugar-sweetened beverages, sweets, baked products, salty snacks) (20, 223, 224).

In 2013, Mensink *et al.* (225) compiled the results from national representative dietary surveys performed in Belgium, Denmark, France, Germany, the Netherlands, Poland, Spain and the United Kingdom (UK). Dietary intake information was evaluated for intakes of the vitamins A, D, E, C, B_6 and B_{12}, thiamin, riboflavin, folate, iodine, calcium, iron, magnesium, copper, selenium and potassium. The reference values were obtained primarily from values originally established in the UK in1991. Where reference values were not available from the UK tables, they were drawn from the Nordic Nutrient Recommendations 2004. All the surveys, except from Belgium and Poland, covered all seasons of the year. For the majority of nutrients evaluated, the proportion of children with daily intakes below the EAR, suggesting a potential for a health risk, was measurable in at least some countries in one or more of the five pediatric age ranges evaluated (ages 1-3 years, 4-6 years, 7-10 years, 11-14 years, 15-17 years). Vitamin D is one example. Its usual intakes were below the EAR in almost all children in every age group and country. Iron is another example. For this nutrient, 94% of girls in Denmark and 85% of girls in the Netherlands aged 11-17 years had usual intakes below the EAR. Magnesium is a third example. In this case, 85.6% of girls and 58.1% of boys aged 11-17 years had usual intakes below the EAR. Even when deficiencies were uncommon across most countries, there were exceptions. For example, the proportion of suboptimal vitamin E intake was consistently low across all countries, with the exception of children aged 1-10 in Belgium (there were no available data for adolescents aged 11-17 years), where more than 30% had intakes below the reference standard.

In 2009, the European Nutrition and Health Report of the European Academy of Nutrition Sciences grouped together the nutritional data from sixteen countries across four geographical regions of Europe (226). The north region included countries from Scandinavia. The east region included Germany, Austria, and countries of Eastern Europe. The south region included Greece, Italy, and the Iberian countries. The west region included Belgium, France, Ireland, The Netherlands, and the UK. In this report reference values for adequate nutrient intake were drawn from 2003 WHO guidelines. When no reference values were given by WHO guidelines, the intake was compared to the D-A-CH reference values. It should be noted that not all countries included in this report provided data for all micronutrients. Across the four age groups evaluated (4-6 years, 7-9 years, 10-14 years, 15-18 years) the most commonly observed deficiencies involved vitamin D and folate. Intake of iron was sufficient for boys and girls aged up to 10 years, but very much below the recommended levels among girls at fertile age apart from Portuguese girls aged 13 years. Insufficient magnesium intake was reported among adolescents of all countries apart from Germany, Norway (only male) and Slovenia (both male and female). In all countries the recommendations for calcium intake were met better by younger children, while the intake levels were insufficient in adolescents from Austria, Belgium, France, Ireland, Poland and the UK. For most of the remaining micronutrients, such as the trace minerals phosphorus and zinc, and the vitamins B_6 and C, deficiencies were observed among some age groups of certain countries.

1.6 The epidemiology of micronutrient deficiencies among children and adolescents in Greece

Literature on micronutrient intakes in Greek children and adolescents is scarce with the exception of certain studies carried out either at regional level (227, 228) or at national level but in limited age ranges (220). Dietary information utilized in the 2009 European Report did not come from a representative Greek sample but from schoolchildren aged 6-15 years residing in Thessaloniki (226, 228). In 2014, Manios *et al.* (220) assessed nutrient intake of schoolchildren aged 9-13 years; it was a representative sample at a country level. The EAR cut-point method proposed by IOM was used to assess the adequacy of the micronutrient intake of the sample. Usual intake of vitamin D and Potassium was insufficient in practically all individuals. Vitamin E, folate, and calcium were insufficient, especially in girls. Finally, more than half of the subjects had usual selenium intake that did not meet the reference values.

1.7 Evaluating meal and snack patterns

In the past, several epidemiological studies that have examined diet and health relationships have concentrated primarily on single nutrients and/or specific foods (229-231). These traditional methods have provided valuable evidence on how individual dietary factors influence human metabolism. Indeed, also the role of foods and nutrients in disease prevention has become clearer. However, the “single-nutrient” analysis has several conceptual and methodological limitations (231). First, the high inter-correlations that exist among some nutrients mean that the effects of a single nutrient may be hard to detect or distinguish from other nutrients. Furthermore, people consume combinations of foods as meals and snacks rather than as isolated foods and nutrients. Findings of dietary patterns reflect the overall nutritional behavior and are more amenable to translation and public health practice (232). These findings can be translated into dietary guidelines and policy applications even before the mechanisms underlying the observed associations are fully understood. Consequently, there has been a gradual shift in the last decade towards exploring the whole diet by using dietary patterns (229-231). However, there has been relatively little focus on dietary intake at the level of “meal and snack patterns”. Meal and snack patterns describe how people eat at the level of an eating occasion (EO) and may include a range of indicators such as frequency, timing and skipping of meals, and frequency and timing of snacks (230).

Epidemiologic evidence suggests that skipping breakfast is associated with increased risk of becoming overweight or obese (233), with other cardiovascular disease risk factors

(234) and with reduced intakes of several vitamins and minerals among children and adolescents (235). Increasing number of meals is associated with more favorable lipid profiles in adults (236, 237). Experimental dietary studies show variable responses in lipid profiles and carbohydrate tolerance to changes in meal and snack patterns in different study populations (238, 239). Many of those studies adjusted for energy and macronutrient intakes but research examining how micronutrient intakes or diet quality are associated with meal and snack patterns is equivocal or limited (230). Specifically, nutrient intake, in association with meal and snack frequency, as well as total EOs, have yielded conflicting results in adults. In 2006, Kerver *et al.* (240) provided descriptive information on the meal and snack patterns of US adults who participated in the Third National Health and Nutrition Examination Survey (NHANES III), and examined how the identified meal and snack patterns were associated with selected nutrient intakes. Results showed that groups reporting breakfast, lunch, dinner and 1 snack, and breakfast, lunch, dinner and ≥2 snacks had the highest intakes of all micronutrients examined except vitamin B6. Breakfast skippers (lunch, dinner and ≥2 snacks) had the lowest intakes of all micronutrients examined. Similarly, Murakami et al (241) observed positive association of meal and snack frequency with diet quality. The researchers assessed diet quality of US adults using the Healthy Eating Index-2010 (HEI-2010). The associations were stronger for meal frequency than for snack frequency; one additional meal per day increased HEI-2010 by 2.14 to 5.35 points, and one additional snack per day increased HEI-2010 by 1.25 to 1.97 points. Conversely, in a recent research of Australian adults, findings suggested that the frequency of meals, but not of snacks, was positively associated with the intake of micronutrients and diet quality (242). A higher snack frequency was positively associated only with calcium intake. Respectively, in another study conducted with British adults, a higher diet quality was associated with higher meal frequency and lower snack frequency (243). To date, these relations of meal and snack patterns with nutrient intake and diet quality have not been investigated in younger population, other than effect of skipping

breakfast (235) and meal patterns (244) on nutrient adequacy. Understanding the nutritional composition of meals and snacks and the way in which different meal and snack patterns make an impact on diet quality of Greek children and adolescents could inform the development of practical meal and snack-based strategies to help the population follow dietary guidelines and achieve the recommended daily intakes of foods and nutrients (231, 245).

2. Scope of the current PhD thesis

Based on the above gaps the aims of the current PhD thesis were: (a) the assessment of the usual dietary intake of twenty micronutrients; (b) the identification of their food sources; and (c) the examination of how different meal and snack patterns are associated with micronutrient intakes and diet quality in an national representative sample of Greek children and adolescents from the HNNHS (Hellenic National Nutrition and Health Survey) survey.

The specific aims of each scientific paper in support to PhD thesis are:

1st Paper: To present the aims of the HNNHS, its design and the methodology that was followed for data collection.

2nd Paper: To provide updated estimates of the usual intake of twenty micronutrients – vitamins A, D, E, K, C, B_6, B_{12}, thiamin, niacin, riboflavin, pantothenic acid, folate, calcium, phosphorus, magnesium, iron, zinc, copper, selenium and potassium – and to get insight into their main food sources. Secondary to evaluate nutrient intake adequacy in the population.

3rd Paper: To examine the relation between the frequency of EOs and specific meal and snack patterns with energy-adjusted micronutrient intakes and overall diet quality.

3. Presentation of papers

PAPER I

Dimakopoulos, I., Magriplis, E., **Mitsopoulou, A.V.,** Karageorgou, D., Bakogianni, I., Micha, R., Michas, G., Chourdakis, M., Ntouroupi, T., Tsaniklidou, S.M., Argyri, K., Panagiotakos, D.B., Zampelas, A. (2018). Aims, Design and Preliminary Findings of the Hellenic National Nutrition and Health Survey (HNNHS) (Submitted manuscript in the BMC Public Health)

Title Page

Aims, Design and Preliminary Findings of the Hellenic National Nutrition and Health Survey (HNNHS)

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Abstract

Background: The aim of the Hellenic National Nutrition and Health Survey was to assess nutritional intake, health status and various behaviors in a representative sample of the Greek population.

Methods: Data collection took place from 01.09.2013 to 31.05.2015. Random stratified sampling was performed by (a) geographical density criteria of Greece (7 regions), (b) age group of the reference population (<19, 20-64 and >65 years) and (c) gender distribution. The final population enrolled included (throughout Greece), 4,574 individuals (42.5% men; 57.5% women of who 47.2% were from Athens metropolitan area, 18.5% from Central Macedonia, and the remaining 34% almost equally scattered throughout the country (p for the comparisons with official statistics by region, age group and sex >0.7). Interviewer's and self-reported questionnaires developed were based on extensive review of the literature, following a validation procedure when necessary.

Results: Preliminary analyses revealed that 32% of the adult population were overweight and 15.5% were obese, with significant gender differences in total and per age group (p<0.001, for all). The majority of the adult population reported being active smokers (50.4%) or regular alcohol consumers (72.4%); with significant gender differences (p<0.001, for all). Prevalence of hyperlipidemia was 16.7%, cardiovascular disease 13.9%, hypertension 13.3%, thyroid disease 13.8%, and Diabetes Mellitus 3.6%. Significant gender and age group differences were found in various diseases.

Conclusions: Study's preliminary results provide valuable information about the Hellenic population's health. Findings from this survey could be used to detect disease risk factors for public health prevention policies and programs.

Keywords

Diet, Public health, Nutrition survey/ methods, cross-sectional study, Greece.

Background

Public health promotion, ensures behavioral and nutritional conditions necessary for the mental and physical health of the population [1]. A basic prerequisite for the promotion of public health is first to evaluate current population's health and nutrition status and second to determine the most important modifiable risk factors for disease prevention and treatment [2-7].

During the last few decades, a pharmacological approach for public health promotion was widely used, hence focusing mostly on disease treatment rather than prevention. This approach allowed increased prevalence of various health risk factors, and led to an increase in health care costs, and a decrease in gross production. World data have shown that 8 out of the 20 main causes of morbidity and mortality are due to unhealthy nutrition [5-7] while recent findings showed that the three leading factors of global disease burden were high blood pressure, smoking, and high alcohol consumption [7, 6]. Overweight and obesity, physical inactivity and other modifiable risk factors represent substantial risk factors too. Although such risk factors were globally assessed, substantial variations have been found when different geographical areas were evaluated. Furthermore, differences have been observed also between gender and different age groups (children, adults, elderly).

Therefore, well-designed country specific studies are necessary for the assessment and evaluation of major modifiable risk factors in different geographic regions, which will enable a focused (per region's needs) promotion of public health. Additionally, data should also focus in gender and age specific differences.

Efficiently performed well-designed nationally representative cross-sectional studies have adequately evaluated the population's health and nutrition habits. Examples from such programs include the National Health and Nutrition Examination Survey (NHANES) and the National Diet and Nutrition Survey (NDNS). During the last several decades, findings from NHANES have been used in the United States of America (USA) for status and the development of health policies to safeguard public health, including policies for prevention of lead poisoning and folic acid deficiency through compulsory food fortification [8, 9]. In Greece, such research is lacking especially given the economic crisis of the past few years, which lead several subgroups of the population to become most vulnerable and/or have reduced access to health services [10-12].

The present Hellenic National Nutrition and Health Survey (HNNHS) is the first national cross-sectional study that takes place in Greece, which encompasses a representative sample of all ages, and following standards established by NHANES (USA) and NDNS (United Kingdom).

The aim of the HNNHS was to assess nutritional intake, health status and various behaviors in the Greek population, which could be used to help promote public health through the design and implementation of related policies and intervention programs. In the present paper, the aims, the design and some preliminary findings of the HNNHS are explained below in detail.

Methods

Study design

This is a cross-sectional observational survey. Responders' selection was performed with a random stratified design based on the 2011 census data. Stratification was made according to (a) geographical density criteria by Greek region (7 regions), as provided by the Hellenic Statistical Authority, (b) age group of the reference population (<19, 20-65 and >65 years) and (c) gender distribution. A random selection of more than one individual per household was possible but no more than one individual from the same age group could be enrolled in the study. If households had children <6 years of age, one (if more were present) was randomly selected to be included in the study, upon consent. The sample required to accurately evaluate measures of effect for common risk factors and prevalence of chronic diseases (a priori estimated to equal to 1.2), at 0.05 level (alpha) was 3634 individuals, to achieve a statistical power equal to 85%. To maintain 85% power in the estimation of prevalence rates of chronic diseases or morbidities equal to 15%, with 1 standard deviation (SD) of the referent population (N=11,000,000), at 0.05 significance, a sample size of 4,658 was needed.

Sample

Invitations were sent to approximately 6,000 individuals (anticipating a 70-75% response rate) in to achieve the required sample size, based on a feasibility and volunteer basis in all Greek regions, by the study's investigators from 01.09.2013 to 31.05.2015**.** A total 4574 (42,5% men and 57.5% women) finally agreed to participate. The sample was distributed throughout Greece, with 47.2% of it residing in the Athens Metropolitan area, 18.5% in the region of Central Macedonia, whereas the rest was almost equally scattered throughout the country (**Table 1**; p for the comparisons with Official statistics by region, age group and sex

>0.7). Posthoc assessment, accounting for large population (N>10,000) resulted in a 92% study power, for an effect size of 1.2 (OR=1.2). When the 15% probability of chronic disease was accounted for, the power was reduced to 84%.

Age standardization was performed using the 2011 Census as the reference population's data to check a-posteriori that the sampled population was representative of the Greek population, as per the aim of the study. The population was stratified by 10 years and statistical analyses were performed. The sampled population was representative for the age groups 0-19, 20-65 and 65+, and, hence were used in the analysis. Furthermore, the prevalence of chronic diseases (surveyed) of the actual Greek population (as per census), through direct standardization, was compared to the prevalence found in the study population. The crude and adjusted odds ratios (OR) calculated by age group in total and by gender did not significantly differ, hence allowing increasing generalizability of the results (all supplementary tables placed in **Appendix**).

Inclusion – exclusion criteria

Total HNNHS sample population included volunteers ≥6 months old that reside in Greece. Exclusion criteria included individuals (i), that did not speak Greek, (ii) women who were at that time breastfeeding or pregnant, (iii) members of the armed forces (including those that are currently undergoing their compulsory military service), (iv) individuals that reside in institutions (e.g. nursing homes, rehabilitation centers, hospice centers, psychiatric institutions, prisons, monasteries), (v) those that were unable to provide informed consent due to any cause (e.g., mental impairment, psychiatric condition, drug abuse, vision or hearing loss) unless a first degree relative was able to assist in the process.

Data collection

Information was collected via a series of interviewer and self-reported questionnaires, from the entire population sampled. Clinical examinations were performed on a subsample. More specifically, an initial interview took place at the volunteer's house, with the use of a specially designed computer software (i.e. Computer Assisted Personal Interview (CAPI)), to minimize response biases and misclassification (minimize volunteer burden and maximize reliability of collected data). The list of questionnaires applied can be seen in the Appendix (Supplementary Tables 1-2). In summary, the interviewing process included data on (i) demographics, (ii) quality of life (QoL), (iii) medical history (i.e. chronic & autoimmune diseases, depression, anxiety), (iv) breastfeeding, (v) vitamin and subscribed drug intake, (vi) memory impairment, (vii) eating habits, (viii) alcohol intake, (ix) smoking habits, (x) physical activity, (xi) sleeping habits, (xii) overall patient health, and (xiii) effects of economic crisis. The questionnaires were chosen according to the volunteer's age, as designated by the study's protocol (Supplementary Tables 1-3).

A detailed 24-hour dietary recall was obtained during this process. The volunteers were also interviewed for a second 24-hour dietary recall via telephone 8-20 days after the first interview and on specific days as specified by HNNHS study-protocol. Specific questionnaire structure and validated food atlases for food quantification were used depending on volunteer's age (≥1.5-4 years old, ≥4-<10 years old, ≥10-<12 years old and ≥12 years old) in order to maximize response accuracy. More specifically, dietary intake data were collected using two automated multiple-pass 24-hour dietary recalls and a Food Propensity Questionnaire (FPQ). To harmonize data collection, we based our food classification and description system on FOOdEx2 developed by EFSA [13], based on volunteers age (<2 years old and ≥2 years old). Main differences between the two versions was the food list, (was shorter for the <2 year old's), as well as the frequency response section. The latter referred to the frequency of food intake over the last 30 days for volunteers <2 years old, or to the past year for those ≥2 years old. Both FPQs were developed based on the Hellenic, European and International guidelines. Overall, the methods of dietary assessment were chosen as per EFSA recommendations for the harmonization of data across countries member states of the European Union [13]. Data on eating patterns and behaviors were also collected (timing of food intake, number of meals, activities performed during food consumption, place of consumption, and others) to account for their effects on individuals weight status as studies support [14-16]. The Nutrition Data System for Research (NDSR) (developed by the University of Minnesota) was used for nutrient analysis.

At the end of the interview, volunteers were provided with a list of questionnaires (hard copy) with specific instructions, to self-complete, based on the volunteer's age and their primary response to disease state during the interviewing process (Supplementary Table 2). These were to be fulfilled within a specific time period, to further reduce volunteer burden (time related) and to decrease interviewer and response bias because of the nature of the nature of the questionnaires (sensitive personal information). These questionnaires included (i) qualitative FPQ (asked to be completed by all volunteers, as explained above), (ii) perceived stress scale, (iii) perception of health control, (iii) eating behavior (iv) chronic disease specific information (onset, treatment, medical follow ups, and others), (v) pregnancy and infantile information (i.e., smoking during pregnancy, number of children, weight gain per pregnancy, infant's birth weight/length, breastfeeding (type & duration), and others), (vi) environmental exposure, (vii) social readjustment factors due to the economic crisis, (viii) asthma related information, and (vi) gastrointestinal disorders (the Greek version of Rome III FGID questionnaires for both children and adults was completed).

Interview based questionnaires and those to be self-completed were addressed to volunteers ≥12 years old. Questionnaires related to volunteers, less than 12 years old, were addressed to his/her parent or primary guardian.

In the case of volunteers being unable to self-respond (i.e., with inhibiting health complications, adolescents with lack of knowledge in specific questions) a parent/guardian was asked to assist in the interview. The economic crisis questionnaire was answered only by one adult member per household. Information on primary respondent, or on potential help received during the process was recorded ("interviewee assistant"). A small list of questionnaires where exempt from this procedure (where the main respondent has to be the volunteer himself), due to the nature of the related questions. These included questions on (i) memory impairment, (ii) screen time and alcohol use, (≥12 years - <18 years), (iv) smoking habits (≥12 years - <18 years) and (v) patient health questionnaire.

All self-reported questionnaires were handed in a mobile unit close to them or to the interviewer who performed their initial interview, when completed. To achieve a maximum response rate, the study's trained personnel performed kind reminders via phone calls. A total of 3180 volunteers (2682 adults and 498 children and adolescents) returned the questionnaires (67% in total; 71% for adults and 62.6% for children & adolescents). An investigator checked the questionnaires handed in, and a quality control list was completed (checking the completed questionnaires).

Blood samples were taken from a randomly selected sub-sample of one third of the population. Each of these individuals visited one of the 5 mobile units where medical and anthropometrics were completed (please see Supplementary Table 3).

Interviewers were from various scientific fields (dietitians, physicians, sociologists as well as dietetic and medical students), and all were trained by specialized personnel using the HNNHS fieldwork protocol. These specialists were also involved in the development, methodology and application of study questionnaires and procedures to ensure the highest standard of data collection. Once field interviewers begun their fieldwork, quality control testing assured that procedures were kept according to the protocol.

Ethical approval and consent form

The study was approved by the Ethics Committee of the Department of Food Science and Human Nutrition of the Agricultural University of Athens. It was also approved by Hellenic Data Protection Authority (HDPA). All members of the staff signed confidentiality agreements. Adult volunteers were asked to sign the consent form. For minors <13 years of age the parent or primary guardian signed the form and for volunteers between 13 and 18 years of age the consent form was asked to be signed by both (parent/ guardian and volunteer).

Questionnaires in brief

All questionnaires used in HNNHS, were derived based on a priori knowledge and from components of previously validated questionnaires. For this process. the outcome of interest and previous work performed in the Greek population were also considered

For demographic characteristics (marital status, education, health insurance, employment, income and changes in employment and/or income during the economic crisis) components from NHANES [17], Behavioral Risk Factor Surveillance System (BRFSS) study [18] and NDNS [19], questionnaires were used.

The Quality of Life (QoL) questionnaire included components of (i) QoL and chronic pain components of the Healthy Days Module developed by the Center for Disease Control (CDC) [20], (ii) questions with regards to self-reported height, weight and oral health, from the Health Survey for England and the Activity Limitations Module (also CDC developed) [21].

Two questionnaires were developed for alcohol consumption; one for minors and the second for adults. For minors (≥12 years old and <18 years old) the questionnaire was developed based on questions from the Youth Risk Behavior Survey [22], the European School Survey Project and other Drugs [23] and the Global School-based Student Health Survey (GSHS) [24]. For the adult questionnaire data from NHANES study [17], BRFSS [18], Arkansas Cardiovascular Health Examination Survey (ARCHES) [25] and Recommended Alcohol Questions by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) [26] were used. Volunteers were classified as alcohol or non-alcohol consumers, based on their intake over the past 30 days. Frequency of alcohol intake among “consumers” was categorized as daily, weekly or monthly, based on their response on (i) total drinks per month consumed, (ii) drinks per week and/or (iii) drinks per month. For minors, the total number of individuals that reported having consumed an alcoholic drink at some point in life (and not just few sips) was reported.

As in the case of alcohol consumption, smoking habits questionnaire(s) were also based on volunteer's age. In particular, for adults questionnaires used were from the NHANES [17] and BRFSS [18] studies; for minors from the Youth Behavior Survey [22], NHANES [17] and the European School Survey Project on Alcohol and other Drugs [23]. Volunteers were grouped into (i) current smokers, if they responded that they had smoked the past month, (ii) ever smokers, if they had smoked at any point in their life, and (iii) non-smokers, if they had never smoked. Frequency of smoking, among current smokers, was also recorded as "daily" or "sometimes". Among minors, the question referred as to whether they had ever tried to smoke (aged up to 19 years).

Physical activity has a well-known role as a health determinant hence the aim was to assess physical activity levels in all ages. Questionnaires on physical activity were modified based on age groups as per a priori knowledge, including (i) ≥2-<12 years old of the questionnaire was based on questions from the NHANES survey [27] and Preschool-aged Children Physical Activity Questionnaire (Pre-PAQ Home Version) [28] (ii) ≥12-<18 years old, the International Physical Questionnaire – Adolescents (IPAQ-A) [29], (iii) ≥18 years-<65 years old the IPAQ short form was used [30] and (iv) for ≥65 years of age a modified version of the IPAQ has been suggested [31]. Preliminary results reported in this study include level of physical activity as perceived by the adult volunteers (sedentary, low, moderate and active) or by the primary care giver if the volunteer was <12 years old.

Information about medical history for disease prevalence among the Greek population, related medical treatment and insurance coverage were collected. The synthesis of this questionnaire was based on the National Health Survey [32], NHANES [17], ARCHES [25], and the Million Women Study [33]. Further details on specific disease states (hypertension, dyslipidemia, diabetes) with specific questionnaires [25], were collected once the volunteer declared as having such a condition. In particular, data on prevalence of Chronic Obstructive Pulmonary Disease (COPD) was obtained using the COPD Population Screener [34] and Asthma using the questionnaire from the Hellenic Thoracic Society [38] (for adults), and the Greek version of the questionnaire International Study of Asthma and Allergies in Childhood (ISAAC) [35] (minors 6 - 18 years old). Furthermore, cardiovascular disease (angina, coronary events) include coronary heart disease, stroke and peripheral arterial disease and represent the main cause of morbidity and mortality [36]. Following a literature review the Rose Questionnaire for Angina [37] and the Edinburgh Claudication Questionnaire were used in HNNHS [38].

Additional the types of questionnaires used in the study can be viewed in the Appendix and they included information on breastfeeding, drug and supplement use, memory impairment (≥45 years old), eating habits and behavior (as previously reiterated), sleeping habits, data on depression, stress (acute and chronic) & health locus of control, gestational & child- birth related questions, environmental exposure, functional gastrointestinal disorders, vitamin D intake status & sun exposure, and economic crisis, to acquire adequate and substantial information on the population's exposures and risks. Details for each of these questionnaires will be provided upon analysis.

Clinical/ Physical evaluation and Biochemical variables

HNNHS also included physical examination (temperature, spirometry, blood pressure, etc.), anthropometry (weight, height, waist and hip circumference, body composition, and grip strength), and several blood tests (glucose, HbA1c (diabetics), insulin, total lipid profile, thyroid hormones, thyroglobulin, PTH, complete blood count, folic acid, iron, ferritin, B_{12}, 25OH-vitamin D, creatine, urea, albumin, total protein, ALT, AST, bilirubin, uric acid, calcium, magnesium, manganese, selenium, hs-CRP, cortisol, and heavy metals, namely As, Cd, Co, Hg, Mo, Pb, Pt, Sb, W, Zn, Ce, La, Th, U) in a subsample of the population, to examine correlations with various health indices in later analyses (Supplementary Table 3).

Statistics

Prior to analysis, data were cross checked for missing values and outliers. Missing information was corrected if the information was derived from other questionnaires and/or measurements (non-reported values of weight and height were completed if the individual was measured at the CAPI). Also, individuals responding as non-diseased but reported taking a disease related medication, were classified as with disease outcome. Baseline socio-demographic are presented as frequencies and percentage (N, %) per gender. Variables of interest are presented in total and per gender and age-group (i.e., population's weight status, smoking, alcohol, physical activity, prevalence of chronic disease), while physical activity is presented by specific age groups (as per questionnaires). Chi-square test was used to assess gender differences by age group for weight status, smoking and alcohol intake, and for total prevalence of chronic disease by gender. Tukey's paired means test was used to detect differences between age groups (for each chronic diseases). All reported p-values were based on two-sided hypothesis tests, with significance level at 5%. The statistical models were computed using STATA 12.0 (STATA corp. Texas).

Results

Demographic data

The sample was distributed in all different regions of Greece (**Table 1**). 47.2% was in the region of Attica, 18.5% Central Macedonia, and the rest of the sample being scattered through various regions of Greece (1.3% Epirus, 4.2 % Eastern Macedonia and Thrace, 3.1% Peloponnese, 2.2% Western Macedonia, 5.2% Thessaly, 2.3% Central Greece, 4.8% Western Greece, 5.7% Crete, 1.1% Ionian islands, 2% North Aegean and 1.9% South Aegean).

The total number of participants is 4574 volunteers of which 1943 were males and 2629 females. **Table 2** shows distribution per gender, age and socioeconomic parameters. Age distribution was representative of the 2011 Census, with 19% (N=869) of the sampled population being 0-19 years old, 67% (N=3064), 20-64 years old, and 14% (N=639) were ≥65 years old. Marital status was as follows: 40.6% of the population was unmarried (43.3% males and 38.5% females), 48.4% married (51.4% males and 46.4% females) and 0.1% having a cohabitation agreement 6.2% were widowers (2.2% males and 9.2% females), 3.8% divorced and 0.7% separated.

Educational level greatly varied with approximately 32% having a University degree or greater, 7.1% had completed secondary education. Approximately 17% of the population had limited to low education, 27.1% completed lyceum (12 years of schooling), 5% technical secondary school and 8.3% private post-lyceum college. A large percentage of the population (78.3%; 77.8% males and 78.8% females) reported having public health insurance whereas only 4.3% had private insurance and 8.9% both types. A total of 8% males and 6.2% females were not insured (Table 2).

In terms of net monthly income (Table 2), 13,5% had low income (<€300-850), 11.4% had €851-1050, approximately 18% had moderate high income (€1051-1500), 10.6% had €1501-1900, 9.1% had €1901-2400, and 10.7% had high income (€2401-3800 and >€3801).

Weight status and behavioral data

Sample's self-reported weight status in total by age group (>20 years old) and gender based on Body Mass Index (BMI) can be found in **Table 3**. A total prevalence of 47.5% of the adult population was overweight (32%) and obese (15.5%), with the prevalence increasing with age in both genders. A significant body weight status difference was found in each age group, with males having a higher prevalence of overweight compared to females (p<0.001) in all age groups.

Frequency of alcohol consumption among adults was 72.4% (**Table 4**), with approximately 7% reporting daily consumption, 33% weekly and 60% on a monthly basis. A significant greater percentage of males reported of being alcohol consumers than females (81.1% compared to 67%, respectively; p<0.001) and being more frequent alcohol consumers as well (p<0.001). Among minors (12 to 19 years of age, inclusive), 111 out of 340 individuals (32.6%) reported as having consumed an alcoholic drink at some point before, and not only a few sips (Table 4). No significant differences were found between genders among minors in alcohol consumption (p=0.121).

Smoking frequency in the total population among adults and minors, per gender, is being shown in **Table 5**. Approximately 34% of the population were current smokers, whereas 50.9% reported on having smoked at some point in their life. Significant gender differences were found in both cases with a higher proportion of males reporting to have smoked (59% compared to 44%) or of being current smokers (38.3% compared to 30.8; p<0.001 for all). Among current smokers 87.3% reported to smoke daily with a borderline difference found between genders (p=0.046). A total of 22% of minors (up to 19 years of age, inclusive) reported of having tried to smoke at some point. No significant gender differences were found (p=0.229).

Preliminary results of physical activity level were self-reported as sedentary, low activity, moderately and very active (**Table 6**). The highest proportion of the population being very active was in young children (2-12 years old, 68.6%) and among adolescents (48.5%). Twenty – five percent (25%) of adults aged 18-65 and >65 years old reported being very active whereas 20% of the elderly (>65) reported of having a completely sedentary lifestyle.

<u>Prevalence of chronic disease</u>

In Table 7, the prevalence of various chronic diseases is presented in total and per age group (20-39, 40-64, and 65+) in adults. In each category, gender specific rates can also be viewed. The highest prevalence (16.7%) was reported for hyperlipidemia (increased cholesterol or triglycerides), with prevalence increasing in both genders with age (Tukey's test p<0.001 between groups). The same pattern was found for hypertension with the prevalence mounting to 56% (51.2% in males, 61% in females; p<0.05) in the elderly compared to 1.7% in adults aged 20-39 and 17.3% in the 40-65 age group (Tukey's test not significant). Accordingly, age patterns were seen in all CVD (CHD, angina, MI, heart failure, arrhythmia and stroke), with significant age group differences found only in heart failure (Tukey's test p=0.014 for 65+ compared to 20-39 years). Diabetes prevalence and osteoporosis was also considerably higher in the older age group (16,8%) compared to 3.8% in total population and 16.2% compared to 5.4%, respectively. Only osteoporosis was significantly different between age groups (p<0.001 for 65+ and 20-39 and 40-64). The prevalence of thyroid disease was high in all age groups, especially in females and significantly different between the 65+ and 20-39-year-old age groups (Tukey's test p=0.026). A significant difference was also found in cancer prevalence between the older and younger adult age groups (Tukey's test, p=0.033)

<u>Gender differences and chronic disease</u>

Significant gender differences were found in hyperlipidemia, arrhythmia, cancer, thyroid disease, osteoporosis, arthritis/rheumatoid arthritis, irritable bowel syndrome, depression, and chronic stress, with females having a significantly higher proportion in each one of them. Prevalence of asthma and cancer was also higher in females, more specifically in the 40-64 age group (4.8% vs. 1.7%; p<0.05 and 3.7% vs. 0.6%; p<0.01, respectively). Gender difference was also found in CHD with males having a higher prevalence in the total adult sample and in the older group (p<0.001, for all). The prevalence of MI did not differ in the total sample but was significantly higher in males over 65 years old than females in the same age group (9.1% vs. 1.9%; p<0.001). Diabetes mellitus was significantly higher in males aged 40-64 years old than females of the same age group (5.9% vs. 2.7%; p<0.01).

Discussion

The HNNHS was set up in 2013 with the aim to provide comprehensive, cross-sectional information, on a representative sample of the Greek population, including prevalence of various chronic diseases, nutritional intake and dietary habits, physical activity, various health related behaviors and lifestyle changes and psychosocial health burden. HNNHS had the support of national institutions, such as the Hellenic Statistical Authority, local medical associations and the Central Union of Municipalities of Greece. In addition, the advisory committee was composed of professors from the National Kapodistrian University of Athens Medical School and the Harokopio University, as well as representatives from the Medical Research Council (MRC) and NatCen Social Research (NatCen). The study's main goal was to provide sufficient data to develop health and nutrition policies at a national level for the increase of the population's health and well-being, hence, improving public health.

Data from NHANES showed Increased levels of obesity in adults that varied by sex, age and ethnicity. Increased levels of hypertension and hyperlipidemia were also noted and relevant policies targeting their reduction were developed [39, 40]. Preliminary results of the HNNHS study also showed an elevated prevalence of overweight and obesity in adults that significantly varied by gender. In both sexes, it increased with age; males had overall a higher weight status than females. Detailed analyses of these findings are needed to determine associated risk factors.

Baseline demographic and lifestyle characteristics of a representative sample of the Greek population (age, area of residence and gender) were reported. It is noteworthy that of the 4,574 participants, 3,944 (86.2% of total sample and 100% of individuals >16 years of age) answered that their income was negatively affected due to the economic crisis, with 23.3% of the population reporting a significant reduction in particular in 2012.

The proportion of alcohol consumption and current smoking status was high. An alarming proportion of minors had tried alcohol or had smoked at some point. Smoking is a known risk factor for many chronic diseases, including cardiovascular disease, many forms of cancer, asthma and COPD. Alcohol, on the other hand, has been found to have protective effects when consumed in moderation. The associations between these factors, along with various covariates that may confound or modify potential effects, remain to be further investigated.

Preliminary results on prevalence of chronic diseases, many of which are currently characterized as epidemics, showed that Greek adults had a high prevalence of hypertension (13.3%), hyperlipidemia (16.7%) and all types of thyroidism (13.8%). Hyperlipidemia prevalence in 2011 in Greece was 15% [41], and results from the ATTICA study reported that 1 in 2 adults (45±15) years old was dyslipidemic [42].This is in accordance with current results from HNNHS (44,8% in total; 39.9% in males and 48.3% in females).

Regarding arterial hypertension, the present study's preliminary results are comparable with other studies where hypertension was self-reported (13.1% vs. 13.3%, respectively, n=5,003) [43]. As hypertension is a common risk factor of cardiovascular disease, data on level of awareness is warranted. Efstratopoulos et al., found an awareness level of 60.2% among Greek hypertensive individuals [44], therefore, further investigation is warranted. The prevalence of hypertension in the NHANES study, for those ≥20 years old was also close to the EPIC and HYPERTENSHELL studies (33.5%) [45].

A 4% self-reported prevalence of diabetes mellitus was found in this study as compared to other studies in Greece which have reported values between 7% and 11% [46, 42, 47] HNNHS included information on thyroid and renal function, for which there are no data available in the Greek population. Respective prevalence levels of 13.7% and 0.6% of those ≥20 years old were reported. The increased prevalence in all types of thyroid conditions (hypothyroidism, hyperthyroidism, Hashimoto thyroiditis), especially among women, underlies the value of HNNHS and stresses the need to further investigate risk factors linked to this outcome, such as iodine and vitamin D status, as well as nutritional intake and search for deficiencies. The prevalence of cardiovascular disease, the leading cause of mortality worldwide, found in the study population was 13.9%, in total. This included 7.7% of the total sample that reported having arrhythmia, 1.8% coronary heart disease, 1.3% myocardial infarction, 0.9% angina, 1.1 heart failure, and 1.1% had suffered a stroke.

Furthermore, an increased level of stress-associated disorders including chronic perceived stress (11.6%), depression (4.2%), Crohn's disease (0.4%) or ulcerative colitis (0,4%), and irritable bowel syndrome (6.9%) were found. These outcomes may be associated with the economic crisis seen in Greece over the past years but can also be linked to various nutritional and behavioral factors, that need to be examined. Interestingly, data with regards to perceived change in household budget show that most volunteers perceived change being more severe in 2012 (23.2%) than 2011 (18.3%) and 2013 (12.6%). Details that may have affected these stress-associated disorders, remain to be investigated.

Limitations

Due to the cross-sectional nature of the study, no causal relationships can be formulated. Also, the data presented and analyzed in this first report are from self-reported data. However, many questionnaires were interview-based by trained professionals, and sensitive personal questions were self-completed to decrease selection bias. Furthermore, self-reported data were cross-checked with other related questions during the statistical analysis in an effort to detect potential misclassification. Reporting of data in more depth and comparison with other past small, non-nationally representative surveys in Greece are beyond the scope of this first methodological publication and will be described elsewhere.

Strengths

This is the first national representative study performed in Greece to assess nutrition and health status of the population including all age groups. Questionnaires used in data collection were constructed after performing an extensive literature review and based on other validated questionnaires that have been used in other large national studies and in the Greek population. Another strength is the synergistic action of multiple health care specialists in study design, filed work and data analysis. Furthermore, the use of the especially designed computer software, CAPI, increases reliability of collected data, since it reduces response bias, misclassification and volunteer burden. Measurements, clinical assessment and blood tests performed in a subsample of the population will be used to further validate the preliminary results presented here.

Conclusions

The HNNHS study aims to evaluate the health of the Greek population. The data presented provide a preliminary overview of demographic and lifestyle data of the population. We envision that this study will provide valuable information regarding the health of the Greek population and that it will become a rolling program that will facilitate the development and evaluation of public health policies addressing key risk factors that impact on the health of the Greek population.

Abbreviations

ARCHES: Arkansas Cardiovascular Health Examination Survey; BRFSS: Behavioral Risk Factor Surveillance System; CAPI: Computer Assisted Personal Interview; CDC: Centre for Disease Control; CHD: Coronary Heart Disease; CVD: Cardiovascular disease; COPD: Chronic Obstructive Pulmonary Disease; DM: Diabetes Mellitus; EFSA: European Food Safety Authority; EPIC: European Prospective Investigation into Cancer and Nutrition; FGID: Functional Gastrointestinal Disorders; FPQ: Food Propensity Questionnaire; GSHS: Global School-based Student Health Survey; HDPA: Hellenic Data Protection Authority; HNNHS: Hellenic National Nutrition and Health Survey; IPAQ: International Physical Activity Questionnaire; ISAAC: International Study of Asthma and Allergies in Childhood; MI: Myocardial Infarction; MRC: Medical Research Council; NatCen: NatCen Social Research; NDNS: National Diet and Nutrition Survey; NDSR: Nutrition Data System for Research; NHANES: National Health and Nutrition Examination Survey; NIAAA: National Institute on

Alcohol Abuse and Alcoholism; OR: Odds ratio; QoL: Quality of Life; USA: United States of America.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Department of Food Science and Human Nutrition of the Agricultural University of Athens. It was also approved by Hellenic Data Protection Authority. All members of staff signed confidentiality agreements and all participants, as well as the parent/ guardian when required, were asked to sign a consent form.

Consent to publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ Contributions

AZ conceptualized, designed and was the Principal Investigator of the study. AZ and RM coordinated the design of the data collection instruments, coordinated and supervised data collection. GM supervised medical data collection. ID, DK, AVM and IB were involved in every step of the study and made substantial contributions to the design and methodology of data collection as well as the acquisition of data and training of field workers. TN coordinated mobile unit data collection. SMT and KA contributed to the mobile unit data collection and analysis. DBP coordinated sample collection methodology. ID drafted the manuscript equally with EM. EM also supervised the design and the preparation of the data base and carried out the statistical analyses and revised the manuscript. GD, CG, EF, EMT, ET, TES, AV, ES, MC, AK, GK, SZ and AP contributed to parts of methodology. All the authors approved the final manuscript as submitted.

Contributors: EF, ET, TS, AV, ES, MC, AT, GK, SZ, AP contributed to the writing of the protocols and the data collection on the field. All contributors approved the final manuscript as submitted.

Advisory Committee: GC, GD, GD, IM and ER acted as external advisory committee. All the Advisory Committee members approved the final manuscript as submitted.

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Tables

Table 1: Distribution of the sample within Greece.

Prefecture	N	%
Attica	2160	47.2
Central Macedonia	844	18.5
Epirus	59	1.3
Eastern Macedonia, Thrace	193	4.2
Peloponnese	144	3.1
Western Macedonia	99	2.2
Thessaly	238	5.2
Central Greece	104	2.3
Western Greece	219	4.8
Crete	262	5.7
Ionian islands	51	1.1
North Aegean islands	92	2
South Aegean islands	87	1.9

Table 2: Volunteer baseline socio-demographic characteristics by gender.

	Males		**Females**	
	N	%	N	%
	1943	42.5	2629	57.5
Age				
0-19	426	21.9	443	16.9
20-64*	1259	64.8	1805	68.7
20-39	*797*	*41.0*	*1040*	*39.6*
40-65	*462*	*23.8*	*765*	*29.1*
65+	258	13.3	381	14.5
Marital status				
Unmarried	841	43.3	1012	38.5
Married	998	51.4	1217	46.3
Cohabitation agreement	2	0.1	2	0.1
Widower	43	2.2	241	9.2
Divorced	47	2.4	127	4.8
Separated	10	0.5	23	0.9
Don’t know	-	-	1	0
Refused	-	-	4	0.2
Educational level				
No or little education	25	1.6	90	4
Primary school	128	8.2	224	9.9
Gymnasium	81	5.2	99	4.4
Lyceum	418	26.7	621	27.3
Technical school	133	8.5	57	2.5
Private college (Post Lyceum)	114	7.3	204	9
University degree (AEI)	336	21.5	517	22.7
University degree (TEI)	144	9.2	219	9.6
Master’s degree	109	7	188	8.3

PhD	31	2	22	1
Refused	4	0.3	3	0.1
Net monthly income (€)				
≤300	76	3.9	106	4
301-650	148	7.6	285	10.8
651-850	171	8.8	264	10
851-1050	237	12.2	283	10.8
1051-1250	172	8.9	236	9
1251-1500	178	9.2	237	9
1501-1900	222	11.4	264	10
1901-2400	183	9.4	231	8.8
2401-3800	177	9.1	202	7.7
>3801	51	2.6	59	2.2
Don't know	122	6.3	214	8.1
Refused	204	10.5	246	9.4
Health insurance				
Uninsured	156	8	162	6.2
Insured, private	91	4.7	105	4
Insured, public	1511	77.8	2071	78.8
Insured, both private and public	157	8.1	252	9.6
Don't know	10	0.5	20	0.8
Refused	4	0.2	3	0.1

*the sampled population (N%) in the age group 20-64, was further categorized to 20-39 years and 40-65 to cross-reference with further analysis performed in these sub-categories.

Table 3: Population's weight status in total by age group and gender based on Body Mass Index (BMI) categorization.

Weight Status Categorization ±	**Total**		**By age group* and gender**					
	Total Adult Population*		**20-39**[a] **N (%)**		**40-64**[b] **N (%)**		**65+**[c] **N (%)**	
	N	%	M	F	M	F	M	F
Underweight	175	4.7	12 (1.5)	88 (8.5)	5 (1.1)	25 (3.3)	8 (3.1)	37 (9.8)
Normal weight	1772	47.9	420 (52.7)	722 (69.5)	139(30.2)	335 (43.8)	60 (23.3)	94 (24.8)
Overweight	1183	32.0	285 (35.8)	160(15.4)	212 (46.0)	244 (31.9)	127 (49.2)	154 (40.6)
Obese total	572	15.5	80 (10.0)	69 (6.6)	105 (22.8)	161 (21.1)	63 (24.4)	94 (24.8)

N (%), Frequency (percentage); M, males; F, Females

By gender: % of males or females in question compared to total number of males or females, respectively

± based on BMI (kg/m^2) categorization: <18.5 = underweight; 20-25=normal weight; >25-30=overweight; >30=obese

*Study population ≥20 years of age; Chi square test for difference in weight status between age groups in total (p<.001) and per gender (p<.001)

[a] Chi square test for difference in weight status between genders in 20-39-year-old group (p<0.001)

[b] Chi square test for difference in weight status between genders in 40-65-year-old group (p<0.001)

[c] Chi square test for difference in weight status between genders in 65+ year-old group (p<0.006)

Table 4: Frequency of alcohol consumption habits in minors and adults in total and by gender.

	Alcohol consumption*						
Adults (20+ years)	Total		Males		Females		Level of significance [a]
	N	%	N	%	N	%	
The past 30 days*							
No	998	26.9	285	18.8	713	32.8	p<0.001
Yes	2685	72.4	1229	81.1	1454	67.0	
Frequency							
Everyday	183	6.8	128	10.4	55	3.8	p<0.001
Weekly	874	32.6	456	37.1	418	28.8	
Monthly	1628	60.6	645	52.5	981	67.5	
**Minors (12-19 years) ** **							
Ever consumed							
No	229	67.4	142	89.3	153	84.5	p=0.121
Yes	111	32.6	17	10.7	28	15.5	
Don't know	1	0.5					
Refused	-	-					

**for adults (20 + years of age: N= 3705 in total, 7 missing): any alcohol consumption the past 30 days and frequency of consumption; ** for minors (12-20 years of age, N=340, 159 males and 181 females): whole alcoholic drink consumed at some point in life (and not just few sips). 66 minors were <18 years and 45 18 &19 years old*

[a] tested via chi square test for gender differences in adult population (20 years +) and in minors (up to 19 years); *p<0.05; **p<0.01; ***p<0.001;

Table 5: Frequency of smoking habits in total population among adults and minors by gender.

	Smoking**						
Adults (20+ years)	Total		Males		Females		Level of significance [b]
	N	%	N	%	N	%	
Ever smoked							
No	1935	52.4	620	41.0	1215	56.0	p<0.001
Yes	1878	50.9	893	59.0	955	44.0	
The past 30 days*							
No	2433	65.7	934	61.5	1497	68.5	p<0.001
Yes	1252	33.8	580	38.2	672	30.8	
Frequency							
Every day[a]	1093	87.3	519	89.5	574	85.4	P=0.046
Some days [a]	158	12.6	60	10.3	98	14.6	
Don't know	1	0.1					
Refused	-	-					
Minors (10-19 years)							
Ever smoked							
No	100	76.3	33	70.2	67	79.8	p=0.229
Yes	29	22.1	12	25.5	17	20.2	
Don't know	1	0.8					
Refused	1	0.8					

**for adults (>19 years of age: 3705 in total): ever smoking; for minors specified if they even tried it (then response yes)*

[a] for adults smoking the past 30 days (frequency (%) of smoking for smokers N=1252)

[b] tested via chi square test for gender differences in adult population (20 years +) and in minors (up to 19 years); *p<0.05; **p<0.01; ***p<0.001;

Table 6: Physical activity levels among different age groups based on self-reported data.

Physical activity	**≥2 - <12 years**		**≥12 - <18 years**		**≥18 - <65 years**		**≥65 years old**	
	N	%	N	%	N	%	N	%
Sedentary way of life	-	-	-	-	-	-	128	20
Low activity	15	3.2	24	11.7	584	18.3	117	18.3
Moderate active, average	126	26.7	74	35.9	1357	42.4	205	32.1
Very active	324	68.6	100	48.5	812	25.4	160	25
Don’t know	-	-	1	0.5	2	0.1	1	0.2
Refuse to respond	-	-	-	-	2	0.1	-	-

* Individuals were asked to report their perceived physical activity status or to state their child’s if they responded on their behalf.

Table 7: Prevalence of chronic disease in adult population sampled, in total, by gender and by gender and age group.

Presence of disease/ condition	**Total**				**By gender and age group[a]**					
	Total Sample		**By Gender [b]**		**20-39**		**40-64**		**65+**	
			N (%)		**N (%)**		**N (%)**		**N (%)**	
	N	%	M	F	M	F	M	F	M	F
Increased cholesterol or triglycerides[1]	765	16.7	297*** (15.2)	468 (17.8)	62** (7.8)	48 (4.6)	127 (27.6)	226 (29.5)	103* (39.9)	183 (48.3)
Don't know	175	3.8								
Hypertension	608	13.3	241 (12.4)	367 (14.0)	21* (2.6)	11 (1.1)	88 (19.1)	124 (16.2)	132* (51.2)	231 (61.0)
	47	1.0								
Coronary Heart Disease	69	1.8	53*** (3.4)	16 (0.7)	0 (0)	1 (0.1)	17 (3.7)	1 (0.1)	36*** (14.0)	14 (3.7)
Don't know	32	0.8								
Angina	36	0.9	19 (1.2)	17 (0.8)	6 (0.8)	4 (0.4)	6 (1.3)	2 (0.3)	7 (2.7)	10 (2.6)
Don't know	31	0.8								
Myocardial Infarction (Heart attack)	49	1.3	37 (2.4)	12 (0.5)	0	0	16** (3.3)	5 (0.7)	21*** (8.1)	7 (1.9)
Don't know	13	0.3								
Heart failure	42	1.1	16 (1.0)	26 (1.1)	0	3 (0.3)	2 (0.4)	8 (1.1)	14 (5.0)	15 (4.0)
Don't know	27	0.7								
Arrhythmia	295	7.7	91** (5.8)	204 (9.0)	21 (2.6)	48 (4.6)	25 (5.4)	71 (9.3)	45 (17.4)	78 (20.6)
Don't know	42	1.1								
Stroke	41	1.1	18 (1.1)	23 (1.0)	1 (0.1)	2 (0.2)	3 (0.7)	4 (0.5)	14 (5.4)	17 (4.5)
Don't know	11	0.3								
Cancer	53	1.2	14** (0.7)	39 (1.5)	3 (0.4)	1 (0.1)	3** (0.6)	28 (3.7)	8 (3.1)	10 (2.6)

Don't know	8	0.2								
Diabetes (Type I & II)[2]	162	3.6	73	89	3	4	27**	21	42	64
			(3.8)	(3.4)	(0.4)	(0.4)	(5.9)	(2.7)	(16.3)	(16.9)
Don't know	24	0.5								
Thyroid (any type of condition) [3]	629	13.8	93***	536	36***	160	26***	248	24***	113
			(4.8)	(20.4)	(4.5)	(15.4)	(5.6)	(32.4)	(9.3)	(29.8)
Don't know	102	2.2								
Asthma	184	4.0	69	115	40	48	8*	37	6	20
			(3.6)	(4.4)	(5.0)	(4.6)	(1.7)	(4.8)	(2.3)	(5.3)
Don't know	16	0.4								
Chronic Obstructive Pulmonary Disease (COPD)	63	1.6	25	38	5	8	9	15	11	15
			(1.6)	(1.7)	(0.6)	(0.8)	(2.0)	(2.0)	(4.3)	(4.0)
Don't know	77	0.6								
Chronic kidney disease	27	0.6	13	14	3	1	2	4	8	9
			(0.7)	(0.5)	(0.4)	(0.1)	(0.4)	(0.5)	(3.1)	(2.4)
Don't know	3	0.1								
Osteoporosis[4]	206	5.4	13***	193	1	3	4***	95	8***	95
			(0.8)	(8.3)	(0.1)	(0.3)	(0.9)	(12.3)	(3.1)	(25.8)
Don't know	79	2.1								
Arthritis/ Rheumatoid disease	324	7.1	65***	259	9*	23	28***	106	28***	128
			(3.3)	(9.9)	(1.1)	(2.2)	(6.1)	(13.9)	(10.8)	(33.8)
Don't know	83	1.8								
Crohn's disease or Ulcerative colitis	16	0.4	6	10	2	3	1	6	3	1
			(0.3)	(0.4)	(0.2)	(0.3)	(0.2)	(0.8)	(1.2)	(0.3)
Don't know	6	0.1								
Irritable Bowel Syndrome (IBS)	317	6.9	53***	264	25***	105	18***	121	9*	35
			(2.7)	(10.1)	(3.1)	(10.1)	(3.9)	(15.8)	(3.5)	(9.2)
Don't know	46	1.0								
Depression	180	4.2	42***	138	15	33	12***	62	15	42
			(2.3)	(5.6)	(1.9)	(3.2)	(2.6)	(8.1)	(5.8)	(11.1)
Don't know	63	1.5								
Chronic Stress	495	11.6	128***	367	56***	143	42***	134	25**	78

			(7.1)	(14.9)	(7.0)	(13.8)	(9.1)	(17.5)	(9.7)	(20.6)
Don’t know	39	0.9								

[a] tested via chi square test for gender differences by age group; [b] tested via chi square test for gender differences in total sample; *p<0.05; **p<0.01; ***p<0.001;

By gender: % of males or females who reported as having the outcome in question compared to total number of males or females, respectively

By age-group: Number of outcomes reported per gender in each age-group (%)

[1]3.5 % of the sample replied that they do not know for cholesterol; <1% for hypertension, coronary heart disease, angina, myocardial infarction (0.3), stroke (0.3), heart failure, ; arrhythmia (1.1%), diabetes (0.53), 2.2% for any thyroid disease, asthma (0.35%), chronic obstructive pulmonary disorder (0.63%). Kidney failure (0.1%), 2.1% for osteoporosis, 1.8% for arthritis, 0.1% for Crohn’s disease, 1.0% for irritable bowel syndrome, 1.5% for depression, 0.91% for chronic stress

[2] prevalence for type I diabetes: 3/4754

[3] 0-19 age group: for thyroid disease: Males (1.6%) and females (3.4%); For asthma: Males (3.5%), Females (2.3%); For chronic stress: Males 1.55%, females 4.3%

[4] out of which 13 osteopenia

PAPER II

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Title Page

Micronutrient Intakes and their Food Sources among Children and Adolescents in Greece: Data from the Hellenic National Nutrition and Health Survey (HNNHS)

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Abstract

Background and Aims: Adequate dietary intake of vitamins and minerals in childhood and adolescence is important for growth and prevention of negative implications to health. To help design effective dietary interventions, it is important to know what foods contribute to micronutrient intakes. Recent analyses of data on vitamin and mineral intakes and on their dietary sources in Greek children and adolescents are lacking. Therefore, the purpose of this study was to assess the usual dietary intake of twenty micronutrients and to identify their food sources in a representative sample of Greek children and adolescents.

Methods: Vitamin and mineral intakes were estimated from two 24-h recalls in 598 participants 1 to 19 years old in the Hellenic National Nutrition and Health Survey (HNNHS). Estimates were calculated using the National Research Council method and the statistical software package Stata13 to account for within- and between- person variation. The prevalence of nutrients' inadequacy among sample was estimated with the use of Estimated Average Requirement (EAR). The contribution of 38 food groups to nutrient intake was estimated to identify micronutrients food sources.

Results: A substantial percentage of children and adolescents had insufficient intakes of numerous micronutrients. Usual intake of vitamins D, K and potassium was inadequate in practically all individuals. Vitamin A, folate, calcium, magnesium were also insufficient in a considerable percentage, especially in girls aged 14-18 years. Pantothenic acid was highlighted as nutrient of interest since only one out of ten boys 9-13 y and girls 14-19 y had intake above the EAR. Data demonstrated that food groups highly ranked in energy contribution were not necessarily important sources of micronutrients.

Conclusions: Results suggest that micronutrient density of children and adolescents' diet should be improved in Greece. These findings might be used by public health policy makers to help young people optimize their food choices in Greece.

Keywords: usual intake; nutrients; children; adolescents; food groups; Greece

Abbreviations used: HNNHS, Hellenic National Nutrition and Health Survey; EAR, Estimated Average Requirement; AUA, Agricultural University of Athens; HDPA, Hellenic Data Protection Authority; AMPM, Automated Multiple-Pass Method; NDSR, Nutrition Data System for Research; EIrep, reported Energy intake; BMR, Basal Metabolic Rate; CV, Coefficient of variation; NRC, National Research Council method; IOM, Institute of Medicine; AI, Adequate Intake.

1. Introduction

Childhood and adolescence are critical periods in the life course in terms of appropriate nutrition due to the increased nutritional needs for growth and development. Unhealthy dietary habits are of concern as they contribute to the epidemic of obesity, but can also contribute to inadequate micronutrient intakes. The impact of prolonged low micronutrient intakes can have negative health effects [1, 2], such as vitamin A deficiency as a cause of xerophthalmia, impaired immunity, retarded growth and anemia [3]; insufficient intakes of iron and of the B vitamins association with different types of anemia and with lower cognitive performances [4]; calcium and vitamin D intakes as determinants of bone mineral content and density [5-7] granted that approximately 40% of total bone mass is accumulated [8, 9]. Additionally, in the longer term, food choices and eating patterns are established early in life [10, 11]. Adopting thus a healthful diet in childhood and adolescence may further help reduce the risk of non-communicable chronic disease in adulthood [12, 13].

Although the European food environment supplies an overabundance of energy and nutrients, several national dietary surveys, such as the Dutch National Food Consumption Survey [14], the German Health Interview and Examination Survey for Children and Adolescents [15] and the Spanish National Dietary Survey on the Child and Adolescent Population (ENALIA) [16], indicate inadequate intakes for several nutrients. It has been suggested that this phenomenon could be explained by changes observed in many industrialized countries regarding food consumption patterns and an increase toward the consumption of foods with a low micronutrient density (i.e., fast-food, sugar-sweetened beverages, salty snacks) [5, 17, 18]. To help design effective interventions for achieving nutrient adequacy, it is valuable to know the types of foods that contribute substantially to micronutrient intakes.

In Greece, with the exception of certain studies carried out either at regional level [19] or at national level but in limited age ranges [20], a survey examining dietary intake among representative sample of children and adolescents has not been conducted to date. The objectives of this study were to: (a) provide updated estimates of the usual intake of 20 micronutrients – vitamins A, D, E, K, C, B_6, B_{12}, thiamin, niacin, riboflavin, pantothenic acid, folate, calcium, phosphorus, magnesium, iron, zinc, copper, selenium and potassium – for Greek children and adolescents aged 1 to 19 years using dietary data from the Hellenic National Nutrition and Health Survey (HNNHS); (b) evaluate nutrient intake adequacy in the population; and (c) get insight into the main food sources of nutrients.

2. Materials and Methods

2.1 Study population

HNNHS was a cross-sectional study launched in Greece between September 2013 and June 2015, designed to assess health and nutritional status of a nationally representative, for both sex and age, sample. The survey methodology included a multistage probability sampling design stratified by geographical region, age group and gender. Briefly, the total sample of the population included men and women aged 6 months of age and over residing in Greece; recruitment of breastfeeding and/or pregnant women, and individuals residing in institutions (e.g., nursing homes, prisons) was not allowed. HNNHS was conducted by the Department of Food Science and Human Nutrition of the Agricultural University of Athens (AUA), and all study procedures were approved by the Ethics Committee of the Department of Food Science and Human Nutrition of AUA and by Hellenic Data Protection Authority (HDPA). Written informed consent for inclusion was obtained from all participants or proxies before they participated in the study.

Of the 4574 survey respondents, 842 children and adolescents aged 1-19 years provided at least one 24-h recall (18.4% of the total population studied). After excluding recalls with extreme energy intakes (<600 and >6,000 kcal per day; n=87), participants were classified as plausible, under-reporters and over-reporters; a total of 244 children were excluded from the analysis due to over- and under- reporting (n=57 and n=172, respectively). The identification of misreporters of energy intake is explained in section "*2.3 Identification of plausible reporters*". The final sample included 598 (71% of the original sample) children and adolescents.

2.2 Dietary Intake

Detailed information on dietary intake was collected by trained interviewers using a computerized 24-h recall which was designed based on the validated USDA Automated Multiple-Pass Method (AMPM) [21]. Proxies, most commonly a parent, were used to collect dietary data for children aged 1-6 years, and assisted with the dietary interview for children aged 7-11 years; adolescents aged 12-19 years reported their own dietary intakes. Estimations of the portion size were facilitated through use of appropriate by age food atlases and of standardized household measures (e.g., cups, glasses, grids). Two 24-h recalls, the first in-person and the second by telephone, were conducted ~10 – 14 days apart. Of the 598 plausible children and adolescents, 416 (69.6%) provided two dietary recalls. HNNHS was conducted for 22 months and thus, encompassed all seasons. Values for energy and micronutrients were estimated using mainly the Nutrition Data System for Research (NDSR) (developed by the University of Minnesota), as well as Greek food composition tables for traditional Greek recipes (e.g., moussakas) [22].

2.3 Identification of plausible reporters

Misreporting (over- and under- reporting) was assessed using the Goldberg cut-off method [23] at the individual level, according to the ratio of the reported energy intake (EIrep) and the predicted Basal Metabolic Rate (BMR). In particular, EIrep was the average value of energy intakes for each 24h dietary recall. BMR was estimated using Schofield's age- and sex-specific equations [24], taking into account body weight and height (when the latter was reported). Lower and upper cut-off values were calculated to classify subjects as plausible, under-reporters and over-reporters using the following equations:

$$EI_{rep}: BMR > PAL * \exp\left(SD_{min} * \frac{S/_{100}}{\sqrt{n}}\right)$$

and

$$EI_{rep}: BMR < PAL * \exp\left(SD_{max} * \frac{S/_{100}}{\sqrt{n}}\right)$$

where

$$S = \sqrt{\frac{CV_{wEI}^2}{d} + CV_{wB}^2 + CV_{tP}^2}$$

The within-subject coefficient of variation (CV) for EI (CV_{WEI}), the within-subject CV for BMR (CV_{WB}) and the total between-subject CV for physical activity (PA, CV_{tP}) were substituted by the revised factors of Black [23]. The number of subjects (n) was set to one. The number of days (d) was set to one or two, depending on whether we had one or two 24-h dietary recall, respectively. PAL of 1.55 was applied for all participants, assuming moderate activity levels [25, 26]. According to these criteria, 172 participants (20.4%) were considered under-reporters and 57 participants (6.8%) were considered over-reporters and were therefore excluded from the analysis reducing the sample size to 598 children and adolescents.

2.4 Food grouping

To determine food sources of nutrients, it was first important to design the appropriate food grouping scheme. Food items were classified in 38 food groups (**Figure 1**), each of which included foods of similar nutrient composition and culinary use. Composite foods (e.g., sandwiches) and recipes (e.g., moussakas) were disaggregated to their ingredients, which were further allocated to their appropriate food group; pizza, souvlaki and burger were not disaggregated, but instead they formed the *"fast-food"* group due to their high energy intake and low micronutrient intake. An assumption was made that the ingredients of a composite food or a recipe, equally contributed to the total nutrients' value of the specified food.

The percentage contribution made by any one food group to the intake of a given nutrient was estimated in the total sample. This was calculated by dividing the sum of all subjects' intakes from the specific food group by the sum of all subjects' nutrient intake from all foods (and multiplying this by 100) [27].

2.5 Assessment of non-dietary variables

A general questionnaire, interview based, was used to record demographic data. Those data included sex, age and country of origin; family income was not assessed due to a high percentage of missing data (21.6%). Furthermore, body weight and height were reported; when the participant was unable to self-respond (i.e., young age), a parent/ guardian was asked to respond.

2.6 Statistical Analysis

The normality of the distribution of the continuous variables was tested using the Kolmogorov–Smirnov test. Continuous variables are expressed as the medians, 25th and 75th percentiles, whereas categorical variables are expressed as percentages (%).

Nonparametric Man-Whitney test was used to compare gender differences for skewed variables and Student t test for continuous, normally distributed data. Chi square tests were conducted for categorical variables.

The National Research Council method (NRC) [28] was applied to estimate the usual intake distribution of 20 vitamins and minerals, after removing the effects of day-to-day (within subject) and subject-by-subject (between-subject) variability in dietary intakes. In brief, the NRC consists of four steps: (1) examining normality of intake distribution (log transforming if required); (2) estimating the within- and between-person variance; (3) adjusting individual subjects' mean intakes to estimate the distribution of usual intakes; and (4) back transforming the adjusted data (if original data were transformed in step 1). The usual (adjusted) dietary intake (step 3) was calculated by the following equation [28]:

$$Adjusted\ intake = \left[(subject's\ mean - group\ mean) * \frac{SD_{between}}{SD_{observed}}\right] + group\ mean$$

where $SD_{between}$ is the square root of true variance of the distribution of usual intakes, and $SD_{observed}$ is the square root of the variance in the observed daily intakes that is not accounted for by between-subject differences, both divided by the mean number of days of intake data per participant. In case that someone had only one 24-h dietary recall (26.92% of plausible participants), the mean number of days of intake data was set to one.

The Estimated Average Requirement (EAR) cut-off point method proposed by the Institute of Medicine (IOM) was used to assess the adequacy of vitamin and mineral intakes of children and adolescents [29]. The IOM DRIs were used since they are regularly updated and frequently used compared to reference values provided by other scientific bodies of organizations [20]. The EAR is the average daily nutrient level estimated to meet the requirements of 50% of all healthy individuals in a particular life stage and group. The proportion of individuals in a group with usual intakes below the EAR provides an estimate of the prevalence of inadequate intakes in the group [29]. For vitamin K, pantothenic acid and potassium, the proportion below the Adequate Intake (AI) was used to assess nutrient adequacy of groups, as there are no established EAR values. The AI is defined as a recommended average daily nutrient intake level, based on experimentally derived intake levels or approximations of observed mean nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

All analyses were performed using the statistical software package Stata13 and a p value < 0.05 was considered statistical significant.

3. Results

Characteristics of the studied sample are presented in **Table 1**. Participants were equally distributed to males and females (48% and 52%, respectively, P=0.3), had a Greek nationality (96%), and gave a second 24h recall (response rate 69.6%).

The usual dietary intakes of vitamins and minerals are summarized in **Tables 2** and **3**, respectively.

Vitamins

In both sexes and all age groups, the percentage of the participants with a usual intake of vitamins D and K below EAR reached 100%. Exceptions to this conclusion were males aged 19 years for vitamin D (71.43% inadequacy), males aged 14-18 years for vitamin K (75% inadequacy) and females aged 19 years for vitamin K (75.76% inadequacy). Nearly all children aged 1-13 years met the EAR for the fat-soluble vitamin A. Inadequate intake in vitamin A (>60%) was recorded in the oldest age groups, with the subset of female adolescents 14-18 being far below the acceptable range (98.57%). Similarly, the prevalence of vitamin E and pantothenic acid inadequacy was quite high among the sample. Greek children and adolescents met intake recommendations for the remaining water-soluble vitamins in general. However, more than 50% of the participants aged 9-18 years had folate intakes below the specific sex and age EAR. Additionally, more than 50% of females aged 14-19 and males aged 19 years had low vitamin C intake.

Minerals

The prevalence of inadequacy was extremely high for potassium (K) in every subset of the population ranging from 85.71% to 100%. Dietary inadequacy was also high for calcium (Ca) and magnesium (Mg), in the subset of adolescents aged 14-18 years. Also, approximately

1 out of 10 girls aged 14-18 y reported intakes of Ca and Mg below the EAR (88.57% and 87.14%, respectively). Regarding zinc (Zn), the prevalence of inadequacy was in general low, except the subset of male adolescents aged 14-18 years (72.31%).

Dietary intake of phosphorus (P) was sufficient for most age groups other than 9-18 year old female participants and for 9-13 year old males. Similarly, only the subset of females aged 14-19 showed a slight inadequacy of copper (Cu) intake. Finally, the inadequacy percentages for iron (Fe) and selenium (Se) were small ranging from 0%-1.54% in boys and 1.43%-6.06% in girls, and 0%-14.29% in boys and 0%-11.43% in girls, respectively.

Tables 4 and **5** show the percentage contribution of food groups to the intake of energy and the studied vitamins and minerals, respectively, for all Greek children and adolescents. In **figures 2, 3** and **4**, the three main predominant food sources are presented.

Energy and vitamins

Processed cereals were the major source of energy (17.7%) for 1- to 19-year olds, followed by milk (9.1%) and fast-food (8.9%). In general, processed cereals together with milk were the two highest ranked dietary sources of many vitamins. More specifically, processed cereals alongside milk had the highest contribution to vitamin A - alongside milk- (21.2%), thiamin (36.2%), riboflavin (23.1%), niacin (33.1%), vitamin B_6 (21.7%) and folate (41.9%). Processed cereals were also among the top three sources of vitamins D (10.6%), E (11.7%), K (6.5%), and B_{12} (21.3%), and pantothenic acid (11.9%). Milk was found to be the prevailing source of vitamin D, reporting the highest contribution of a food group to a vitamin, of pantothenic acid (25.6%) and of vitamin B_{12} (25.3%). Furthermore, the dairy products, milk and cheese, were important contributors to riboflavin (22.9% and 6.8%, respectively). Cheese was also ranked as the third food source for vitamin A (8.3%).

As far as wholegrain cereals are concerned, the specific food group was ranked as the second or third highest contributor of certain vitamins: thiamin (7.4%), pantothenic acid (7.2%), folate (19.4%), vitamins B_6 (13.4%) and B_{12} (16.5%). Non-starchy vegetables provided less than half of vitamin K intake (39.9%); this group also provided 14.5% of vitamin C. Fruit and fruit juices predominated as dietary sources of vitamin C (17.4% and 16.6%, respectively). Lastly, salty snacks were the main food source of vitamin E (12.7%).

Minerals

Processed cereals were the highest ranked dietary source of Mg, Fe, Zn, Cu and selenium (Se) (15.2%, 40.2%, 20.4%, 17.8% and 30.9%, respectively). This food group was also ranked as second major source of P (12.5%) and third major source of Ca (11.5%). Milk was the first contributor to Ca, P and K (30.6%, 20.6% and 16%, respectively) and was also the second highest food source of Mg and Se. In addition, the dairy product cheese was placed among the top three contributors of Ca and P (13.4% and 9.1%, respectively). Wholegrain cereals, red meat, water and fruits were recorded as the second higher food source of Fe (12.7%), Zn (12.3%), Cu (11.1%) and K (11.1%), respectively.

4. Discussion

This study provides recent estimates of the usual dietary intakes for 20 micronutrients in a representative sample of Greek children and adolescents. Except for vitamin D, for which the major source is exposure to sunlight, a well-balanced and healthy diet should provide for most of vitamins and minerals. However, a high prevalence of inadequate micronutrient intake is highlighted, as more than half of the population consumes most vitamins and minerals below the EAR reference values used here. Additionally, this study provides insight in the major dietary sources of those nutrients.

Our study highlights the poor mean vitamin D intake, and the fact that the prevalence of inadequacy was over 93% across all age and sex groups, with the exception of the nineteen year old boys. Our results are in line with other European epidemiologic surveys conducted in Spanish children and adolescents [16], in Belgian adolescents and in European adolescents

[30]. This is due to the fact that vitamin D occurs naturally in very few foods (oily fish, such as salmon, cod liver oil and egg yolks) [31]. However, the low consumption of those foods (**Table 4**) makes it unrealistic that individuals would achieve the recommended dietary intake. Food fortification and the use of supplements have been reported to impact the %<EAR for vitamin D in Northern European countries [32, 33], USA [27] and Canada [33], and could set the basis for improving vitamin D status in Greece.

Vitamins K and E were also identified as nutrients of public health concern from this analysis (rates of inadequate prevalence ranging from 62% to 100%). Regarding vitamin K, almost all individuals had intakes that were at risk. This prevalence is quite high relative to the HELENA study [30], although differences may be due to different eating patterns. More specifically, Manios et al. [34] concluded that 86% - 93% of boys and girls in Greece aged 9-13 years did not meet the recommended portions for vegetables, which are the primary food source of vitamin K [35]. Regarding vitamin E, the findings of the current study are consistent with those reported by NHANES 2003-2006, which showed that 83% of children and adolescents failed to meet the recommendations, except for those taking supplements [36]. Similar data were observed in Spanish adolescents [16]. Vitamin E deficiency is more frequently found in children compared to adults due to low body reserves and intense growth and development, which lead to a faster appearance of deficiency symptoms [37]. Dietary consumption of nuts, oils and whole grains is positively associated with increasing alpha-tocopherol concentrations [38].

As for vitamins A and C, a substantial portion of adolescents had usual intakes below the EAR. While the adequacy in the intake of those two vitamins has been reported to several European studies [14, 16, 39], data from the current analysis are comparable to the percentages reported for children and adolescents in the United States [27]. The relative high rates of vitamins A and C inadequacy could be related to the lower adherence to the

Mediterranean diet [18]. Increasing fruit and vegetable consumption may improve the intake of those micronutrients and lower the risk of deficiency [40].

The low levels of folate among children and adolescents over 9 years old raises concerns, especially for female adolescents of whom only 6% had intakes above the EAR. Other nutritional studies have also described folate dietary intake by adolescents as problematic [16, 30]. Adequate intake among women of reproductive age is essential not only for the prevention of feta neural tube defects during pregnancy [41], but also for the synthesis of nucleic acids (DNA and RNA) [42], for essential functions of cell metabolism [43] and for the prevention of hyperhomocysteinemia [44] and megaloblastic anemia [43]. Recently, Martiniak et al. [45] investigated the potential intake of dietary folate of the population based on the data of the German National Dietary Survey II under consideration of different food fortification level and evaluated the percentage of the population meeting the recommended intake. The consumption of foods with a high fortification level did lead to a substantial decrease in the prevalence of inadequacy.

Children and adolescents aged over 9 years old did not meet the recommendations of the water-soluble vitamin pantothenic acid, with boys aged 9-13 years and girls aged over 14 years showing the lowest adequacy. Surveys providing data on pantothenic acid are scarce in Europe [46]. Foods rich in this nutrient include meat products, eggs, nuts and vegetables [47], and the low consumption of those foods by Greek children and adolescents (**Table 4**) could explain the prevalence of suboptimal pantothenic acid intake.

Regarding minerals, the low intakes of K in all age and gender categories are a cause of concern, taking into account the beneficial effect of potassium intake on blood pressure and cardiovascular outcomes [48]. Considering that dietary patterns established in childhood can set the scene for future food choices, it is important to encourage a diet for children that is high in potassium-rich foods. Similar results have been observed in other European preschoolers [49], children and adolescents [16, 30].

Regarding Ca and Mg, the proportion of the usual intake below EAR showed an increase by age and the highest proportions were observed in adolescents, especially girls. Several published studies [16, 27, 50] indicate that those micronutrients are under-consumed by adolescents relative to the EAR. Adequate intake of the two most important minerals for skeletal development during adolescence is crucial for decreasing the incidence of osteoporosis in adult life [51]. Further, there is more concern on bone health for female children and adolescents aged 9-18 years and male children aged 9-13 years, since one quarter of those subgroups combine a low phosphorus diet [52].

The EAR recommendations for Fe, Se, Zn and Cu were met by almost all children and adolescents. The exceptions to this were Zn and Cu. Data showed that approximately one third of adolescent males had low usual Zn intakes. In agreement with this study, similar median daily intakes of Zn have been reported by adolescents in the NDNS survey [8]. This mineral is primarily found in animal products and seafood [53], and the low consumption of those food groups could explain the low intakes. As for Cu, findings indicated that girls over 14 years old were the subgroup less likely to reach the recommendations.

Faced with high prevalence of inadequate nutrient intakes among children and adolescents, it was important to identify principal sources of energy and examined micronutrients. This analysis showed that major sources of energy in young people’s diet are not necessary the same food groups that provide rich sources of nutrients. Five food groups including fast-food, desserts and sweets, baked products, salty snacks, and sugar-sweetened beverages provided cumulatively 25.6% of energy intake, with no noticeable contribution to other nutrients. In contrast, dairy products (milk, yogurt and cheese) accounted for 15.9% of energy intake and provided a variety of nutrients, such as vitamin D, calcium and pantothenic acid. The conclusion that foods providing empty calories are highly ranked as sources of energy is consistent with reports that many children and adolescents do not follow dietary recommendations [5].

One of the main strengths of this analysis is that HNNHS is the first nationally representative survey in Greece, to our knowledge, and therefore its dataset can provide a robust basal analysis of the nutrient status of the Greek population. Adjustments for the effects of day-to-day (within subject) and subject-by-subject (between subject) variability to estimate usual intakes using the NRC method also strengthens this report. Further, with regards to the above, the assessment period of HNNHS covered all months of the year and all days of the week were included, thus the effects of intakes' variability by season and of day-to day variability could be removed. Since 24-hour recalls are prone to under- and over-reporting, misreporters were identified and excluded from the analysis. The age- and sex-groups differed significantly in their adherence to the EAR recommendations in nearly all nutrients (data not shown). Moreover, the use of standardized and validated procedures to record data (dietary intake, anthropometric and demographic characteristics) could be considered as another asset of the survey.

Some limitations of the study are worth noting when implementing the findings. First, the small HNNHS sample size in young ages, besides its representativeness, could be listed as one of the main limitations. Further, self-reporting of food intake data via 24-hour recall introduces bias (e.g., such as misreporting because of respondent memory lapses, or miss-estimation of portion size consumed) to the dietary intake data [54], despite the fact that misreporters were excluded. Another important issue is the lack of detailed Greek food consumption tables and the use of NDSR food and nutrient database for this analysis.

The present paper provides a detailed analysis of the current dietary intake of vitamins and minerals, and their food sources among Greek children and adolescents. Identification of micronutrients of concern and awareness of food sources can help public health policy makers design and promote effective population-based strategies to reduce energy consumption and increase nutrient density of the diet. Overall, health professionals should educate children and adolescents establish balanced food choices (possibly with the addition of fortification policy or supplements for some crucial nutrients).

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Statement of Authorship

AZ conceptualized, designed and was the Principal Investigator of the study. AZ and RM coordinated the design of the data collection instruments, coordinated and supervised data collection. GM supervised medical data collection. AVM, DK, ID, and IB were involved in every step of the study and made substantial contributions to the design and methodology of data collection as well as the acquisition of data and training of field workers. TN coordinated mobile unit data collection. SMT and KA contributed to the mobile unit data collection and analysis. DBP coordinated sample collection methodology. AVM carried out the statistical analyses and drafted the manuscript. EM supervised the design and preparation of the database, supervised the statistical analysis, and revised the manuscript. GD, GG, EF, EMT, ET, TES, AV, ES, MC, AK, GK, SZ and AP contributed to parts of methodology. All authors approved the final manuscript as submitted.

Contributors: EF, ET, TS, AV, ES, AT, GK, SZ, AP contributed to the writing of the protocols and the data collection on the field. All contributors approved the final manuscript as submitted.

Advisory Committee: GC, GD, GD, IM and ER acted as external advisory committee. All members of the Advisory Committee approved the final manuscript as submitted.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Table 1 Characteristics of 1-19 year old Greek children and adolescents providing plausible dietary intake data in HNNHS.

	Total	Boys	Girls	P[a]
n (%)	598 (100)	287	311 (52)	0.328
Age (years), X±SD[b]	9.8±5.6	9.5±5.3	10.1±5.8	0.108
Age group, n (%)				
1-3 years	96 (16)	43 (15)	53 (17)	0.788
4-8 years	186 (31.1)	95 (33.1)	91 (29.3)	0.576
9-13 years	134 (22.4)	70 (24.4)	64 (20.6)	0.599
14-18 years	135 (22.6)	65 (22.6)	70 (22.5)	0.989
19 years	47 (7.8)	14 (4.9)	33 (10.6)	0.530
Dietary Information (%)				
1st recall	598 (100)	287 (100)	311 (100)	0.051
2nd recall	416(69.6)	212 (73.9)	204 (65.6)	0.065
Nationality (%)				
Nationals	574 (96)	277 (96.5)	297 (95.4)	0.505
Non Nationals	24 (4)	10 (3.5)	14 (4.6)	0.894

Data are expressed as n (%), unless indicated.

[a]P-values are derived from t-test to detect significant differences (P< 0.05) between sex groups.

[b]X is referred to mean age and SD to its standard deviation.

[c]The symbol "-" indicates that the test could not be implemented because there were insufficient data.

Table 2 Usual intakes of vitamins stratified by sex and age group in 1-19 year old Greek children and adolescents, and percentage of the population having usual micronutrient intakes below the Estimated Average Requirement: HNNHS.

	Boys			Girls		
	Median (P25-P75)	**EAR**	**<EAR(%)**	**Median (P25-P75)**	**EAR**	**<EAR(%)**
Vitamin A (mcg)						
1-3 years	403.7 (340.1-536.4)	210	4.65	409.8 (291.1-469.9)	210	7.55
4-8 years	462.4 (367-582.9)	275	4.21	472.1 (399.5-593.6)	275	6.59
9-13 years	534.4 (381.2-825)	445	32.86	564.5 (415-772.2)	420	25
14-18 years	552.1 (410.8-699)	630	67.69	455.9 (447.7-460.4)	485	98.57
19 years	505 (412-1003.3)	625	64.29	476.8 (276.5-814.3)	500	51.52
Vitamin D (mcg)						
1-3 years	5.9 (3.8-6.9)	10	93.02	4.6 (4.1-4.9)	10	98.11
4-8 years	5.1 (3.7-6.7)	10	95.79	4.7 (3.6-6.2)	10	98.90
9-13 years	4.7 (3.9-5.8)	10	100	4.7 (3-6.7)	10	93.75
14-18 years	4.2 (4.1-4.3)	10	100	3.9 (3.2-4.3)	10	100
19 years	5.6 (2.7-11.8)	10	71.43	3.9 (2.6-5.3)	10	93.94
Vitamin E (mg)						
1-3 years	4.1 (3.5-5)	5	74.42	4.6 (3.8-5.6)	5	62.26
4-8 years	5.6 (5-6.1)	6	68.42	5.4 (4.9-6.2)	6	69.23
9-13 years	6.2 (5-8)	9	87.14	6.9 (5.2-8.1)	9	84.38
14-18 years	7.6 (6-8.9)	12	96.92	6 (5-7.1)	12	100
19 years	7.4 (6.2-10.1)	12	92.86	7.4 (5.5-8.5)	12	100
Vitamin K (mcg)						
1-3 years	15.3 (10.4-20.5)	30*	93.02	14.5 (11-21.2)	30*	90.57
4-8 years	18 (16.1-20)	55*	100	19 (13.1-24.3)	55*	100
9-13 years	20.1 (12.6-28.6)	60*	92.86	21 (10.1-26.8)	60*	96.88
14-18 years	25.8 (17-44.8)	75*	95.38	19.8 (16.1-25.6)	75*	100
19 years	33.2 (8.9-50.1)	120*	85.71	36.4 (16.8-61.5)	90*	75.76

	Boys			Girls		
	Median (P25-P75)	EAR	<EAR(%)	Median (P25-P75)	EAR	<EAR(%)
Vitamin C (mg)						
1-3 years	47.6 (24.1-84.6)	13	6.98	37.5 (27.6-65.9)	13	9.43
4-8 years	50.2 (33.5-66.4)	22	14.74	48.9 (34.6-67.5)	22	6.59
9-13 years	45.8 (39.5-53.7)	39	18.57	55.7 (35.2-87.5)	39	28.13
14-18 years	66.5 (45.2-96.5)	63	44.62	50.1 (35.7-60.1)	56	67.14
19 years	59.2 (43.8-77.4)	75	71.43	59.6 (41.5-65.8)	60	51.52
Thiamin (mg)						
1-3 years	1.3 (1.2-1.4)	0.4	2.33	1.3 (1.1-1.3)	0.4	0
4-8 years	1.4 (1.3-1.5)	0.5	0	1.4 (1.3-1.5)	0.5	0
9-13 years	1.5 (1.4-1.7)	0.7	0	1.5 (1.3-1.8)	0.7	0
14-18 years	1.4 (1.4-1.5)	1	0	1.4 (1.3-1.6)	0.9	0
19 years	1.8 (1.4-2)	1	0	1.7 (1.3-2.2)	1	3.03
Riboflavin (mg)						
1-3 years	1.7 (1.4-1.9)	0.4	0	1.5 (1.3-1.7)	0.4	1.89
4-8 years	1.7 (1.5-2)	0.5	0	1.6 (1.5-1.9)	0.5	0
9-13 years	1.8 (1.6-2)	0.8	0	1.8 (1.6-2.1)	0.8	1.56
14-18 years	1.8 (1.8-1.9)	1.1	0	1.7 (1.5-1.9)	0.9	0
19 years	2 (1.7-2.6)	1.1	0	2.1 (1.6-2.5)	0.9	0
Niacin (mg)						
1-3 years	12.1 (11.7-12.7)	5	2.33	11.1 (9.3-12.1)	5	5.66
4-8 years	12.1 (10.1-15.9)	6	1.05	11 (9-14.4)	6	9.89
9-13 years	13.5 (10.9-18.8)	9	14.29	13.7 (11.6-16.5)	9	3.13
14-18 years	19.8 (14.4-24.9)	12	13.85	13.9 (11.6-16.4)	11	15.71
19 years	19.8 (14.7-34.6)	12	14.29	16.6 (14.7-22.7)	11	3.03

	Boys			Girls		
	Median (P25-P75)	**EAR**	**<EAR(%)**	**Median (P25-P75)**	**EAR**	**<EAR(%)**
Pantothenic acid (mg)						
1-3 years	3.1 (2.6-3.5)	2*	2.33	3.2 (2.9-3.5)	2*	1.89
4-8 years	3.2 (2.5-4.1)	3*	36.84	3.1 (2.5-3.8)	3*	43.96
9-13 years	3.2 (3.1-3.4)	4*	97.14	3.3 (2.6-4.1)	4*	67.19
14-18 years	3.9 (3.2-4.7)	5*	80	3.1 (2.5-3.8)	5*	97.14
19 years	4.2 (2.7-5.4)	5*	71.43	3.5 (2.5-4.1)	5*	90.91
Vitamin B_6 (mg)						
1-3 years	1.2 (1.1-1.5)	0.4	2.33	1.1 (0.9-1.3)	0.4	1.89
4-8 years	1.4 (1.2-1.6)	0.5	1.05	1.4 (1.2-1.5)	0.5	0
9-13 years	1.6 (1.3-1.8)	0.8	0	1.6 (1.3-2)	0.8	3.13
14-18 years	1.8 (1.6-2.1)	1.1	3.08	1.4 (1.2-1.7)	1	10
19 years	2.1 (1.6-2.6)	1.1	7.14	1.6 (1.4-2.1)	1.1	6.06
Folate (mcg)						
1-3 years	190.2 (146.3-225.1)	120	16.28	207.5 (190.6-218.9)	120	5.66
4-8 years	213.5 (171.1-306.2)	160	17.89	214.9 (155.1-268.6)	160	26.37
9-13 years	242 (208.6-285.3)	250	62.86	247.5 (191.8-317.6)	250	51.56
14-18 years	257.7 (222.8-336.9)	330	73.85	237.2 (196.2-269.8)	330	94.29
19 years	411.5 (177.9-619.2)	320	35.71	342.2 (254.5-539.1)	320	42.42
Vitamin B_{12} (mcg)						
1-3 years	2.7 (2.3-3.4)	0.7	2.33	2.9 (2.5-3.1)	0.7	1.89
4-8 years	3 (2-4.7)	1	3.16	2.8 (2.5-3.2)	1	1.10
9-13 years	3.2 (2.6-4)	1.5	0	3.4 (2.3-4.5)	1.5	9.38
14-18 years	3.4 (2.7-4.4)	2	9.23	2.4 (1.9-3.9)	2	28.57
19 years	4.3 (2.4-9)	2	21.43	3.6 (1.6-5.7)	2	30.30

Abbreviation: EAR= Estimated Average Requirement.

*Adequate intake (AI) and therefore, the percentages of subjects below AI have been calculated.

Table 3 Usual intakes of minerals stratified by sex and age group in 1-19 year old Greek children and adolescents, and percentage of the population having usual micronutrient intakes below the Estimated Average Requirement: HNNHS.

	Boys			Girls		
	Median (P25-P75)	**EAR**	**<EAR(%)**	**Median (P25-P75)**	**EAR**	**<EAR(%)**
Calcium (mg)						
1-3 years	902.8 (686.7-1027.4)	500	4.65	813.7 (641.2-1050)	500	11.32
4-8 years	957.4 (796.7-1080.9)	800	25.26	912.8 (779.5-1039.5)	800	28.57
9-13 years	1066.2 (786.8-1343.4)	1100	52.86	992.7 (752.3-1189.5)	1100	64.06
14-18 years	1032.2 (913.3-1200.9)	1100	60	905.6 (814.5-985.9)	1100	88.57
19 years	1076.6 (911.2-1342.9)	800	21.43	891.5 (748.6-1073.8)	800	30.30
Phosphorus (mg)						
1-3 years	995.4 (832.1-1109.3)	380	0	953.3 (794.7-1046.5)	380	1.89
4-8 years	1057.9 (1018.7-1089.9)	405	0	990 (879.7-1140.2)	405	0
9-13 years	1095.3 (1019-1200.5)	1055	37.14	1123.4 (980*1233.9)	1055	42.19
14-18 years	1355.4 (1169.5-1648)	1055	9.23	1061.3 (921.6-1202.7)	1055	50
19 years	1156.4 (1089.5-1187.1)	580	0	1065.2 (1056.6-1078.4)	580	0
Magnesium (mg)						
1-3 years	188.9 (165.2-217.8)	65	2.33	198.9 (187.1-207.5)	65	1.89
4-8 years	210.7 (201.7-219.9)	110	0	206.3 (190.7-217.7)	110	1.10
9-13 years	209.2 (19.2-237)	200	28.57	215.2 (183.4-241.9)	200	40.63
14-18 years	277 (240.6-333.8)	340	76.92	219.5 (195-247.1)	300	87.14
19 years	334 (241-423.7)	330	50	246.7 (212.9-341.1)	255	51.52
Iron (mg)						
1-3 years	9.9 (9.4-10.9)	3	0	10.1 (9.7-10.6)	3	1.89
4-8 years	10.2 (8-13.5)	4.1	0	10.1 (8.2-12)	4.1	2.20
9-13 years	11.5 (10.8-12.3)	5.9	0	12.5 (10.3-15.4)	5.7	1.56
14-18 years	13.6 (11.9-16.9)	7.7	1.54	11.3 (10.3-12.2)	7.9	1.43
19 years	17.7 (12.6-20.5)	6	0	14.1 (12.6-20)	8.1	6.06

	Boys			Girls		
	Median (P25-P75)	EAR	<EAR(%)	Median (P25-P75)	EAR	<EAR(%)
Zinc (mg)						
1-3 years	7.5 (6.8-8.6)	2.5	0	7 (5.9-8.3)	2.5	1.89
4-8 years	8.2 (8-8.5)	4	0	7.9 (7.4-8.6)	4	0
9-13 years	8.4 (8.1-8.9)	7	2.86	8.9 (7.5-10.2)	7	17.19
14-18 years	8.4 (8.3-8.5)	8.5	72.31	8.2 (7.1-9.2)	7.3	30
19 years	10.9 (8.4-14.6)	9.4	35.71	8.9 (7.3-11.5)	6.8	18.18
Copper (mg)						
1-3 years	0.6 (0.5-0.7)	0.26	2.33	0.7 (0.6-0.7)	0.26	0
4-8 years	0.7 (0.6-0.7)	0.34	1.05	0.7 (0.6-0.8)	0.34	2.20
9-13 years	0.8 (0.6-0.9)	0.54	11.43	0.7 (0.6-0.8)	0.54	6.25
14-18 years	0.8 (0.9-1.1)	0.685	10.77	0.7 (0.6-0.8)	0.685	38.57
19 years	1 (0.8-1.2)	0.7	21.43	0.8 (0.7-0.8)	0.7	30.30
Selenium (mg)						
1-3 years	59.8 (44.4-76.6)	17	0	57 (43-71.2)	17	1.89
4-8 years	66 (65.2-67)	23	0	62.8 (50.2-76.9)	23	3.30
9-13 years	72.2 (60.3-80.9)	35	0	67.2 (65.1-69.6)	35	0
14-18 years	80.4 (70.5-96)	45	3.08	77.5 (56.-95)	45	11.43
19 years	96.6 (62.5-130.1)	45	14.29	67.1 (65-68.4)	45	0
Potassium (mg)						
1-3 years	1938.5 (1603.7-2137.6)	3000*	97.67	1886.7 (1584.8-1992.2)	3000*	98.11
4-8 years	1915.5 (1736.2-2160.9)	3800*	98.95	1958.4 (1759.5-2145.8)	3800*	100
9-13 years	2053.9 (1836.1-2289.3)	4500*	100	2050.8 (1731.4-2296.2)	4500*	100
14-18 years	2691.1 (2196.1-3165.2)	4700*	93.85	2000.4 (1896.8-2083.6)	4700*	98.57
19 years	3057.5 (1985.4-3864.9)	4700*	85.71	2125.1 (1831.2-2801.1)	4700*	90.91

Abbreviation: EAR= Estimated Average Requirement.

*Adequate intake (AI) and therefore, the percentages of subjects below AI have been calculated.

Table 4 Percentage contribution of food groups to energy and vitamins in the diets of 1-19 year old Greek children and adolescents: HNNHS.

Food Groups	Energy	Vitamin A	Vitamin D	Vitamin E	Vitamin K	Vitamin C	Thiamin	Riboflavin	Niacin	Pantothenic acid	Vitamin B_6	Folate	Vitamin B_{12}
	%	%	%	%	%	%	%	%	%	%	%	%	%
Fruits	4.0	2.0	0.0	3.7	7.0	17.4	2.2	2.4	2.7	6.3	8.1	4.4	0.0
Fruit juice	0.8	0.4	0.0	0.1	0.1	16.6	1.2	0.4	0.5	1.2	0.5	2.1	0.0
Non-starchy vegetables	4.0	5.9	0.1	8.0	39.9	14.5	3.6	2.0	0.9	1.4	5.7	3.3	0.2
Starchy vegetables	0.4	0.1	0.0	0.8	0.9	1.4	0.5	0.2	0.1	0.1	0.4	0.3	0.0
Potato	3.6	0.1	0.0	4.8	6.2	6.3	2.8	0.8	3.3	6.3	7.2	1.1	0.0
Wholegrain cereals	2.1	6.0	1.3	8.8	0.6	4.0	7.4	6.4	8.2	7.2	13.4	19.4	16.5
Processed cereals	17.7	21.2	10.6	11.7	6.5	10.2	36.2	23.1	33.1	11.9	21.7	41.9	21.3
Legumes	0.5	0.0	0.1	0.6	0.3	0.3	0.5	0.2	0.1	0.2	0.6	0.8	0.1
Nuts	0.4	0.0	0.0	2.1	0.2	0.1	0.4	0.2	0.3	0.3	0.3	0.4	0.0
Milk	9.1	21.2	57.4	2.4	1.9	0.6	6.9	22.9	1.4	25.6	5.2	3.9	25.3
Yogurt	1.3	2.9	0.6	0.6	0.3	0.1	0.5	1.7	0.1	1.8	0.4	0.2	1.7
Cheese	5.5	8.3	2.1	2.0	2.4	1.2	3.1	6.8	2.4	4.2	3.3	2.8	7.3
Eggs	2.0	2.7	2.6	2.3	0.8	0.6	0.9	2.7	0.1	2.5	1.6	1.0	1.7
Fish and Shellfish	1.3	0.9	9.7	2.3	0.9	0.2	1.1	1.1	3.0	2.1	1.9	0.6	5.7
Red meat	4.9	1.0	5.0	3.1	2.8	1.4	5.5	4.5	9.3	5.9	6.0	1.0	7.5
White meat	2.7	0.7	0.4	1.5	1.8	0.1	1.1	1.9	8.7	5.5	4.6	0.6	1.9
Processed red meat	2.5	3.1	1.2	0.9	1.0	0.1	2.9	1.9	2.7	2.0	1.3	1.6	2.3
Processed white meat	0.4	0.0	0.0	0.6	0.4	0.2	0.2	0.3	0.7	0.6	0.4	0.1	0.2
Processed fish	0.1	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.3
Olive oil and Olives	4.4	1.1	0.0	9.3	3.5	7.9	2.6	1.4	0.0	0.1	4.0	0.1	0.0
Vegetable fat	1.0	1.3	0.0	2.0	1.2	0.1	0.3	0.4	0.0	0.1	0.3	0.1	0.1

Food Groups	Energy	Vitamin A	Vitamin D	Vitamin E	Vitamin K	Vitamin C	Thiamin	Riboflavin	Niacin	Pantothenic acid	Vitamin B_6	Folate	Vitamin B_{12}
	%	%	%	%	%	%	%	%	%	%	%	%	%
Animal fat	2.3	3.1	0.9	1.1	1.7	0.1	1.9	1.5	1.9	1.5	0.8	1.5	1.4
Alcoholic beverages	0.5	0.0	0.0	0.1	0.2	1.0	0.1	0.2	0.2	0.2	0.3	0.3	0.0
Sugar-sweetened beverages	1.6	2.0	0.0	0.8	0.7	9.9	0.5	0.6	0.6	1.1	1.2	0.7	0.2
Artificially-sweetened beverages	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salty snacks	3.2	0.1	0.0	12.7	4.1	1.1	1.9	1.2	1.6	0.8	1.5	2.4	0.1
Desserts and Sweets	6.5	4.1	2.9	5.2	3.2	0.7	2.2	3.8	1.2	4.0	1.4	2.0	3.0
Condiments and Spices	0.1	0.0	0.0	0.1	0.2	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Salt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Coffee	0.8	0.3	0.8	1.6	0.3	0.0	0.2	1.5	9.6	0.8	0.2	0.1	0.5
Tea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Artificially sweeteners	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sugar	0.5	0.1	0.0	0.2	0.2	0.5	0.0	0.1	0.1	0.1	0.1	0.1	0.0
Baked products	5.4	3.3	2.2	2.7	5.2	0.0	4.4	3.4	3.1	2.6	0.8	4.8	1.6
Artificially-sweetened Fruit juices	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fast-Food	8.9	6.7	0.6	5.6	4.9	1.7	7.2	5.0	3.2	2.7	4.7	2.0	0.6
Baby food	0.3	0.3	1.0	0.4	0.5	0.2	0.6	0.8	0.5	0.7	0.3	0.2	0.5

Table 5 Percentage contribution of food groups to minerals in the diets of 1-19 year old Greek children and adolescents: HNNHS.

Food Groups	Calcium	Phosphorus	Magnesium	Iron	Zinc	Copper	Selenium	Potassium
	%	%	%	%	%	%	%	%
Fruits	1.1	1.8	6.7	2.0	1.4	8.8	0.6	11.1
Fruit juice	0.3	0.4	1.1	0.4	0.1	1.3	0.0	2.1
Non-starchy vegetables	2.4	3.4	4.6	4.2	4.2	2.4	0.5	7.1
Starchy vegetables	0.2	0.3	0.5	0.5	0.3	0.2	0.0	0.7
Potato	0.6	2.3	4.7	2.7	2.3	9.4	0.5	8.7
Wholegrain cereals	0.8	1.9	2.9	12.7	4.7	3.5	3.8	1.1
Processed cereals	11.5	12.5	15.2	40.2	20.4	17.8	30.9	7.6
Legumes	0.2	0.5	0.8	1.0	0.6	0.6	0.1	0.8
Nuts	0.1	0.5	1.0	0.3	0.4	1.8	0.3	0.3
Milk	30.6	20.6	11.6	0.8	10.7	7.3	10.9	16.0
Yogurt	2.6	1.6	0.9	0.1	0.8	0.4	0.7	1.3
Cheese	13.4	9.1	3.5	2.4	8.0	1.9	5.7	2.1
Eggs	1.1	2.3	1.0	1.5	2.7	0.2	2.6	1.3
Fish and Shellfish	0.5	2.2	1.8	1.0	1.0	1.6	5.7	1.7
Red meat	0.8	6.3	3.2	3.2	12.3	3.1	8.8	5.0
White meat	0.4	3.4	2.1	2.2	4.8	2.0	6.6	2.2
Processed red meat	3.0	2.8	1.7	1.8	2.8	1.5	3.2	1.2
Processed white meat	0.0	0.6	0.3	0.4	0.6	0.6	1.0	0.3
Processed fish	0.0	0.1	0.1	0.0	0.1	0.2	0.3	0.0
Olive oil and Olives	1.5	2.6	2.5	3.1	3.3	0.2	0.1	4.3
Vegetable fat	0.7	0.7	0.6	0.6	0.6	0.8	0.1	0.5
Animal fat	2.8	2.2	1.4	1.5	1.9	1.2	2.6	0.8
Alcoholic beverages	0.1	0.2	0.3	0.1	0.1	0.3	0.1	0.3
Sugar-sweetened beverages	0.4	0.4	1.1	0.6	0.3	1.8	0.1	1.6
Artificially-sweetened beverages	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Salty snacks	0.6	1.2	1.7	1.3	0.7	0.9	2.1	1.2
Desserts and Sweets	3.8	4.0	5.0	2.9	3.7	9.3	3.4	3.2
Condiments and Spices	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Water	5.1	1.4	6.1	1.4	2.8	11.1	0.0	1.6
Coffee	1.2	1.8	9.1	1.7	0.4	1.2	1.0	8.7
Tea	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1
Artificially sweeteners	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sugar	0.1	0.1	0.2	0.1	0.1	0.4	0.1	0.3
Baked Products	1.8	3.2	2.2	3.6	1.5	4.2	4.6	1.4
Artificially sweetened Fruit juices	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fast-Food	11.3	9.0	5.2	4.0	5.7	3.3	3.2	4.8
Baby food	0.8	0.5	0.5	1.9	0.6	0.4	0.3	0.4

Fig.1. Food groupings used when determining food sources of nutrients.

Fruits	**Egg**	**Artificially-sweetened beverages**
Fresh fruits, cooked or dried	Eggs	Carbonated artificially-sweetened beverages
Fruit juice	**Fish and Shellfish**	**Salty snacks**
Natural fruit juices unsweetened	Fish fresh and frozen	Chips, crackers, pop corn
Non-starchy vegetables	Shellfish	**Desserts and Sweets**
Green leafy vegetables	**Red meat**	Sweets, candy, chocolate
Tomatoes, carrots, lettuce	Lamb, pork, veal, game	Milk desserts
Mixed and other vegetables	**White meat**	Sugary foods (i.e., baklavas)
Vegetable juice	Poultry	**Condiments and Spices**
Starchy vegetables	**Processed red meat**	Condiments
Corn, beans, green beans	Sausages, ham, salami, beacon of red meat origin	Spices
Pumpkin	**Processed white meat**	**Salt**
Sweet potatoes	Sausages of white meat origin, Chicken nuggets	Salt
Potato	**Processed fish**	**Water**
Potatoes	Smoked, canned and salted fish	Water natural, mineral and carbonated
Wholegrain cereals	Fish sticks	**Coffee**
Wholegrain cereal products	**Olive oil and Olives**	Coffee
Processed cereals	Olive oil	**Tea**
White cereal products	Oils	Tea
Legumes	**Vegetable fat**	**Artificially sweeteners**
Legumes (i.e., beans)	Vegetable oils, vegetable fat, vegetable oil-based salad dressing	Artificially sweeteners
Meat alternatives, soy, tofu	**Animal fat**	**Sugar**
Nuts	Butter, mayonnaise	Sugar, honey, syrup
Nuts, almonds, seeds	White sauce, cream	**Baked products**
Peanut butter	**Alcoholic beverages**	Cake, biscuit, pie, muffin, doughnuts
Almond milk	Alcoholic beverages	**Artificially sweetened Fruit juices**
Milk	**Sugar-sweetened beverages**	Artificially sweetened fruit juices
Milk and milk drinks	Carbonated and non-carbonated sugar-sweetened beverages	**Fast-Food**
Yogurt		Pizza, souvlaki, burger, fried potatoes
Yogurt		**Baby food**
Cheese		Baby food
Cheese		

Fig.2. Top 3 food sources of energy and fat-soluble vitamins in the diets of 1-19 year old Greek children and adolescents: HNNHS.

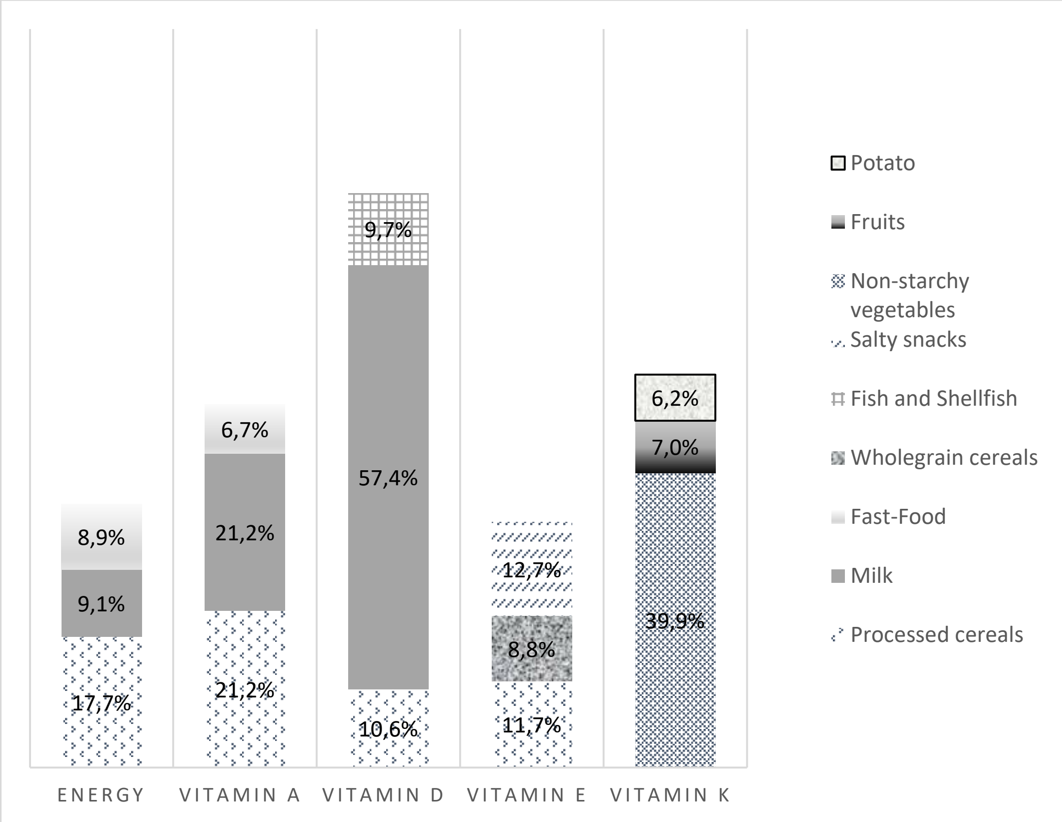


Fig.3. Top 3 food sources of water-soluble vitamins in the diets of 1-19 year old Greek children and adolescents: HNNHS.

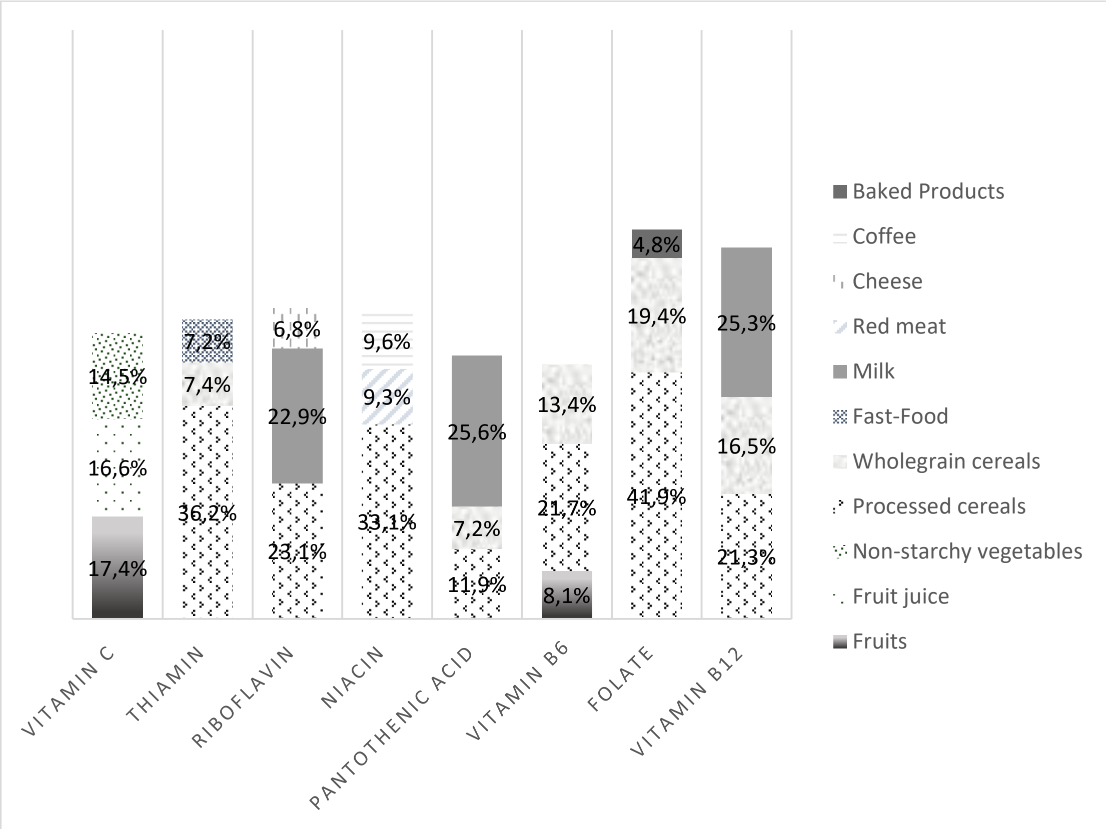
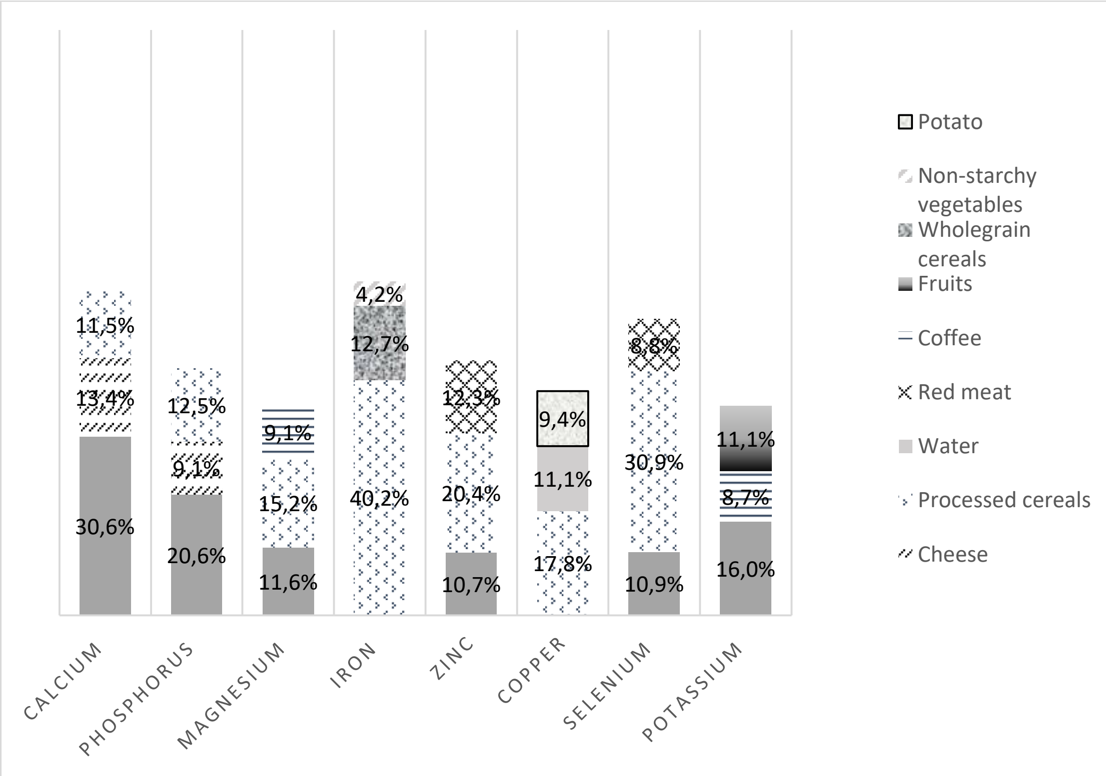


Fig.4. Top 3 food sources of minerals in the diets of 1-19 year old Greek children and adolescents: HNNHS.



PAPER III

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Title page

Association of Meal and Snack Patterns with Nutrient Intakes among Greek Children and Adolescents: Data from the Hellenic National Nutrition and Health Examination Survey (HNNHS)

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Abstract

Purpose: To examine how different meal and snack patterns are associated with micronutrient intakes and diet quality in a representative sample of Greek children and adolescents.

Methods: This cross-sectional analysis included 598 children and adolescents aged 1-3 and 4-19 years from the Hellenic National Nutrition and Health Survey. Meal and snack patterns were derived using 24-h dietary recalls. Mean Adequacy Ratio (MAR) was used as an overall measure of diet quality. Multiple linear regression adjusted for covariates was conducted to examine associations between eating patterns, energy-adjusted nutrient intakes and MAR.

Results: Four most frequently reported eating schemes were identified including breakfast (B), lunch (L), dinner (D) and 2 snacks (S) (22.8%); B, L, D and 1S (17.6%); B, L, D and 3S (11.2%); and B, L and D (7.9%). Based on these schemes, the daily consumption of all main meals from the majority of the sample was highlighted. In children and adolescents aged 4-19 years, increasing snack frequency was positively associated with intakes of vitamins A, D, K, riboflavin, pantothenic acid, folate, magnesium, and copper. An inverse association was recorded for vitamins E, B_6, calcium and iron. Among children aged 1-3, only copper was significantly associated with number of snacks, with the group of “B-L-D-2S” presenting the highest intake. As for the overall diet quality, among all participants there was no significant association of MAR with the type of meal and snack pattern, and thus the snack frequency.

Conclusions: These findings suggest that snacking behavior is a common practice among children and adolescents. Modifying current snack foods with nutrient-rich choices could lead to an improvement of their diet’s nutritional quality.

Key words: meals, snacks, eating occasion frequency, nutrients, children, adolescents

Introduction

It is widely recognized that optimal dietary intake of micronutrients wards off deficiency-related diseases [1,2], and contributes to the prevention of non-communicable chronic diseases [3,4]. Micronutrient intake among children and adolescents are of particular interest, as it has been shown that eating behaviors are developed early in life and tend to track into adulthood [5,6]. Even though these age groups represent a nutritional-bridge into adulthood, a great percentage of children and adolescents have suboptimal micronutrient intakes [7-9], despite adequate food intake in most cases [8]. Other studies demonstrated that children acquired adequate micronutrients, but from ultra-processed foods; however, healthy food consumption was below the recommendation in these specific populations [10,11].

Evidence suggests that research should move beyond specific foods and concentrate on eating patterns, including frequency of eating occasions [12], to account for diet heterogeneity and nutrient interactions. More specifically, although several epidemiologic studies conducted among young populations have related micronutrient intake with amount and types of foods children and adolescents consume [13,8,14], humans acquire these micronutrients via the consumption of a variety of foods within meals and snacks; they do not eat such nutrients in isolation [15,16]. Understanding the nutritional composition of meals and snacks, the total eating occasions, irrespectively of the time these have been consumed, and the way such different meal and snack patterns affect diet quality may be more informative, leading to the notion of eating occasions. In fact, developed dietary guidelines based on already established meal and snack patterns can more easily be adapted by the public as opposed to those regarding a number of different nutrients [17,18].

Research examining the associations of meal and snack patterns with nutrient intake is limited. Specifically, nutrient intake, in association with meal and snack frequency, as well as total eating occasions (EO), have yielded conflicting results in adults [16]. Some studies reported that the type of meals, as well as the total number of snacks consumed daily, is an important determinant of nutrient intake and diet quality [19,20], while others found that the frequency of meals, but not snack consumption is beneficial in meeting nutrient needs [21,22]. To date, these relations have not been thoroughly investigated in younger populations, to our knowledge. One study assessed meal patterns of adolescents and nutrient adequacy [23], while there are few other studies mainly focusing on associations between breakfast consumption and diet quality [24-26]. A better understanding of the relation between EOs and meal and snack patterns with micronutrient intake is necessary, especially in the young population, because intervening early in childhood and adolescence could improve health indicators in the long-term [27,5].

In this context, this study used data from the Hellenic National Nutrition and Health Survey (HNNHS) to examine the relation between the frequency of all EOs and specific meal and snack patterns with energy-adjusted nutrient intakes and overall diet quality among a representative sample of Greek children and adolescents aged 1-19 years old.

Methods

Study design & Sample

HNNHS was a population-based, nationally representative, cross-sectional survey conducted in Greece between September 2013 and July 2015 and the target population consisted of people living in the country and aged ≥6 months. Recruitment of pregnant and lactating women was not allowed, nor included individuals that (i) were not able to speak the national language, (ii) were residing in institutions (e.g. nursing homes, prisons) and (iii) were not able to provide informed consent due to any cause (e.g. mental impairment). The HNNHS 2013-2015 sample selection employed a stratification scheme based on the 2011 census data. Stratification was made according to geographical density by country periphery, and age group and gender distribution. Of the 4,574 respondents, a total of 842 children and adolescents 1–19 years old participated in the survey. HNNHS was approved by the Ethics Committee of the Department of Food Science and Human Nutrition of the Agricultural University of Athens, and by Hellenic Data Protection Authority. All participants or proxies gave their written informed consent for inclusion.

Recalls with extreme energy intakes (<600 and >6,000 kcal per day) were excluded from this analysis (n=15), and subsequently, the plausibility of energy intakes for the remained 827 children and adolescents was assessed with the use of the Goldberg cut-off method updated by Black [28] according to the ratio of EI:BMR. In particular, EI (energy intake) was the average value of energy intakes for each 24h recall, and BMR (basic metabolic rate) was estimated using Schofield's age- and sex-specific equations [29], taking into account body weight and height (when the latter was reported). After the exclusion of misreporters, (over-reporters=57; under-reporters=172), the final size of the sample was reduced to 598 children and adolescents. Of those 598, 182 participants provided one recall day and 416 participants provided two recall days. Thus, 1014 available recall days from 598 unique participants were used for the study analyses.

Dietary assessment

Detailed information on dietary intake from 2 non-consecutive days covering both weekdays and weekends was collected by trained interviewers using a computerized 24-h recall which was designed based on the validated USDA Automated Multiple-Pass Method (AMPM) [30]. The first 24-h recall was collected in person at the time of the interview at the participant's house and the second recall was collected 8-20 days latter over the telephone. Proxy responders (most commonly a parent) were used to collect dietary intake for children aged 1-11 years, and for children older than 11 years the 24-h recall was self-reported. Portion size was mainly estimated by appropriate by age food atlases participants [31,32] and by standardized household measurements (e.g. glasses, cups) in order to ensure quality.

Respondents were asked to indicate the type of each EO and the time when that EO was consumed. The name of each EO was self-reported from a predefined list of possible labels that included breakfast, lunch, dinner, any type of snack, specifically morning snack and/or afternoon snack, beverage, alcohol, extended consumption, other, and do not know. Values for energy and nutrients were calculated using the Nutrition Data System for Research (NDSR) (developed by the University of Minnesota) and the Composition Tables of Foods and Greek Dishes [33].

Eating patterns

Proxies' and participants reported data from the 24-h recalls were used to calculate the total number of EOs per day and to identify meal and snack patterns. For the present analysis, an EO was defined as "*any distinct occasion during which a food and/or beverage was consumed that contained a minimum energy content of 50 kcal*" and was separated in time from the preceding and succeeding EO by >15 minutes [21]. All EOs not reported as breakfast (B), lunch (L) or dinner (D) were classified as snacks (S). Based on the aim of the study, number of snacks consumed per day, not timing, were addressed. In total seventy-nine different meal and snack patterns were identified, however, four of these explained 59.5% of the population (B-L-D; B-L-D-1S; B-L-D-2S; and B-L-D-3S) based on the sample distribution. The rest of meal and snack patterns were classified as "Other". Daily frequency of all EOs, based on the sample distribution, was subsequently calculated and respondents were divided into four categories (1-3, 4, 5, and ≥6).

Nutrients

Mean total dietary intakes of 20 micronutrients were calculated for each available recall day. The residual method was used to adjust all nutrient intakes for total energy, by regressing nutrient intakes on self-reported total energy intake derived from the 24-h recall [34].

Diet quality

Mean Adequacy Ratio (MAR) was calculated as an overall measure of nutrient adequacy of the different meal and snack patterns [35]. Recommended Daily Allowance (RDA) and Adequacy Intake (AI) (when the formers were not available) cut-offs, proposed by the Institute of Medicine (IOM), were used to compute for the Nutrient Adequacy Ratio (NAR). NAR was truncated at 100% so that a nutrient with a high NAR could not compensate for inadequate intake of other nutrient. The MAR was calculated by averaging the NAR for the 20 examined micronutrients.

Confounding variables

Demographic information collected from the respective HNNHS questionnaires included age, sex and ethnicity. Family income was not assessed due to a high percentage of missing data (21.6%). Age was categorized as 1-3, 4-8, 9-13, 14-18, and 19 y, consistent with the categories used in the Nutrient Reference Values [36]. Ethnicity of children and adolescents was categorized into nationals and non-nationals.

Self-reported weight and height were used to calculate body mass index (BMI) (weight/ height^2 in kg/m^2). Participants' classification according to their BMI level as normal, overweight or obese was based on the International Obesity Task Force (IOTF) age- and gender- specific BMI cut-offs points [37].

Screen-related sedentary behavior (SED) in the last week was assessed for ages 4-19 years with the use of questions from the HNNHS questionnaires on physical activity and it was defined as time spent watching television, using computer or playing with electronic devices. The average daily time (min/d) was calculated and participants were divided into two categories: ≥2 hours per day and <2 hours per day as per recommendations from American Pediatric Association [38].

Statistical Analysis

Statistical analyses were performed using the statistical software package Stata version 13.1 and data were stratified by age into two groups (1-3 and 4-19 years), to account for differences in recommended intakes by IOM [36] and for the non-existence of information on sedentary behavior of young children aged 1-3years. After checking the distribution of the micronutrient intakes, these were found highly skewed, hence they were log-transformed to approach normality. All nutrients were energy adjusted prior to analysis.

Bivariate analysis (Pearson's chi-squared test) was performed to assess categorical variable associations ($P<0.05$). Multiple linear regression analyses were implemented to test for associations between eating patterns (daily eating frequency and meal and snack pattern groups) and energy-adjusted nutrients. A series of models were applied with each energy-adjusted nutrient intake being the dependent variable and the daily eating frequency group as the independent variable, after adjusting for the confounding variables ($P<0.05$). An identical series of models were applied having each meal and snack pattern group as the independent variable. All models were tested for multicollinearity issues, using Variance Inflation Factor (VIF).

Mean energy-adjusted nutrient intakes were reported after back-transforming (i.e exponantiating) the log-transformed data. Also, additional adjustment for the confounding variables was made for the calculation of mean nutrient intakes. Analysis of variance (ANOVA) adding Bonferroni adjustment for pairwise multiple comparisons was performed to determine significant differences in nutrients and diet quality between groups of daily eating frequency and meal and snack patterns. Those differences between groups of meal and snack patterns were considered significant if ANOVA's P-value was less than 0.01 (0.05/5 categories), whereas differences between groups of eating frequency were considered significant if ANOVA's P-value was less than 0.0125 (0.05/4 categories). Wald test was used to test the overall significance of eating occasion frequency and of meal and snack pattern variables ($P<0.05$).

Additional analyses were performed to compute the contribution of the food groups to energy intake of snacks by the prevailing categories of meal and snack patterns. Foods and beverages were classified in 38 food groups (listed in **Online Supplemental Table 1**) based on nutrient composition and culinary use. The percentage contribution of each food group to energy intake of snacks was calculated by dividing the sum of energy from the specific food group by the total energy intake of snacks, and multiplying this number by 100.

Results

Tables 1 and 2 display sociodemographic and lifestyle characteristics by categories of meal and snack patterns, and daily eating frequency (stratified by age), respectively. The prevailing meal and snack pattern of the total population (13.9% of children aged 1-3y and 24.4% of children and adolescent aged over 3 years old) was B-L-D-2S. The majority of 1-3 years old participants reported consuming ≥6 eating occasions per day, whereas the majority of older children reported 5 dailies eating occasions. In younger children, BMI categories were associated with type of meal and snack pattern (P=0.019) and daily eating frequency (P=0.008). In older children, sedentary life (P=0.001) and age group (P=0.002) played a significant role on meal and snack pattern, whereas EO frequency was related to only sex (P=0.03) and age group (P=0.001).

Tables 3 and 4 refer to multivariate-adjusted micronutrient intakes for categories of meal and snack patterns and daily eating occasion frequency. For 1-3 years old participants, there was no consistent pattern (increase or decrease) of the mean micronutrient intakes between type of eating pattern and frequency of EOs. Overall, differences in nutrient intakes between eating patterns and frequency of EOs were insignificant (using Bonferroni correction), except for copper. A significant difference of its intake was identified between categories “B-L-D-1S” and “B-L-D-2S”. Regarding 4-19 years old participants, consuming “B-L-D-3S” and eating more than six times a day resulted in reporting the highest mean intake for many micronutrients. Mean intake differences between meal patterns and eating occasion frequencies were identified and deemed significant (P<0.01 and P<0.0125, respectively) for the 60% and 65% of micronutrients, respectively.

Tables 5 and 6 present the results from the multiple linear regression, after adjusting for confounders. Overall, among young children no significant differences were found between energy-adjusted micronutrient intake and meal patterns or frequency of EOs. Significant associations were found between meal and snack patterns with magnesium and copper intake (P=0.049 and P=0.005 respectively), and between EOs’ frequency and vitamin C intake (P=0.035). As for older children and adolescents aged

4-19 years, eating 3 main meals and 3 snacks and having ≥6 EOs daily was positively associated with intakes of eight micronutrients (vitamins A, D, K, riboflavin, pantothenic acid, folate, magnesium and copper). A higher frequency of EOs was also associated with higher intakes of niacin, vitamin B_{12}, calcium, selenium and potassium.

Fig. 1 displays the mean adequacy ratio for adjusted micronutrient intakes by categories of meal and snack patterns and sex. Based on the findings, differences for MAR between meal patterns in the age and sex groups were not deemed significant, for both 1-3 and 4-19 years old participants (P>0.01). For 1-3 years old participants, the MAR of "B-L-D-2S" was higher in males than in females (95.6% vs. 91.2%, respectively). The former value was the highest MAR being calculated. Also, males had higher MAR when reporting "B-L-D" and "B-L-D-3S" meal pattern than in females (95.1% and 94.4% vs. 38.1% and 89.7%, respectively). On the contrary, females had higher MAR in "B-L-D-1S" and "Other" than in males (94.3% and 94.6% vs. 84.8% and 93.5%, respectively). Meanwhile, for 4-19 years old participants, the MAR of "B-L-D-3S" was higher in males (and the highest value of this age group) than in females (88.7% vs. 86.8%, respectively). Females had higher MAR only in "B-L-D" meal pattern category than in males (82.8% vs. 80.6%, respectively). For the rest of eating pattern categories, males' MAR ranged from 82.3% to 87.4%, whereas females' MAR ranged from 81.2% to 87%.

Figs. 2 and **3** illustrate the five predominant food groups that contributed to the energy intake from snacks by categories of meal and snack patterns and age group. Regarding young children aged 1-3 y, "Fruits" contributed most to the energy in the "B-L-D-1S" scheme (39.2%), followed by "Baked Products" (11.5%) and "Yogurt" (8.2%). As snack frequency increased, a reduction in the consumption of "Fruits" was recorded. Among those who reported the "B-L-D-2S" scheme, "Fruits", "Processed cereals" and "Desserts and Sweets" were the most-energy providing foods. In the "B-L-D-3S" category "Desserts and Sweets", "Milk" and "Processed cereals" were the top three reported foods and beverages among young children, whereas in the "Other" category "Baked Products" replaced "Processed cereals" and occupied the third place.

Across all meal and snack patterns, the two most popular snacking food groups in Greek children and adolescents aged 4-19 y were "Desserts and Sweets" and "Baked Products". The highest contribution of "Desserts and Sweets" was observed for the participants belonging to the "B-L-D-1S" category (19.4%) and the highest contribution of "Baked products" was observed for the participants belonging to the "B-L-D-3S" category (17.1%). "Fast-food" was the third most commonly consumed snack food among 4-19 y olds, except for those reporting the “B-L-D-3S” scheme. The ranking of “Fruits” in the top five food groups ranged from third to fifth place and the highest contribution to energy intake was reported in the “B-L-D-3S” scheme (11.9%). Salty snacks and processed cereals also topped the list in certain meal and snack patterns.

Discussion

This study provides a new insight for Greek children and adolescents eating patterns suggesting that specific meal and snack patterns are associated with certain micronutrient intakes. To our knowledge, this is the first study among young people to evaluate the relation between meal and snack patterns (beyond simple EO frequency) and dietary intake.

Next to identifying the most commonly reported schemes (B-L-D; B-L-D-1S; B-L-D-2S; and B-L-D-3S), it was also shown that eating daily all three main meals (breakfast, lunch and dinner) was reported in the majority of the recalled days (59.5% of the total sample). It is widely acknowledged that a regular consumption of meals in the day is associated with healthier food choices and higher nutrient density [39], with the most consistent finding being an inverse relation of skipping breakfast and lower intake of many nutrients [16]. Ovaskainen and colleagues [40] also concluded that a meal-dominating pattern (defined as the majority of daily energy intake derived from meals rather than snacks) was characterized by higher intake of micronutrients (vitamins A, C, E, K, calcium, iron and magnesium), as opposed to a snack-dominating pattern.

In our study, the overall number of snacking episodes was the feature that differentiated the four distinctly examined eating schemes (0-3 snacks per day); hence snacking frequency was the key feature when evaluating the association of eating patterns with daily nutrient intakes. After adjustment for the potential confounders, our findings suggest that snacking frequency among young children aged 1-3 years was not significantly related to micronutrient intakes, except for copper. In contrary, in children and adolescents aged 4-19 years, as snacking level increased, intake of some nutrients increased too. Micronutrients positively and significantly affected by each additional snack episode were vitamin A, vitamin D, vitamin K, riboflavin, pantothenic acid, total folate, magnesium and copper. Additionally, an inverse association between a higher snack frequency and intakes of vitamin E, vitamin B_6, calcium and iron was reported. Our findings are not precisely consistent with those from previous studies that have examined the association between the number of snacks per day with nutrient intakes. For instance, in a study conducted among US adolescents by Sebastian et al. [41], a higher snack frequency was associated with higher mean daily intakes of vitamin C in both sexes, and of vitamin A, vitamin E and magnesium among boys. Intakes of vitamin B_6, folate, calcium, iron and phosphorus were not influenced by more snacking. Conversely, in another study conducted with US adults, positive associations between overall snacking episodes and intakes of vitamin C, magnesium, folate, calcium, iron and potassium have been reported [19]. Furthermore, in a recent research of Australian adults, findings suggested that the frequency of meals, but not of snacks, was positively associated with the intake of the four micronutrients that were also examined in our study [21]. Specifically, snack frequency was related to substantially higher intakes of calcium only.

The conflicting findings for snack frequency across studies may be in part explained because of the lack of consistency in the definitions used as to what constitutes a meal and a snack [16]. The main approaches to label EOs are: participant-identified, time-of-day, types and/or amount of food consumed, and interviewer assessment [16]. The present study relied on participants to label their eating occasion; however, different perceptions within and between populations of what meals and snacks constitute could explain the non-consistent findings [19]. Before the effects of eating patterns (frequency of meals, snacks and overall EOs) on health outcomes can be fully explained, a universally accepted definition of meal and snack is crucial [16]. Furthermore, comparison of the HNNHS results and the findings of the aforementioned studies may be difficult, because of the differences in the ages of study participants. Food and beverage choices made by adults during snacking episodes (and therefore their nutritional value) are different for younger people. Finally, a recent study compared foods consumed by children during snacking between Australia, China, Mexico and the United States, and researchers concluded that food items at snacks may differ by country [42]. In agreement with this study, contributions of fruits, dairy products, bread, sweetened beverages and fatty-sweet products to energy intake for overall snacking was different in French adults as compared to US and Finish adults [43].

Snacking incidence for all age groups has increased over the last years in Western countries [43] and the present study confirms that this behavior is a common practice also among Greek children and adolescents; indeed 51.6% of the total sample population reported an eating scheme that consisted of the main meals and at least one snack. After assessing the overall diet quality with the use of MAR, there was no significant association of MAR with the examined eating schemes, and thus the number of snack episodes. Our findings suggest that snack choices made by children and adolescents can be improved. Despite differences in the prevalence of snacking, there was a common feature in the foods and beverages consumed as a snacking event. The main sources of snacking energy in the population were “Desserts and Sweets” and “Baked Products”. “Fruits” was the most reported item in younger children who consumed one or two snacks daily, but dropped among older children. Our results are consistent with trends from previous studies in children in the United States, in Mexico and in China [42,44,45], and suggest that snacking reflects less healthy choices. Similarly, among Greek adults, a small study (n=200) concluded that two of the most popular snack items were desserts (chocolates, cakes, ice cream) and savory pies [46].

Substitution of the current snack foods with nutrient-rich choices should be explored further. Some countries have developed specific snack recommendations [47]. For instance, Switzerland provides a comprehensive list of foods and beverages that should be consumed for snacks between meals. Identification of the nutrient insufficiencies and recording the current snack food preferences of the population could be used to develop national dietary guidelines and even promote the development of snacks rich in nutrients of concern.

A major strength of our study lies in the assessment of meal and snack patterns of a national representative sample of Greek children and adolescents. Another strength of this analysis is that it examined energy-adjusted nutrients and that associations of eating patterns, nutrient intakes and diet quality were tested for multiple confounders.

On the other hand, we acknowledge that some limitations of our study need to be mentioned. A major limitation is that HNNHS is a cross-sectional study; as such, it does not allow us to establish causal relations. In addition, it is generally accepted that 24-h recalls are prone to misreporting dietary intake [48]. However, the exclusion of misreporters that was applied in our analysis might have created a biased sample, since under- and over-reporters might have a special food choice or eating behavior. Furthermore, when recalling foods and beverages during the interview, items consumed between meals are more likely to be forgotten by participants than items consumed at the main meal time [49]. The AMPM method, though, that was used to collect dietary intake data is designed to address these concerns. Another limitation is the use of the NDSR food and nutrient database when calculating nutrient intake, due to the lack of a detailed national food composition database. Finally, the lack of a consistent definition of "meal" and "snack" in the literature raises issues when comparing the findings of the current study with others assessing the impact of eating patterns [16].

In conclusion, this study takes a first step toward describing the construction of meal and snack patterns consumed by Greek children and adolescents and understanding the nutritional impact of these patterns. Results highlight the common practice of the three main meals, but also indicate the intake of high prevalence of non-nutrient beneficial snacks. As snacking represents a great opportunity for children and adolescents to meet their nutritional needs and improve the quality of their diet, future studies should examine which food groups are already consumed between meals. Better knowledge of how the food profile of snacks affects nutrient intakes will provide valuable information for practitioners when developing strategies to improve young people's dietary profile.

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Statement of Authorship

AZ conceptualized, designed and was the Principal Investigator of the study. AZ and RM coordinated the design of the data collection instruments, coordinated and supervised data collection. GM supervised medical data collection. AVM, DK, ID, and IB were involved in every step of the study and made substantial contributions to the design and methodology of data collection as well as the acquisition of data and training of field workers. TN coordinated mobile unit data collection. SMT and KA contributed to the mobile unit data collection and analysis. DBP coordinated sample collection methodology. AVM carried out the statistical analyses and drafted the manuscript. EM supervised the design and preparation of the database, supervised the statistical analysis, and revised the manuscript. GD, GG, EF, EMT, ET, TES, AV, ES, MC, AK, GK, SZ and AP contributed to parts of methodology. All authors approved the final manuscript as submitted.

Contributors: EF, ET, TS, AV, ES, AT, GK, SZ, AP contributed to the writing of the protocols and the data collection on the field. All contributors approved the final manuscript as submitted.

Advisory Committee: GC, GD, GD, IM and ER acted as external advisory committee. All members of the Advisory Committee approved the final manuscript as submitted.

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Compliance with ethical standards

The study was approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Demographic and lifestyle characteristics of Greek children and adolescents by type of meal and snack pattern: HNNHS

	1-3 years						4-19 years					
	B-L-D	**B-L-D-1S**	**B-L-D-2S**	**B-L-D-3S**	**Other**	**P[a]**	**B-L-D**	**B-L-D-1S**	**B-L-D-2S**	**B-L-D-3S**	**Other**	**P**
Population (%)	6.33	12.7	13.9	12.7	54.4	-	8.18	18.5	24.4	11.0	38.0	-
Sex												
Female	3	12	11	10	48	0.57	38	79	99	40	175	0.28
Male	7	8	11	10	38		32	79	110	54	150	
Age group												
4-8 years	-	-	-	-	-	-	19	48	80	51	124	0.002
9-13 years	-	-	-	-	-		21	46	68	19	68	
14-18 years	-	-	-	-	-		24	49	43	19	96	
19 years	-	-	-	-	-		6	15	18	5	37	
Ethnicity												
Nationals	10	20	22	19	85	0.57	64	147	203	93	310	0.07
Non nationals	0	0	0	1	1		6	11	6	1	15	
BMI levels												
Normal	3	15	18	17	70	0.019	54	116	143	73	245	0.65
Overweight	3	3	0	1	8		9	25	44	12	52	
Obesity	4	2	4	2	8		7	17	22	9	28	
Sedentary Life												
>=2 hours	-	-	-	-	-	-	58	126	163	77	217	0.001

<2 hours	-	-	-	-	-	12	32	46	17	108

Values are n, unless indicated

[a]Significant differences assessed using Pearson chi-squared test; P<0.05 was considered statistically significant

Table 2 Demographic and lifestyle characteristics of Greek children and adolescents by daily eating occasion frequency: HNNHS

	1-3 years					4-19 years				
	1-3 times	**4 times**	**5 times**	**>=6 times**	**P[a]**	**1-3 times**	**4 times**	**5 times**	**>=6 times**	**P**
Population (%)	14.6	22.8	22.8	39.9	-	5.42	27.6	33.3	23.7	-
Sex										
Female	9	20	19	36	0.51	81	121	136	93	0.030
Male	14	16	17	27		51	115	149	110	
Age group										
4-8 years	-	-	-	-	-	32	69	111	110	0.001
9-13 years	-	-	-	-		34	65	85	38	
14-18 years	-	-	-	-		49	78	62	42	
19 years	-	-	-	-		17	24	27	13	
Nationality										
Nationals	23	36	36	61	0.38	122	222	275	198	0.09
Non nationals	0	0	0	2		10	14	10	5	
BMI levels										
Normal	12	27	30	54	0.008	102	176	200	153	0.51
Overweight	3	5	1	6		20	39	56	27	
Obesity	8	4	5	3		10	21	29	23	
Sedentary Life										
≥2 hours	-	-	-	-	-	97	174	219	151	0.82

<2 hours	-	-	-	-	35	62	66	52

Values are n, unless indicated

[a]Significant differences assessed using Pearson chi-squared test; $P<0.05$ was considered statistically significant

Table 3 Multivariate-adjusted mean nutrient intakes of Greek children and adolescents by type of meal and snack pattern: HNNHS

	1-3 years					4-19 years				
	B-L-D	**B-L-D-1S**	**B-L-D-2S**	**B-L-D-3S**	**Other**	**B-L-D**	**B-L-D-1S**	**B-L-D-2S**	**B-L-D-3S**	**Other**
Vitamin A, mcg/d	712	494	513	520	520	577[a]	465[b]	569[b, c]	589[d]	441[a, c, d]
Vitamin D, mcg/d	8.0	5.9	7.4	7.4	6.0	4.1	3.7	4.4[a]	4.6[b]	3.4[a, b]
Vitamin E, mg/d	6.2	6.6	5.3	4.8	5.5	8.1	7.0	8.0[a]	8.0	6.8[a]
Vitamin K, mcg/d	19.2	17.5	23.9	20.7	21.1	16.3[a, b, c]	19.7[d, e]	27.1[a, d]	28.2[b, e]	25.1[c]
Vitamin C, mg/d	65.7	27.6	38.1	56.7	37.4	43.2	50.9	55.5	58.8	46.8
Thiamin, mg/d	1.6	1.5	1.6	1.7	1.5	1.8	1.7	1.7	1.8	1.6
Riboflavin, mg/d	2.6	2.0	2.1	2.2	2.1	2.1	1.9[a, b]	2.1[a, c]	2.2[b, d]	1.9[c, d]
Niacin, mg/d	9.7	8.9	17.2	14.7	13.7	16.3	15.0	18.2	19.3	16.6
Pantothenic acid, mg/d	3.9	3.7	4.5	3.9	3.8	3.1[a]	3.2[b]	3.6	3.9[a, b]	3.4
Vitamin B_6, mg/d	1.8	1.6	1.3	1.5	1.6	2.0[a]	1.7	1.9[b]	1.9	1.6[a, b]
Total folate, mcg/d	260	196	295	269	218	247[a]	255[b]	306	342[a, b, c]	265[c]
Vitamin B_{12}, mcg/d	4.0	2.9	4.5	4.3	3.8	3.4	2.8[a]	3.6[a, b]	3.6	2.8[b]
Calcium, g/d	1.36	1.09	1.20	1.11	1.03	1.04	0.894	1.02	1.02	0.964
Phosphorus, g/d	1.44	1.24	1.33	1.24	1.26	1.30	1.17	1.23	1.23	1.22
Magnesium, mg/d	298	224	240	237	230	250	238[a, b, c]	269[a]	271[b]	261[c]
Iron, mg/d	14.1	11.4	12.4	12.8	11.5	15.5[a]	14.1	15.2[b]	15.0	12.8[a, b]
Zinc, mg/d	10.3	9.3	11.2	9.3	10.4	10.8	9.4	9.8	10.3	9.5
Copper, mg/d	0.7	0.7[a]	1.1[a]	0.9	0.8	0.7[a, b, c]	0.8[d, e]	0.9[a, d]	0.9[b, e]	0.9[c]

Selenium, mcg/d	69.7	69.8	92.3	75.3	77.0	64.3	74.0	81.3	85.7	76.8
Potassium, g/d	2.54	2.27	2.18	2.11	2.19	2.34	2.23	2.40	2.45	2.27

Values are weighted geometric means. Following the ANOVA table, Bonferroni correction was used for pairwise multiple comparisons between categories of meal and snack pattern. Same superscript letters indicate significant differences between types of patterns

Table 4 Multivariate-adjusted mean nutrient intakes of Greek children and adolescents by daily eating occasion frequency: HNNHS

	1-3 years				4-19 years			
	1-3 times	**4 times**	**5 times**	**>=6 times**	**1-3 times**	**4 times**	**5 times**	**>=6 times**
Vitamin A, mcg/d	449	574	548	578	498	436[a, b]	518[a]	5639[b]
Vitamin D, mcg/d	4.8	6.2	7.7	7.6	3.2[a, b]	3.3[c, d]	4.4[a, d]	4.8[b, c]
Vitamin E, mg/d	6.4	5.3	5.7	5.3	7.4	7.0	7.7	6.9
Vitamin K, mcg/d	21.5	18.5	20.4	21.6	19.5[a, b]	20.1[c, d]	25.7[a, c]	29.8[b, d]
Vitamin C, mg/d	21.4	35.0	47.4	64.7	40.0	47.5	51.8	56.9
Thiamin, mg/d	1.5	1.7	1.5	1.5	1.7	1.7	1.7	1.8
Riboflavin, mg/d	2.1	2.2	2.2	2.2	2.0	1.8[a, b]	2.1[a]	2.2[b]
Niacin, mg/d	13.8	11.6	12.9	12.7	15.8	15.2	17.5	18.6
Pantothenic acid, mg/d	3.6	3.9	4.1	4.0	2.9[a, d]	3.2[b]	3.5[c, d]	4.1[a, b, .c]
Vitamin B_6, mg/d	1.6	1.7	1.4	1.6	1.8	1.7	1.8	1.8
Total folate, mcg/d	206	234	243	246	234[a, c]	246[b]	286[c]	322[a, .b]
Vitamin B_{12}, mcg/d	3.4	3.7	4.2	4.0	2.8[a]	2.7[b, c]	3.5[b]	3.7[a, c]
Calcium, g/d	0.959	1.08	1.20	1.20	0.998	0.889[a, b]	1.03[a]	1.08[b]
Phosphorus, g/d	1.33	1.23	1.33	1.29	1.27[a]	1.16[a, b, c]	1.24[b]	1.26[c]
Magnesium, mg/d	246	230	235	244	246[a]	242[b, c]	268[c]	277[a, b]
Iron, mg/d	13.7	12.5	11.0	11.3	14.0	13.4	14.4	14.1
Zinc, mg/d	11.2	9.6	10.6	9.8	10.8	9.4	9.8	10.0
Copper, mg/d	0.8	0.7	0.9	0.9	0.7[a, b, c]	0.8[a, d, e]	0.9[b, d, f]	1.0[c, e, f]

Selenium, mcg/d	85.8	67.4	82.4	73.8	63.3[a, b]	74.3[c]	80.7[a]	87.5[b, c]
Potassium, g/d	2.08	2.25	2.32	2.35	2.12[a]	2.07[b]	2.35	2.47[a, b]

Values are weighted geometric means. Following the ANOVA table, Bonferroni correction was used for pairwise multiple comparisons between categories of meal and snack pattern. Same superscript letters indicate significant differences between categories of eating occasion frequency

Table 5 Associations between energy-adjusted log-transformed micronutrient intakes and type of meal and snack pattern among Greek children and adolescents: HNNHS

	1-3 years					4-19 years				
	B-L-D-1S	**B-L-D-2S**	**B-L-D-3S**	**Other**	**P**	**B-L-D-1S**	**B-L-D-2S**	**B-L-D-3S**	**Other**	**P**
Vitamin A, mcg/d	-0.365	-0.327	-0.315	-0.315	0.65	-0.216	-0.014	0.021	-0.268	<0.001
Vitamin D, mcg/d	-0.310	-0.076	-0.079	-0.287	0.59	-0.099	0.084	0.122	-0.193	0.008
Vitamin E, mg/d	0.063	-0.152	-0.252	-0.118	0.41	-0.146	-0.006	-0.015	-0.172	0.005
Vitamin K, mcg/d	-0.093	0.216	0.074	0.010	0.86	0.185	0.507	0.547	0.429	<0.001
Vitamin C, mg/d	-0.867	-0.546	-0.148	-0.565	0.52	0.165	0.251	0.310	0.081	0.30
Thiamin, mg/d	-0.066	0.001	0.055	-0.047	0.84	-0.044	-0.039	-0.003	-0.101	0.09
Riboflavin, mg/d	-0.271	-0.172	-0.185	-0.209	0.40	-0.135	-0.003	0.026	-0.118	<0.001
Niacin, mg/d	-0.084	0.569	0.412	0.347	0.09	-0.084	0.106	0.169	0.015	0.07
Pantothenic acid, mg/d	-0.054	0.146	0.006	-0.020	0.47	0.029	0.153	0.238	0.090	0.008
Vitamin B_6, mg/d	-0.080	-0.303	-0.157	-0.122	0.41	-0.190	-0.058	-0.085	-0.247	<0.001
Total folate, mcg/d	-0.283	0.124	0.034	-0.179	0.17	0.047	0.227	0.340	0.084	0.003
Vitamin B_{12}, mcg/d	-0.322	0.103	0.064	-0.070	0.46	-0.192	0.078	0.056	-0.195	0.009
Calcium, mg/d	-0.217	-0.121	-0.201	-0.274	0.38	-0.154	-0.022	-0.026	-0.079	0.034
Phosphorus, mg/d	-0.148	-0.079	-0.149	-0.132	0.60	-0.108	-0.059	-0.061	-0.069	0.06
Magnesium, mg/d	-0.287	-0.216	-0.228	-0.258	0.049	-0.045	0.074	0.082	0.046	0.007
Iron, mg/d	-0.215	-0.131	-0.096	-0.207	0.79	-0.095	-0.019	-0.035	-0.194	0.002
Zinc, mg/d	-0.103	0.081	-0.105	0.011	0.44	-0.132	-0.092	-0.046	-0.120	0.12

Copper, mg/d	-0.080	0.402	0.241	0.156	0.005	0.159	0.311	0.368	0.270	<0.001
Selenium, mcg/d	0.001	0.281	0.078	0.100	0.44	0.141	0.234	0.287	0.177	0.05
Potassium, mg/d	-0.113	-0.154	-0.186	-0.148	0.72	-0.043	0.028	0.047	-0.029	0.31

Values are β coefficients from multiple linear regression models. Dependent variables are energy-adjusted log-transformed micronutrient intakes; the independent variable is meal and snack pattern, adjusted for confounders. A Wald test was used to examine the significance (P<0.05) of type of patterns on log-micronutrient intakes. Reference category is “1B 1L 1D”

Table 6 Associations between energy-adjusted log-transformed micronutrient intakes and daily eating occasion frequency among Greek children and adolescents: HNNHS

	1-3 years				**4-19 years**			
	4 times	**5 times**	**>=6 times**	**P**	**4 times**	**5 times**	**>=6 times**	**P**
Vitamin A, mcg/d	0.245	0.200	0.253	0.42	-0.133	0.041	0.123	0.003
Vitamin D, mcg/d	0.267	0.479	0.469	0.09	0.031	0.313	0.408	<0.001
Vitamin E, mg/d	-0.174	-0.110	-0.179	0.57	-0.056	0.004	-0.066	0.19
Vitamin K, mcg/d	-0.149	-0.050	0.006	0.86	0.033	0.276	0.424	<0.001
Vitamin C, mg/d	0.489	0.794	1.104	0.035	0.171	0.259	0.353	0.08
Thiamin, mg/d	0.105	0.003	0.026	0.66	-0.034	-0.004	0.011	0.67
Riboflavin, mg/d	0.065	0.027	0.050	0.91	-0.087	0.051	0.099	<0.001
Niacin, mg/d	-0.174	-0.066	-0.085	0.89	-0.039	0.103	0.163	0.030
Pantothenic acid, mg/d	0.067	0.126	0.098	0.68	0.086	0.190	0.347	<0.001
Vitamin B_6, mg/d	0.039	-0.145	-0.038	0.39	-0.077	0.011	-0.016	0.35
Total folate, mcg/d	0.129	0.165	0.178	0.75	0.052	0.201	0.320	<0.001
Vitamin B_{12}, mcg/d	0.071	0.203	0.159	0.77	-0.031	0.238	0.306	<0.001
Calcium, mg/d	0.117	0.224	0.224	0.20	-0.115	0.027	0.080	<0.001
Phosphorus, mg/d	-0.082	-0.002	-0.029	0.60	-0.090	-0.023	-0.003	0.001
Magnesium, mg/d	-0.069	-0.045	-0.009	0.64	-0.017	0.088	0.121	<0.001
Iron, mg/d	-0.094	-0.224	-0.193	0.44	-0.038	0.034	0.009	0.54
Zinc, mg/d	-0.149	-0.055	-0.132	0.42	-0.140	-0.091	-0.070	0.020

Copper, mg/d	-0.033	0.214	0.139	0.06	0.140	0.242	0.354	<0.001
Selenium, mcg/d	-0.241	-0.040	-0.150	0.24	0.159	0.242	0.323	<0.001
Potassium, mg/d	0.079	0.083	0.121	0.57	0.034	0.104	0.153	0.003

Values are β coefficients from multiple linear regression models. Dependent variables are energy-adjusted log-transformed micronutrient intakes; the independent variable is eating occasion frequency, adjusted for confounders. A Wald test was used to examine the significance ($P<0.05$) of eating occasion frequency categories on log-micronutrient intakes. Reference category is "1-3 times"

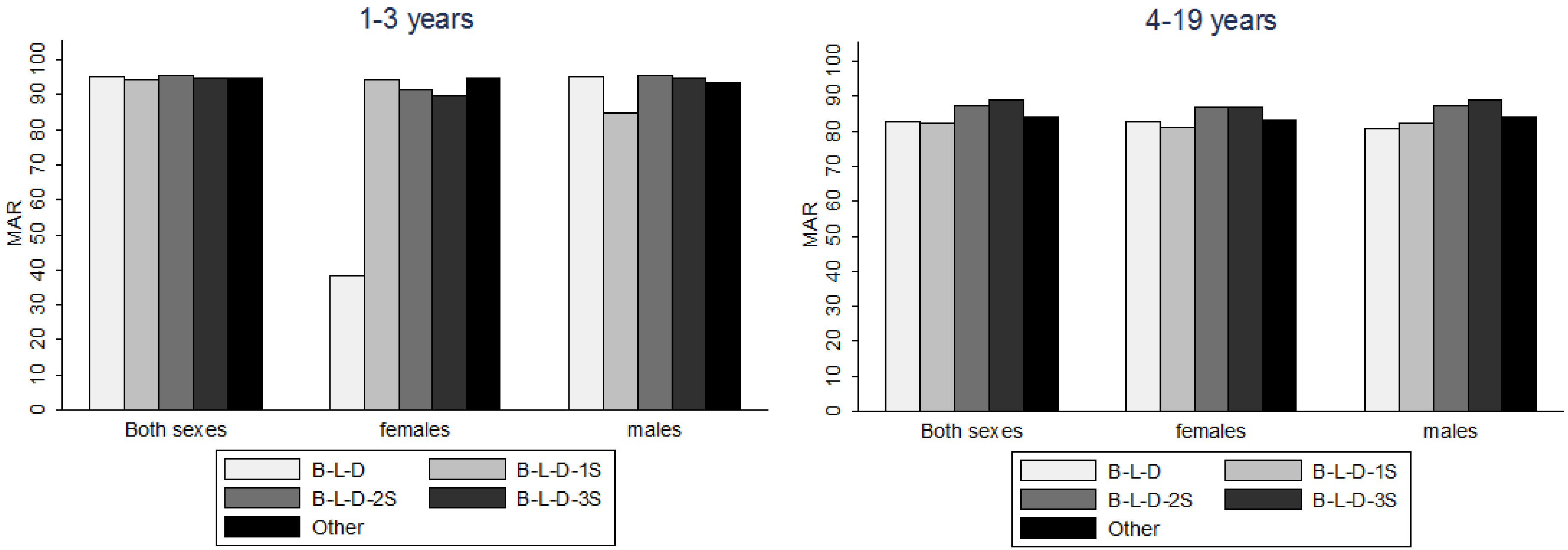

Fig. 1 Mean Adequacy Ratio (MAR) for nutrient intakes by type of meal and snack pattern and sex among Greek children and adolescents from HNNHS. Values are means ± SDs. Intakes of participants aged 1-3 y were adjusted for energy intake and BMI levels, whereas intakes of participants aged 4-19 y were adjusted for energy intake, age group and sedentary life. No significant differences of mean values were found between meal and snack patterns after using a Bonferroni correction ($P<0.01$)

Fig. 2 Contribution of the top 5 food groups to energy intake of snacking episodes by type of meal and snack pattern among Greek children aged 1-3 y from HNNHS. Values are percentages of energy intake

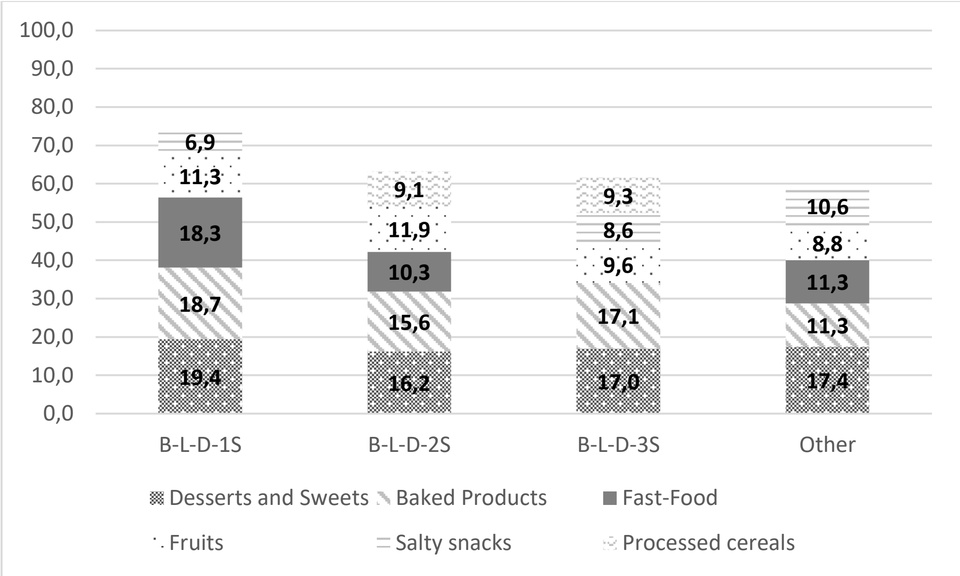

Fig. 3 Contribution of the top 5 food groups to energy intake of snacking episodes by type of meal and snack pattern among Greek children and adolescents aged 4-19 y from HNNHS. Values are percentages of energy intake

4. General Discussion

It is widely recognized that optimal dietary intake of micronutrients prevents deficiency-related problems (246, 247) and contributes to the prevention of NCDs (248, 249). Childhood and adolescence are critical periods in the life course in terms of appropriate nutrition due to the increased nutritional need for growth and development. Furthermore, their dietary habits are of particular interest since it has been shown that eating behaviors are established early in life and tend to track into adulthood (210, 250). Thus, these age groups represent a nutritional-bridge into adulthood and an intervention of policy makers at an early stage may be of great importance. This has been also the basis of this thesis, where data from children and adolescents 1-19 years taking part in the HNNHS survey were used to evaluate micronutrient intake adequacy in the population, provide insight in the major dietary sources of those nutrients and examine the association of meal and snack patterns with micronutrient intakes and diet quality, as stated in the section of Scope.

Theoretically, except for vitamin D, for which the major source is exposure to sunlight a well-balanced diet should provide for most of vitamins and minerals. However, a high prevalence of inadequate micronutrient intake was highlighted, as more than half of the population consumed most vitamins and minerals below the reference values provided by IOM. Our deficiency risk data for individual vitamins and minerals agree with other analyses in European countries and the USA. Specifically, this study showed the highest prevalence of inadequacy (i.e., >80%) in all age groups for the intakes of vitamins D, E and K, and potassium. Vitamin D is a worldwide problem, and similar data have been reported in Spain (251), Belgium (252) and in European adolescents (253). The reduced consumption of oily fish and egg yolks, *i.e.*, the most important food sources of dietary vitamin D, could partially explain the high rates of inadequacy (47-49). Mandatory vitamin D food fortification, as well as a high use of supplements by populations living in Northern European countries (254, 255), USA (256) and Canada (255) could set the basis for improving vitamin D status in Greece. As for vitamin K intake, the prevalence of inadequacy is quite high relative to the HELENA study (253), but at the same time it is consistent with a recent study conducted with children in Greece (257). The authors concluded that 86%-93% of boys and girls in Greece aged 9-13 years did not meet the recommended portion for vegetables, which are the primary food source of this vitamin. Regarding vitamin E, similar results have been shown in other groups of children and adolescents in USA (258) and in Spanish adolescents (251). However, it must be underlined the inability of the participants in the HNNHS survey to precisely account for added oil in salads, so it is likely that vitamin E intake is even greater than that recorded and that the contribution of the food group “Olive oil and olives” to the total vitamin E intake is higher than the one estimated. As for potassium, almost all individuals had intakes that were at risk and our potassium inadequacy estimates are in line with other analyses conducted with European preschoolers (252), children and adolescents (251, 253). Taking into account the beneficial role of potassium in blood pressure and cardiovascular outcomes (200-204) and the consistency of dietary habits from childhood to adulthood (210-212, 215), the observed inadequate potassium intakes are a cause for concern for the health of the Greek children and adolescents as well as of the next generation of adults. It is important to encourage a diet high in potassium-rich foods for children.

Further, folate, pantothenic acid and calcium were identified as nutrients of public health for children and adolescents aged 9-18 years (rates of inadequate prevalence ranging from 51.56% to 97.14%). The finding that the prevalence of inadequacy for folate and calcium increased with age in both genders has been reported by others (251, 253, 256, 259). Recently, Martiniak et al. (260) investigated the potential intake of dietary folate of the population based on the data of the German National Dietary Survey II under consideration of different food fortification level and evaluated the percentage of the population meeting the recommended intake. They found that the consumption of foods with a high fortification level did lead to a substantial decrease in the prevalence of inadequacy. Thus, mandatory folate fortification could shift the prevalence of inadequate intakes lower. As for pantothenic acid intake, the very low consumption of foods rich in this vitamin (*i.e.*, meat products, eggs, nuts and vegetables) (108) by Greek children and adolescents is linked with the high prevalence of suboptimal vitamin intake.

The prevalence of inadequacies for the intakes of magnesium and vitamins A and C were also considerably high (*i.e.*, rates ranging from 44.62% to 98.57%) in adolescents. Regarding magnesium, these figures are similar to those found in other studies (251, 253, 256, 259), and along with the high rates of inadequate calcium intake they are reason for concern for the incidence of osteoporosis in adult life. As for vitamins A and C, data from this analysis are comparable to the percentages reported for children and adolescents in the United States (256). The relative high rates of vitamins A and C inadequacy could be related to the lower adherence to the Mediterranean diet (223). Increasing fruit and vegetable consumption may improve the intake of those micronutrients and lower the risk of deficiency (261). Finally, two third of adolescent males reported intakes at risk for deficiency and these figures are comparable to the percentages reported by adolescents in the NDNS survey (262). The low consumption of the top food sources of zinc (*i.e.*, animal products and seafood) (180) could explain the observed trends.

The link between the high prevalence of inadequate nutrient intakes among Greek children and adolescents with the low consumption of nutrient-rich food groups can be supported by the assessment of food sources of energy and examined micronutrients. This analysis showed that major sources of energy in young people’s diet are not necessary the same food groups that provide rich sources of nutrients. Five food groups including fast-food, desserts and sweets, baked products, salty snacks, and sugar-sweetened beverages provided cumulatively 25.6% of energy intake, with no noticeable contribution to other nutrients. In contrast, dairy products (milk, yogurt and cheese) accounted for 15.9% of energy intake and provided a variety of nutrients, such as vitamin D, calcium and pantothenic acid. The conclusion that foods providing empty calories are highly ranked as sources of energy is consistent with reports that many children and adolescents do not follow dietary recommendations (224).

In confirmation of the above, the analysis of meal and snack patterns of Greek children and adolescents has shown that there was no significant association of the overall diet quality with the identified eating scheme, and thus the number of snack episodes. More specifically, after identifying the most commonly reported schemes (B-L-D; B-L-D-1S; B-L-D-2S; and B-L-D-3S), it was shown that the overall number of snacking episodes was the feature that differentiated the four distinctly examined eating schemes (0-3 snacks per day). After adjustment for the potential confounders, our findings suggest that as snacking level increased there was no improvement in the overall diet quality. This conclusion has prompted us to identify the five predominant food groups that contributed to the energy intake from snacks by categories of meal and snack patterns and age group. Despite differences in the prevalence of snacking, there was a common feature in the foods and beverages consumed as a snacking event. The main sources of snacking energy in the population were “Desserts and Sweets” and “Baked Products”. “Fruits” was the most reported item in younger children aged 1-3 years who consumed one or two snacks daily, but dropped among older children. Our results are consistent with trends from previous studies in children in the United States, in Mexico and in China (263-265), and suggest that snacking reflects less healthy choices. Similarly, among Greek adults, a small study (n=200) concluded that two of the most popular snack items were desserts (chocolates, cakes, ice cream) and savory pies (266).

As for the association of eating patterns with micronutrient intakes, this analysis revealed that snacking frequency among young children aged 1-3 years was not significantly related to micronutrient intakes, except for copper. In contrary, in children and adolescents aged 4-19 years, as snacking level increased, intake of some nutrients increased too. Micronutrients positively and significantly affected by each additional snack episode were vitamin A, vitamin D, vitamin K, riboflavin, pantothenic acid, total folate, magnesium and copper. Additionally, an inverse association between a higher snack frequency and intakes of vitamin E, vitamin B_6, calcium and iron was reported. The findings of the present analysis are not precisely consistent with those from previous studies that have examined the association between the number of snacks per day with nutrient intakes. For instance, in a study conducted among US adolescents by Sebastian et al. (267), a higher snack frequency was associated with higher mean daily intakes of vitamin C in both sexes, and of vitamin A, vitamin E and magnesium among boys. Intakes of vitamin B_6, folate, calcium, iron and phosphorus were not influenced by more snacking. Conversely, in another study conducted with US adults, positive associations between overall snacking episodes and intakes of vitamin C, magnesium, folate, calcium, iron and potassium have been reported (240). Furthermore, in a recent research of Australian adults, findings suggested that the frequency of snacks, was not associated with the intake of the four micronutrients that were also examined in our study, except for calcium (242).

The inconsistency in the findings for snack frequency across studies may be in part explained because of the lack of consistency in the definitions used as to what constitutes a meal and a snack (230). The main approaches to label EOs are: participant-identified, time-of-day, types and/or amount of food consumed, and interviewer assessment (230). The present study relied on participants to label their eating occasion; however, different perceptions within and between populations of what meals and snacks constitute could explain the non-consistent findings (240). Furthermore, comparison of the HNNHS results and the findings of the aforementioned studies may be difficult, because of the differences in the ages of study participants. Food and beverage choices made by adults during snacking episodes (and therefore their nutritional value) are different for younger people. Finally, a recent study compared foods consumed by children during snacking between Australia, China, Mexico and the United States, and researchers concluded that food items at snacks may differ by country

(263). In agreement with this study, contributions of fruits, dairy products, bread, sweetened beverages and fatty-sweet products to energy intake for overall snacking was different in French adults as compared to US and Finish adults (268).

Given the growing contribution of snacks to everyday dietary intake and the need for effective strategies to increase micronutrient intake, it is important to consider whether substitution of the present snack choices with nutrient-rich foods could contribute to an adequate, balanced diet. Some countries have, in fact, developed specific snack recommendations (269). For instance, Switzerland provides a comprehensive list of foods and beverages that should be consumed for snacks between meals. Identification of the nutrient insufficiencies and recording the current snack food preferences of the population could be used to develop national dietary guidelines and even promote the development of snacks rich in nutrients of concern.

5. Conclusion

It is widely recognized that optimal dietary intake of vitamins and minerals wards off deficiency-related diseases, and contributes to the prevention of NCDs. However, diets in the developed countries are usually characterized by low vitamin and mineral content. Further, childhood and adolescence are crucial periods of life for the establishment of eating behaviors which are often carried into adulthood. Therefore, if young individuals learn to eat in a healthy way, they will probably preserve these habits and their health later in life.

This research has generated new knowledge that can help public health policy makers increase nutrient density of the diet of Greek children and adolescents. To begin with, the detailed analysis of children and adolescents' nutrient intake presents valuable information about micronutrients of concern and about the energy and micronutrient contribution of food groups. This knowledge can help policy makers design and promote effective population-based strategies to reduce energy consumption and increase nutrient density of the diet. Nutrition education should be considered to help children and adolescents establish balanced food choices from an early age. Along with increasing the consumption of nutrient-dense foods, food fortification policy and supplement use could be considered to correct imbalances of some crucial nutrients.

In addition, the assessment in the current thesis of meal and snack patterns revealed the common practice of the three main meals (breakfast, lunch and dinner) as well as the fact that snacks constitute a large portion of children and adolescent's total dietary intake. Although the intakes of some micronutrients are positively associated with every additional snack, after examining the impact of the number of snack episodes on the overall diet quality, the high prevalence of non-nutrient beneficial snacks has been highlighted. As snacking represents a great opportunity for children and adolescents to meet their nutritional needs and improve the quality of their diet, future studies should focus on exploring the food groups that are already consumed between meals by the population. Better knowledge of how the food profile of snacks affects nutrient intakes will provide valuable information for public health policy makers when designing strategies to improve young people's dietary profile.

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