MICRONUTRIENT INTAKE AND PREVALENCE OF ADEQUACY IN EUROPEAN CHILDREN, FROM BIRTH TO 8 YEARS. INFLUENCE OF CALCIUM INTAKE ON BONE MINERAL DENSITY

Marta Zaragoza Jordana

UNIVERSITAT ROVIRA I VIRGILI
MICRONUTRIENT INTAKE AND PREVALENCE OF ADEQUACY IN EUROPEAN CHILDREN, FROM BIRTH TO 8 YEARS. INFLUENCE OF CALCIUM INTAKE ON BONE MINERAL DENSITY
Marta Zaragoza Jordana

Marta Zaragoza Jordana

European Doctoral Thesis

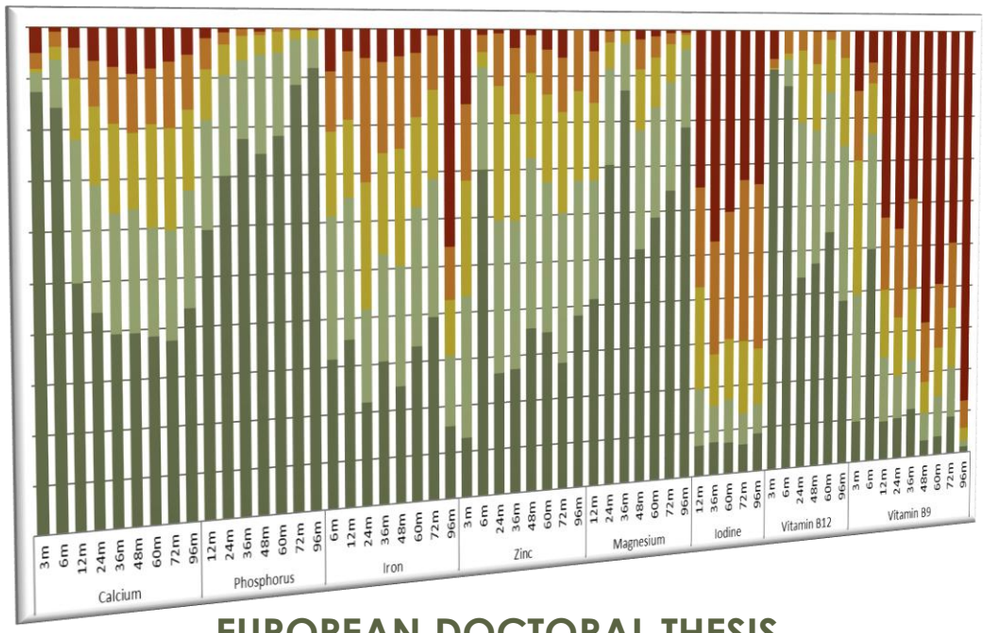
Marta Zaragoza Jordana
Unitat de **R**ecerca en **P**ediatria, **N**utrició i **D**esenvolupament **H**umà
Universitat Rovira I Virgili

UNIVERSITAT ROVIRA I VIRGILI

Marta Zaragoza Jordana

Micronutrient intake and prevalence of adequacy in European children, from birth to 8 years.

Influence of calcium intake on bone mineral density.



EUROPEAN DOCTORAL THESIS
Universitat Rovira i Virgili

Marta Zaragoza Jordana

MICRONUTRIENT INTAKE AND PREVALENCE OF ADEQUACY IN EUROPEAN CHILDREN, FROM BIRTH TO 8 YEARS. INFLUENCE OF CALCIUM INTAKE ON BONE MINERAL DENSITY.

EUROPEAN DOCTORAL THESIS

Directed by Dr. Ricardo Closa Monasterolo,

Dr. Joaquin Escribano Subías and Dr. Natàlia Ferré Pallàs

Departament de Medicina i Cirurgia

Unitat de **R**ecerca en **P**ediatria, **N**utrició i **D**esenvolupament **H**umà

UNIVERSITAT ROVIRA I VIRGILI

Reus 2015

Departament de Medicina i Cirurgia

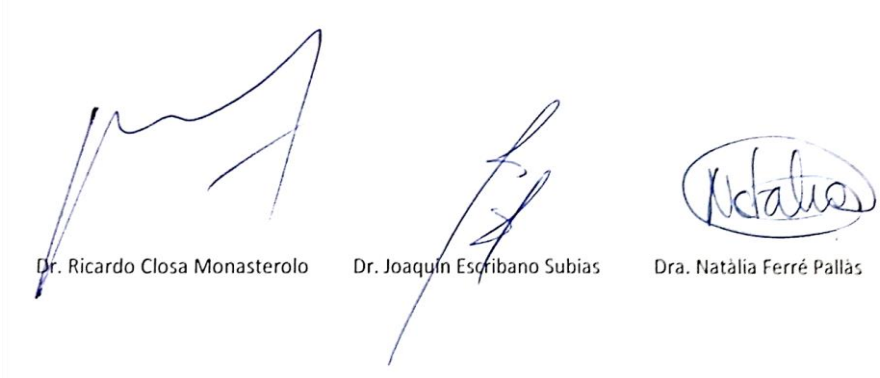
Carrer Sant Llorenç, 21

43201 Reus

FEM CONSTAR que aquest treball, titulat "Micronutrient intake and prevalence of adequacy in European children from birth to 8 years. Influence of calcium intake on bone mineral density", que presenta Marta Zaragoza Jordana per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció al Departament de Medicina i Cirurgia d'aquesta universitat i que acompleix els requeriments per poder optar a Menció Europea.

Reus, 31 d'Agost de 2015

I per a que així consti, firmen aquest certificat.



ACKNOWLEDGMENT

Through these lines I would like to thank all the people that has somehow made this work possible.

First of all I would like to highlight the effort of all the families from five European countries that have been participating in the EU CHOP study since their children were born to 11 years, without them we would not have had the opportunity to collect such valuable data. In addition, all the work carried out from 2002 could never had been done without the exceptional multidisciplinary teams collaborating in this project in Belgium, Germany, Italy, Poland and Spain. And everything was possible thanks to the European Commission dedicated funds (5th Framework Programme from the European Union, project registered at clinicaltrials.gov as NCT00338689; 6th Framework Programme (FOOD-CT-2005-007036) and 7th Framework Programme (FP7-KBBE-2007-1, ref. nº 212652; and FP7-289346-EarlyNutrition).

Of course, one of these extraordinary teams is the *Unitat de Recerca en Pediatria, Nutrició i Desenvolupament Humà* of the *Universitat Rovira i Virgili* to which I belong since 2007 when I was invited to collaborate in it although I did not know much about what research was. To the human team of this unit I deserve my professional and scientific knowledge and skills. I'll never be able to show enough gratitude for the opportunity that you brought me 8 years ago, especially Ricardo, Joaquin and Veronica. Later, the Unit became larger with Natalia, Mariona and Carme. And as time goes by fast, and so does life, from all the time inverted in this wonderful work

environment, I just can be grateful to all the colleagues of the Unit, who with the time became mentors in both professional and personal life. I appreciate you all so much. It was also a pleasure to work with all the other people that sometime took part in the Unit: Mercè, Ainara, Montse and Paloma.

I would like to thank all the staff (nurses, physicians, technicians and administrative staff) from Hospital Universitari Sant Joan de Reus and from Hospital Universitari Joan XXIII de Tarragona that collaborated in some way to make our field work easier. And to those from the laboratory and from the Biobanc, I can only say: thanks for your patience!

I would not like to finish without a few kind words to Veit. Thanks for your dedication to EU-CHOP data bases caring. Data would not be that accurate without your efforts. I have learned a lot from your obstinacy in doing things as best as possible. Although I have to confess that at some point I only wished to go straight forward, when you are confident on your data then you can be really proud on your results. Vielen dank!

I also want to thank to the Division of Metabolic and Nutritional Diseases and Nutritional Medicine in the Dr. Von Hauner Children's Hospital and Ludwig Maximilian's University of Munich, and especially to its responsible Professor Berthold Koletzko, for accepting me for a three-month stage with their team. This stage was funded by the Catalan government through an AGAUR agency grant (2011 BE101007). And in reference to that period of my life, I would like to mention Melissa and Cristina, who made my life far from home easier.

Concerning my personal environment, which has unconditionally supported me in all the things I have done, either hits or errors, during infancy, childhood, youth and currently in adulthood; they will probably never read this work. My parents, family and friends do not need me to mention them because they already know how fortunate I do feel to be part of their lives and how grateful I am for all their help and efforts to make me as I am. They constantly teach me how to appreciate the important things in life in-between of all the disturbing noise.

And to finish I would like to make a consideration. I believe that all the people with whom you come across at some point in life provides you some knowledge, experience or even feeling, either positive or negative. Any of those influences modulate the person you become later.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

AI – Adequate Intake
AMP – Adenosine Mono Phosphate
ATP – Adenosine Tri Phosphate
AR – Average Requirement
ARI – Acceptable Range of Intake

BF – Breastfed
BMA – Bone mineral area
BMC – Bone mineral content
BMD – Bone mineral density
BMI – Body Mass Index

DNA – Deoxyribonucleic Acid
DRI – Dietary Reference Intakes
DRNI – Daily recommended Nutrient Intakes

EAR – Estimate Average Requirement
EFSA – European Food Safety Authority
e.g. – For example
EU – European Union
EU CHOP – European Childhood Obesity Project
EU-SCF – Scientific Committee on Food of the European Union

FAO – Food and Agriculture Organization of the United Nations
FMI – Fat Mass Index
FNB – Food and Nutrition Board
FNB-IOM – Food and Nutrition Board of the American Institute of Medicine

g – Grams

HP – Higher protein content

IGF-1 – Insulin like Growth Factor 1
IOM – American Institute of Medicine
IDA – Iron Deficiency Anaemia

LP – Lower protein content
LS – Lumbar Spine
LTI – Lowest Threshold Intake

mg – Milligrams
µg - Micrograms

PAI – Probability of Adequate Intake
PAI>95% - High Probability of Adequate Intake
PAQ-C – Physical Activity Questionnaire for Children
PII – Probability of Inadequate Intake
PNI – Protective Nutrient Intake
PTH – Parathyroid Hormone
PRI – Population Reference Intakes

RDA – Recommended Dietary Allowances
RNA – Ribonucleic Acid
RNI – Recommended Nutrient Intake

SCF – Scientific Committee on Food
SD – Standard Deviation

UL – Tolerable Upper Intake Levels
UV – Ultraviolet light
UVB – Ultraviolet B light
UNU – United Nations University

WB – Whole Body
WHO – World Health Organization

SUMMARY

Title: Micronutrient intake and prevalence of adequacy in European children, from birth to 8 years. Influence of calcium on bone mineral density.

Background: Micronutrients are essential for development. The objective of dietary intake evaluation is determining the adequacy to nutritional recommendations. Suboptimal intakes for calcium, iron, zinc, thiamine, riboflavin, niacin, folate and vitamin D have been previously described across Europe. No studies have assessed micronutrients intake of children from different European countries using the same methodology. Calcium intake influence on bone mass density (BMD) has been described in adults and children. Bone poor mineralization drives to osteoporosis, which might be prevented from childhood.

Aim: To describe micronutrients intake and adequacy to dietary recommendations of European children during childhood. To analyse the relation between calcium intake and BMD.

Methods: Prospective observational study secondary to the European Childhood Obesity Project (EU CHOP). Dietary intake was collected periodically with 3-day food records. Micronutrients adequacy was calculated following the American Institute of Medicine guidelines. At 7 years, BMD was measured by Dual-energy X-Ray Absorptiometry in a subsample of participants.

Results: EU CHOP study recruited 1679 children at birth. Intake data was available for 904 children at 3 months, decreasing to 396 at 8 years. BMD was measured in 179 children. Sodium, potassium, calcium, phosphorus, iron, zinc, magnesium, iodine, vitaminB_{12}, folate, vitamin A and vitamin D intakes were described at 7 timepoints. Calcium, iron and zinc showed prevalence of adequacy between 60 and 90%; and folate, iodine and vitamin D under 20%. Maintained high probability of calcium adequacy improved BMD at 7 years and reduced more than 12 fold osteopenia risk.

Conclusions: Calcium, iron, zinc, folate, iodine and vitamin D intakes were inadequate within European children. Maintained high probability of calcium adequacy improves lumbar and whole body BMD at 7 years and reduces osteopenia risk.

Introduction

1. INTRODUCTION

Over the last century, nutrition, traditionally defined only as a biological process, became a Science that comprehends the physiological, genomic and medical aspects of nutrition function; but also the social and environmental interactions[1]; in fact, rigorous evidence on the influence of dietary intake on optimal development, health, and disease prevention has been compiled[2].

Nutrition is the Science that studies **food**, the **nutrients** in food and the physiological role of those nutrients; and determines the **requirements** of nutrients for each determined population or group of individuals to ensure health and a correct development.

Nutrition, as a biological process, is defined as the provision of food to a living being, and, it includes ingestion, digestion, absorption, metabolism, transport, storage and excretion. It is the process by which the body converts and uses the substances contained in foods, called nutrients, and synthesizes essential components to sustain life[1].

Digging a little deeper, nutrients are the molecules obtained during the food global digestion process. They are used by the body to develop vital functions like tissue building and reparation, body processes regulation and they are also converted to and utilised as energy; consequently they are essential to maintain health. Some dietary components are synthesised by human and therefore considered non-essential, but most are not synthesised in vivo and, thus, must be consumed through diet.

There are several kinds of nutrients: carbohydrates, fats, proteins, water, minerals and vitamins; and they can be classified using different criteria. For example, as organic or inorganic molecules, taking into account their composition; as essential or not essential nutrients, taking into account if they can be synthesized by the body or not; or, as macronutrients or micronutrients, taking into account their amounts required to maintain health. And, consequently, from this last option of classification, carbohydrates, fats and proteins are considered macronutrients; vitamins and minerals are considered micronutrients, and water is considered as the main nutrient, as it is needed in very big amounts[3].

Good nutrition is essential for health; it is defined as an adequate and well balanced diet which provides optimal energy and beneficial nutrients that can maintain health and have positive effects on development. Furthermore, it prevents or alleviates from many common health problems. On the contrary, excessive intake of energy or insufficient input of some nutrients could lead to health disparities such as reduced immunity, increased predisposition to disease or compromised physical and mental development; as well as to have a health-threatening effect like obesity, metabolic syndrome and other cardiovascular diseases, diabetes or osteoporosis; what is known as the “double burden” phenomenon[2,4].

Then, an adequate diet should provide all the nutrients needed to maintain health and ensure growth and development of individuals, and to do so in the correct amounts to guarantee neither deficiencies nor excesses of any nutrient, whether they are macro or micronutrients.

It has been described that there are critical periods of time during the prenatal, infant, early childhood and adolescent periods, where nutrition plays a key role in determining the health course, either positively or negatively. Nutritional status of the mother and/or child is an important factor in programming the child for healthy development and long-term maintenance of organ systems[2].

This dissertation work will focus on micronutrients consumption description in a sample of healthy European children, and the analysis of the adequacy of this consumption to the dietary intake recommendations. Furthermore, the influence of calcium consumption on bone mineral density will be explored.

1.1. MICRONUTRIENTS

Micronutrients are nutrients needed in very small amounts. Nevertheless, they are essential for health, to arrange a variety of physiological functions and to ensure a proper growth and development as well. There are several kinds of micronutrients depending on their chemical composition, their functions or the amounts needed by the organism[5].

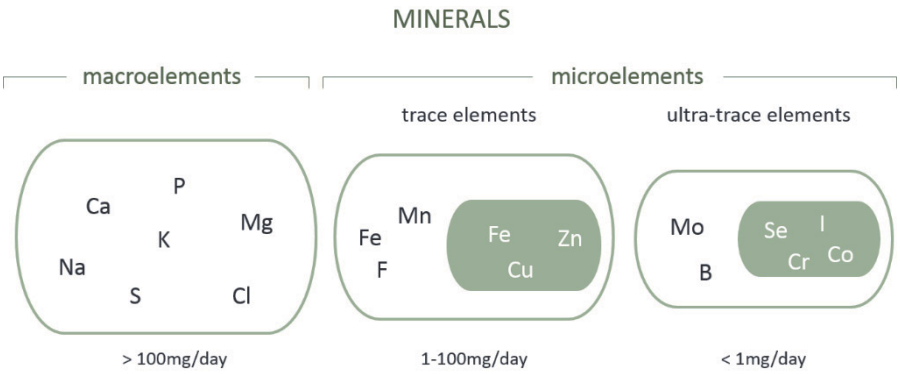
Vitamins are organic compounds that need to be provided in small amounts with the diet in order to maintain some basic body functions like growth, development, metabolism and cellular integrity. During the 20th century 13 vitamins were isolated, identified and synthesized; their metabolism was described, but for some of them, knowledge about their role in biological process is still needed. Vitamins comprise the B complex (thiamine, riboflavin, pyridoxine, niacin, cobalamin, folate, biotin and pantothenic acid), vitamin C and fat soluble vitamins A, D, E and K. Some of them are semi-essential due that they can be synthesized by the body from other molecules; the others are 100% essential[6].

Minerals, unlike vitamins, are inorganic compounds. They are also needed in small amounts and for very specific functions. However, some of them, are considered macroelements because they are needed in daily amounts greater than 100mg (in adults), like calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl) and sulphur (S). Besides, there are the microelements, which can be separated in two groups: trace elements, needed in amounts from 1 to 100mg/day, or ultra-trace elements, needed in amounts smaller than 1mg/day. Iron (Fe), zinc

(Zn), manganese (Mn), copper (Cu) and fluorine (F) are trace elements, while selenium (Se), molybdenum (Mo), iodine (I), chromium (Cr), boron (B) and cobalt (Co) are considered ultra-trace[6].

Furthermore, from the 90 mineral elements found in the nature, 22 of them seem to be essential for humans, those whose deficiency occasions a disorder in a particular function. Iron, zinc, copper, selenium, iodine, chromium and cobalt are considered essential[7]. Figure 1 summarizes all this mentioned differentiation within minerals.

Figure 1. Classification of mineral elements according to daily requirements and essentiality.



Required amounts of macroelements, trace elements and ultra-trace elements are detailed under each category globe. Essential minerals are highlighted into green globes.

Given the wide variety of micronutrients, thenceforward this dissertation will focus on some of them and provide a short description on their molecules, sources, functions and deficiency or toxicity consequences.

1.1.1. Sodium

Sodium is the main cation of extracellular fluid. Over a third part of sodium body pool is fixed in the bones and has low capability to interchange with the sodium in other body fluids[8]. Sodium is rapidly absorbed through the intestine to the extracellular fluid. It is excreted fundamentally by the kidney in urine, and it is also subjected to some neuroendocrine control (aldosterone, ADH)[8].

Sodium amount naturally present in not-processed food is low and could rarely satisfy the minimum requirements of humans. Vegetables and fruits contain practically no sodium; milk, meat and fish contain a little bit more[9]. However, giving that sodium is the main chemical component in common table salt (sodium chloride), almost all our sodium intake comes from the salt added to the food. Furthermore, many condiments and industrially processed food such as sauces, dressings, soups, breads, crackers, meats and snacks often contain high amounts of sodium. The richest foods in sodium are those that undergo salting and curing processes[3,9–11].

Sodium main function is the regulation of extracellular fluid, thus, of blood plasma volume; as well as of several cardiovascular factors like cardiac expenditure or blood pressure. Sodium has an important function in osmolality regulation, acid-base balance and membrane potential of cells (transmission of nerve impulses). It also takes part in the active transport through cellular membranes, and has to be pumped out in exchange with potassium, in order to maintain an appropriate intracellular environment[8,9,12].

The sodium deficiency due to low dietary intake is not frequent, even in individuals on low sodium diets. Data from all over the world indicates that sodium intake is well above that required for physiological functions[9]. Depletion could appear in extreme profuse and persistent sweating situations, or when there is a trauma, chronic diarrhoea or a renal disease that produce an incapacity to retain this electrolyte[13]. Extreme malnourished patients with anorexia nervosa could present hypovolemic hyponatremia due to long-term sodium restriction as a method to control body weight[14].

Excessive sodium intake has a harmfully prevalence worldwide[10]. The chronically excessive intake of sodium produce an increase of extracellular space, due that water goes out of cells to preserve normal levels of sodium concentration in that fluid. The result of increased sodium consume is oedema and elevated blood pressure or hypertension[15], what has been shown to be a risk factor for heart disease, stroke and kidney disease[13]. However, in a cohort study with children, reported by Geleijnse in 1990[16]; there was not significant higher rate of increase in blood pressure over time in the group of children consuming the higher amount of sodium compared with the group consuming the lowest, whereas reduced sodium consumption decreased blood pressure[17–19].

Nevertheless, sodium acute toxicity is not a danger because when sodium requirements are reached the kidney excretes its excess[3].

1.1.2. Potassium

Potassium is the main cation of intracellular space[13,20]. About 98% of potassium is inside cells, whilst only 2% is in the extracellular space[8,13]. Potassium from food is 90% absorbed by passive diffusion, together with water. Once in the bloodstream, potassium concentration remains constant due to the kidney regulation, which retains or excretes potassium depending on physiologic needs[20].

Potassium is widely distributed along foods, but especially in fresh fruits and vegetables and legumes; as well as in potatoes, dried fruits, nuts, avocado, banana, some meats (pork and veal), fish and shellfish[13,21]. Potassium content of food decreases with food processing, consequently, a diet rich in processed foods and poor in fresh fruits and vegetables will often result in a lacking in potassium diet[22].

Due to its location, potassium plays an essential role in the osmotic balance of intracellular fluid[8]. It is also indispensable to maintain body acid-base balance, and for the total body fluid volume maintenance[21]. Furthermore, potassium intervenes in nerve impulse transmission and muscular contraction (smooth, skeletal and cardiac muscles)[13,20].

Potassium deficiency is not common, but present in general population[23,24] and when it occurs entails a high risk for health. Main causes of potassium deficiency are renal and gastrointestinal problems such as diarrhoea, vomiting, diuretics or laxative abuse, increased secretion of potassium by the distal tubule, excessive aldosterone secretion or the use of some

antibiotics[20]. Severe deficiency produces hypokalaemia, what in turn causes cardiac arrhythmias, muscle weakness and glucose intolerance. A moderate deficiency has been associated with elevated blood pressure, hypertension, increased urinary excretion of calcium, therefore with increased risk of kidney stones and increased bone turnover; as well as an increased risk of cardiovascular disease, mainly stroke[13,25].

Nonetheless, higher levels of consumption could be protective against cardiovascular diseases and hypertension[26,27]; however these beneficial effects are not entirely consistent[25,28–30]. In children, a meta-analyses from Aburto et al.[21], stated that increased potassium does not reduce blood pressure significantly; however in a cohort study mentioned in the same review, potassium intake was inversely related to the rate of increase in blood pressure over a seven year period; children with the highest third intake of potassium had 1.00 (0.35 to 1.65) mmHg per year lower increase in blood pressure than children with the lowest third of potassium intake[16]. Anyway, there is a lack of data in children; high quality randomised controlled trials are needed to explore this relation further.

An excessive consume of potassium could produce gastrointestinal discomfort, but not toxicity at all because is readily excreted in the urine, if kidneys function correctly[13]. If kidneys do not work properly, then potassium can cumulate in the body disturbing the cardiac function[8].
The systematic review and meta-analyses from Aburto et al, published in 2013[21]; checked that increased potassium had no adverse effect on blood lipid concentration, catecholamine concentrations or renal function in adults.

1.1.3. Calcium

Calcium is the fifth element in the elementary composition of the human body, after oxygen, carbon, hydrogen and nitrogen. It makes up 1.9% of the body weight and almost all (99%) is located in the skeleton and teeth[31]. The other 1% is located in blood, soft tissues and a few in the extracellular fluid. Calcium absorption depends on the amount of calcium consumed, active vitamin D, Ca/P ratio, and the presence of substances in the diet that favour or interfere absorption, as lactose and casein or phytate, respectively[32,33].

Dairy products such as milk, yogurt and cheese are the most rich in calcium foods. Fortified products like tofu or some beverages, canned fish (eaten with bones), Chinese cabbage, savoy, chard, spinach and broccoli, legumes and nuts have also such important calcium content, but not all have the same bioavailability[13,34]. Although grains are not very rich in calcium, some processed grain-based foods are a good source of calcium due to the use of calcium-containing additives[13].

Calcium's main function in the body is to build and provide rigidity to the bone structure and teeth, and it is also essential to vascular contraction and vasodilation, muscular contraction (skeletal, smooth and heart muscle), neuronal transmission, neurotransmitters formation and glandular secretion (hormone and enzyme)[13,35–37]. To ensure normal growth and development of the skeleton and for adequate bone mineralization, it is necessary that calcium intake is well equilibrated during all growth process, especially along the first 2 years of life and on puberty and adolescence too[37]. Therefore, these are at deficiency risk groups, as well as pregnant

women (mainly during third trimester), lactating women, postmenopausal women, and, possibly, elderly men[31].

Calcium deficiency can be produced due to a low intake, vitamin D deficiency, increased loses or to a low Ca/P relation[38]. Calcium deficiency occasions poor bone mineralization or osteoporosis, what conducts to rickets in childhood and adolescence. Growing rate stop, hypertension, increase of some cancer risk and convulsions are other risks linked to calcium insufficiency[3,13]. Furthermore, a study performed by Zemel et al. in 2000, demonstrated that low calcium diets favour increased adipose tissue energy storage[39], in fact, a previous study in obese African-Americans found that an increase in calcium from 400 to 1000mg/day during 1 year, produced a body fat reduction of 4.9Kg[40], proposed mechanisms and further associations were described later elsewhere[41,42].

On the other side, calcium excess intake has primarily been described through supplementation studies. The main adverse effects are: kidney stones, hypercalcaemia and/or hypercalciuria, renal insufficiency (milk-alkali syndrome), soft tissue calcification, irritability, headache and interaction of calcium absorption with other minerals (Fe, Mg, Mn or Zn)[13,31,43]. Hypercalcaemia can produce constipation, sickness, polyuria, kidney stones; and extremely muscle tone lose, coma and death[3,32]. Furthermore, a review published by Daly on 2010 highlighted that, although more investigations are needed, it could be that a total calcium consumption of 2000-2500 mg/day (adding supplemental and dietary calcium) may increase the risk of vascular disease (myocardial infarction) in postmenopausal women[44].

1.1.4. Phosphorus

Phosphorus makes the 0.8-1.1% of total body weight[45]. Together with the calcium, an 80% of phosphorus can be found in the mineral structure of the bones and teeth; nearly a 20% is in soft tissues and a very small amount in the extracellular fluid[32]. Phosphorus absorption is around a 65-90% in infants and children through passive concentration processes and it is favoured by active vitamin D. Once in the blood, its concentration and cellular availability is regulated by the kidney[38].

Phosphorus is present in most foods as a natural component and as an additive in processed foods[46]. It is mainly found in cheese, pulses, fish, breakfast cereals, nuts, sesame, sunflower seeds, wheat germ, egg yolk and offal[45]. In adults, approximately 20-30% of dietary phosphorus comes from milk and milk products, and 20-30% from meat, poultry and fish, grain products and legumes (all of which are high protein foods)[47].
While protein rich foods are naturally high in phosphorus, bioavailability may vary with the food source[48]. Phosphorus is found in foods as a mixture of organic and inorganic forms, and most absorption occurs as inorganic phosphate[13]. Food-bound phosphorus (constituent of protein and other nutrients) is slowly and inefficiently absorbed; phosphorus absorbed from a vegetarian diet may be tiny because it is present as a phytate, which is not as easily digested as animal protein[49]; and phosphorus in food additives is rapidly and almost completely absorbed[46]. In fact, phosphorus content of the American diet is increasing as a result of the growing availability of and interest for processed foods and the widespread use of phosphate additives by food processors[47].

Phosphorus main function is structural, in the bones and teeth; but it also has a role as a producer of metabolic fuels as, for instance, ATP. Furthermore, phosphorus is part of DNA, RNA and other phosphatides involved in a number of biological processes; as well as part of the cyclic AMP that acts as an intracellular messenger and of other free nucleotides. In addition, phosphorus is part of the nervous tissue and also forms the phospholipids present in biological membranes regulating the acid-base balance[3]. Dietary phosphorus supports tissue growth and restores phosphorus pool lost through excretion and the peeling of skin cells[13].

Phosphorus deficiency is generally not a problem because phosphorus is so omnipresent in the diet. But, if chronic low phosphorus consumption occur, it may lead to bone loss and bone pain, muscle weakness, decreased growth, poor dental development, rickets, anaemia, anorexia, confusion, ataxia, paraesthesia and general debility[3,13].

The consumption of phosphorus has increased and normally exceeds the nutrient requirements of most men and women[50]. Although phosphorus intake is the main contributor to phosphate serum concentrations it has been challenging to show association between high dietary phosphorus intake and fasting serum phosphate (due to the rapid and effective management of increases in serum phosphate by normal kidneys, and due to the underestimation of phosphorus content of foods by the nutrient content databases)[46,50].

Traditionally, high serum phosphate concentrations have been related with cardiovascular and all-cause mortality in patients with chronic kidney disease[51]; but now, the association of cardiovascular disease risk and all-

cause mortality with excess phosphorus consumption and small increases in serum phosphorus within the normal range has been described in the general healthy population[52–56].

The disruption of the endocrine regulation of calcium, vitamin D and phosphorus is the potential toxicity mechanism of phosphorus excessive intake[50]. High phosphorus has been demonstrated to induce parathyroid hormone (PTH) and/or fibroblast growth factor-23 release from bone, occasioning pathogenic cardiovascular effects such as arterial calcification, endothelial dysfunction and left ventricular hypertrophy; as well as contributing to impaired kidney function, disordered mineral metabolism, tetany and bone loss[46,50,57,58].

1.1.5. Iron

Iron can be found in inorganic state as ferrous (Fe^{2+}) or ferric (Fe^{3+}) salts and in organic form, contained in the haem groups. Body needs to convert all inorganic iron from the diet into Fe^{2+} because it cannot use ferric salts[3,13].

Main sources of haem iron are offal, red meats, poultry, fish and seafood. And non-haem iron, which is the most abundant, is found in legumes, whole grains, fruits and vegetables, nuts and oily seeds. Currently there are many processed products fortified with iron[3]. Cooking at high temperatures for long time can convert haem iron into non-haem iron. Calcium negatively influences haem and non-haem iron absorption. As well as phytate inhibits non-haem iron absorption. Ascorbic acid, meat, fish and seafood and

fermented vegetables would enhance absorption of non-haem iron (also found in supplements)[31].

Iron is an element needed for a wide variety of biological functions. It is a key component of several proteins: iron-containing haem proteins (haemoglobin, myoglobin and cytochromes), iron-sulfur enzymes, proteins for iron storage and transport (transferrin, lactoferrin, and ferritin) and other iron-containing or activated enzymes[13]. Haemoglobin in the erythrocytes carries oxygen from the lungs to the tissues. Myoglobin is iron-containing oxygen storage in the muscle cells, and its mission is to transfer energy within the cell. Cytochromes are in charge of the synthesis of steroid hormones and bile acids, detoxification of foreign substances in the liver; signal controlling in some neurotransmitters[31].

Almost two-thirds of body's iron is in haemoglobin, found in circulating red cells. Another 25% is stored in a readily mobilized pool in the liver, as ferritin and hemosiderin. Most of the remaining is found in myoglobin, in the muscles[13].

During the early infancy, especially on the weaning period, iron is very important for the adequate development of brain and other tissues such as muscles, which are differentiated early in life[31,59].

Infants, children, adolescents and women in childbearing age, especially pregnant women, are the population groups most at risk for iron deficiency due to the physiologic increase of requirements. This situation is solved for infants and children in developed countries thanks to the fortification of infant and children cereals[31].

Iron deficiency can be due to low intake, impaired absorption (achlorhydria, gastric surgery, celiac disease) or excessive physiologic loses. Iron deficiency can drive to iron deficiency anaemia (IDA), in which haemoglobin levels are very under the rule, and so are iron stores (ferritin), and in addition, erythropoiesis is impaired (mean corpuscular volume)[3]. Iron deficiency anaemia is the most common nutritional deficiency in the world[13], also in children, particularly at pre-school ages.

Iron deficiency reduces the physical work capacity, especially during endurance activities, as a consequence of the impairment of oxidative metabolism in the muscles, and an improvement has been demonstrated after iron administration. Iron deficiency can also influence negatively the normal function of immune system against infections, being this affectation also reversible after administration of iron for few days[31].

Contrarily, iron is related with brain function and development and iron deficiency during early life may lead to irreparable damage to brain cells. Furthermore, several studies have demonstrated a relationship between iron deficiency and attention, behaviour, memory and learning in infants and children; and no effects was noted from the administration of iron[59–62].

Iron toxicity is not common in healthy individuals due to natural protective system that inhibits iron absorption if necessary. But, if it happens, as in individuals suffering from hemochromatosis; excessive iron intake could produce gastrointestinal distress, secondary iron overload, and acute toxicity[13].

1.1.6. Zinc

Zinc is an essential microelement for proper growth, development and maintenance of health. The presence of zinc is general in all body tissues and fluids[3,13].

Zinc is widely distributed in foods. Zinc-rich sources are lean red meats and offal, some seafood, legumes, whole grains and fortified breakfast cereals. Nuts, oily seeds, broccoli, cauliflower, egg yolk, meat with high fat content and fish, are also zinc sources[3,13,31]. Although unrefined cereals and legumes are a source of zinc, they contain high levels of phytic acid, known to be a potent inhibitor of zinc absorption that might be neutralised by fermentation, for instance[63].

Zinc is an essential constituent of many enzymes participating in most major metabolic pathways, and consequently, is necessary for a number of biochemical, immunological and clinical functions.
Zinc intervenes in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids[63,64], as well as in the regulation of gene expression (polynucleotides transcription), alcohol and proteins metabolism as well as in the metabolism of other micronutrients.
Zinc contributes to cell and organ integrity maintenance procuring stability to the molecular structure of many key tissues, cellular components and membranes (vision, taste perception, brain development, growth, cellular proliferation)[64–66]. It seems that zinc is essential for neurogenesis, neuronal migration and synaptogenesis, so its deficiency could drive to altered neuropsychological behaviour[66,67].

It also plays a central role in the immune system, affecting many aspects from the physical barrier of the skin to cellular and humoral immunity[68].

Inadequate dietary intake of absorbable zinc is the primary cause of deficiency[65], but excessive losses due to diarrhoea or increased requirements during pregnancy and lactation could also be the aetiology of deficiency[63]. Given the importance of zinc functions described above, its deficiency can affect multiple processes, including physical growth, immune competence, reproductive function and neuro-behavioural development[63]. Though, deficiency risk is particularly critical for infants, children, adolescents and pregnant women[63].

The only clear demonstrated signs of mild zinc deficiency in humans are a reduced growth rate and impairments of immunity[68]. Impaired taste and smell, and delayed wound healing are less consistently observed[31,65].

Severe zinc deficiency signs are skeletal growth retardation, delayed sexual maturation, reduced bone mineralisation, acrodermatitis enteropathica (skin lesions, diarrhoea and alopecia), impaired appetite, increased susceptibility to infections mediated via severe defects in the immune system, and the appearance of behavioural changes due to zinc essentiality for neurogenesis, neuronal migration and synaptogenesis[64,67,69–71].

A meta-analysis by Brown et al. in 2002, stated that Zn supplementation results in a positive response in linear growth and weight gain, more effective among children with pre-existing growth failure[72]. Furthermore, in several RCT among high risk infants, young children and pregnant women, zinc supplementation was shown to reduce the incidence of different kind of infections such as diarrhoeal infection and acute lower respiratory

infections[73]; as well as reductions in the incidence of diarrhoea and pneumonia of 18 and 41% respectively. Zn may also reduce the severity of malaria attributable to Plasmodium falciparum parasitaemia[68]. Supplementation also decreased the duration of common cold and reduced the duration of sickness when given during a diarrheal episode in pre-schoolers[64,74].

In addition, although controversial data, zinc supplementation seems to increase activity levels and to improve developmental scores among infants and young children[63]. Supplementation also seems to be associated with improved neuropsychological function among most vulnerable schoolchildren[67,75].

Zinc toxicity is not common, toxic intakes are unlikely to be reached with most diets (except for excessive intakes of some types of seafood). Its symptoms are nausea, vomiting, diarrhoea, fever and lethargy and have been observed after very high ingestions of zinc (4-8g).

However, chronic intakes over the requirements could interact with other trace elements metabolism[31].

1.1.7. Magnesium

Magnesium is the fourth most abundant mineral and the second intracellular cation in the human body[76]. Nearly a 60% of body's magnesium is located in bones linked to calcium and phosphorus; a 30-40% is found at muscles and soft tissues and a 1% is in extracellular fluid[105,133].

Magnesium is widely available in plant and animal foods, although there is not a food that provides an extremely high amount of magnesium[76]. Foods rich in magnesium include most green leafy vegetables, legume seeds, beans and nuts, as some shellfish, spices and soya flour[78]. Unrefined cereals, refined flours, tubers, fruits, fungi and most oils and fats are reasonable sources of magnesium but do not contribute significantly as a dietary input[31].

High intake of fibre is related with lower magnesium absorption, due to phytate. However, consumption of phytate and cellulose rich products also increases magnesium intake, which often compensates for the decrease in absorption. High intakes of phosphorus and zinc may decrease magnesium absorption[31].

Magnesium contributes to bone and other calcified tissues health, gives consistency to bone structure and intervenes in its metabolism, given that is essential to osteoblasts and osteoclasts, as well as for all other living cells[78]. It also takes part in more than 300 enzymatic processes in the body such as lipid, protein and nucleic acid synthesis, cellular energy production and storage, stabilization of membranes; and participates in nervous and cardiac functions[76]. Magnesium is involved in the maintenance of intracellular levels of potassium and calcium. Furthermore, magnesium participates in blood pressure regulation by acting as a calcium antagonist on smooth muscle tone, causing vasorelaxation[3,13,79,80].

Magnesium depletion is naturally present in several neuromuscular and cardiovascular diseases, malabsorption syndromes, diabetes mellitus, renal wasting syndromes, osteoporosis and chronic alcoholism[13]. An increase in

magnesium intake would produce a beneficial effect on these chronic diseases by inhibiting inflammation[81].

Magnesium deficiency produces tachycardia together with weakness, muscle cramps, hypertension, disorientation, sickness, vomiting and seizures. Magnesium deficiency may contribute to osteoporosis; in fact, Mg intake was positively associated with bone mass density in elderly[82] and so it was in young adulthood if intake had been correct during pre-adolescent ages[83]. Moderate to severe magnesium deficiency may cause hypocalcaemia[13,45].

Adverse effects for excess intake of magnesium from food sources are rare, but the use of pharmacological doses can result in toxicity, presenting diarrhoea, metabolic alkalosis, hypokalaemia, paralytic ileus and cardiorespiratory arrest[13]. Anyway, although magnesium toxicity through excess dietary intake is not common in healthy individuals, it can drive to diarrhoeas and weakness if there is an excessive intake in individuals with some renal failure[3].

1.1.8. Iodine

Iodine content of food depends on the iodine content of the soil in which it is grown, being seawater a rich source of iodine due to the iodine drag from the coastal areas to the sea during glaciations and flooding[31,84]. Therefore, marine foods (fish, seafood and seaweed) and milk products from animals reared with iodine-enriched feeds or pastured on iodine-rich soils or treated with iodine cleaners for milk and teats contain high amounts of iodine[85].

Bread and grain products can also be a iodine source due to the use of iodate as bread conditioner[84].

Most foods are low in iodine if we take into account the physiological needs of this mineral[85]. The main strategy for controlling iodine deficiency disorders has been universal salt iodization, adding iodine to all salt destined to human consumption, including salt used in food processing[86], followed by supplementation[87]. Iodised-table-salt and food industry iodised-salt addition to processed foods are expected to be the main source of iodine, but in many countries, the use of iodised-salt is not mandatory in processed foods, and unfortunately more salt intake comes from commercial processed foods than from household salt[88]. In some regions of the world were salt iodization is not possible, other common foods are iodized (bread), or oral iodized oil supplements are administered[84].

Iodine is necessary for the synthesis of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), which at the same time are essential for the development of the central nervous system[87,89]. The physiological actions of thyroid hormones are growth and development and the control of metabolic processes in the body. Thyroid hormones play a major role in the growth and development of brain and central nervous system from 15th week of gestation to 3y of life[31]. Thyroid hormones also regulate the basal metabolic rate, reducing it to 50% in total absence or increasing it over a 100% when they are produced in excess[3]. The metabolic processes controlled by iodine include carbohydrate, fat, protein, vitamin and mineral metabolism (thyroid hormone increases energy production, increases lipolysis, regulates gluconeogenesis and glycolysis)[31].

Iodine deficiency can affect the whole population at all stages of life, but infants and children younger than 3 years and pregnant and lactating women are the most important groups in which to diagnose and treat of iodine deficiency due to the irreversible damage to which leads when occurring during foetal and neonatal growth and development[90]. If iodine deficiency occurs during brain and central nervous system development and results in thyroid hormone deficiency, an irreversible derangement of this development can occur, being the most serious form cretinism[31].

Mild to moderate deficiency occasions goitre and can affect cognitive development in infants and children[84,91,92]. Severe iodine deficiency causes hypothyroidism, with mental and growth retardation, and can result in children born with cretinism related to maternal iodine deficiency[89]. Iodine deficiency can be defined as the world's greatest single cause of preventable brain damage[93].

Although iodine supplementation has decreased the number of people at risk of iodine deficiency, it has also led to concerns of excessive iodine exposure in some individuals. The only potential source of excess intake of iodine through food is consumption of many varieties of seaweed in some parts of the world like Japan[87]. Besides dietary consume, iodine is present in huge concentrations (up to several thousand-fold higher than upper level recommendations) in medications, supplements and in the iodinated contrast agents utilised for some studies[87]. In susceptible individuals the use of these substances can result in thyroid dysfunction as a result of high iodine load, sometimes, only after one single exposure to high doses[87].

Generally, excess iodine exposure does not result in any apparent consequences, because excess is mostly excreted in urine, but could occur in vulnerable patients. Excessive consume of iodine could occasion thyroid dysfunction by inhibiting the synthesis and liberation of thyroid hormones (hypothyroidism)[85] due to the acute Wolff-Chaikoff effect described first in 1948[94], this dysfunction can be transient or permanent. Aversely, in susceptible patients, an excess of iodine load can increase thyroid hormones production (hyperthyroidism)[87]. The incidence of both thyrotoxicosis was increased after period of mandatory salt iodisation, compared with when supplementation was not required[95,96].

1.1.9. Vitamin B_{12} (cobalamin)

Cobalamin is a water soluble vitamin, also known as vitamin B_{12}, and it is the larger of the vitamin B complex vitamins. Cobalamin can be found as methylcobalamin or 5-deoxyadenosilcobalamin (or coenzyme B_{12}) in mammalian cells. In nature, there are two other forms of vitamin $B_{12,}$ hydroxycobalamin and aquacobalamin. Furthermore, the synthetic form of vitamin B_{12} found in supplements and fortified foods is cyanocobalamin. These three last can be enzymatically activated to the mammalian cells forms of cobalamin[31].

Although B_{12} is naturally present only in foods of animal origin, most microorganisms synthesize vitamin B_{12} (including bacteria and algae)[97,98], besides, in many animals, gastrointestinal fermentation support the growth of these vitamin B_{12} synthesizing microorganisms; then, vitamin B_{12}

produced is absorbed and incorporated into the animal tissues. Therefore, viscera such as liver and kidney and, generally, products from herbivorous animals, such as meats, eggs or dairy products are foods rich in vitamin B_{12}. Fish would also be a good source, specially tuna, sardines and clams[13,31,97].

Vitamin B_{12} has essential roles in human health as it is a co-factor in the metabolism of DNA and erythrocytes synthesis[99]. Vitamin B_{12} also intervenes in other several processes of synthesis like RNA, protein and neurotransmitters formation; it is also useful in maintaining the myelin of nerve cells and the energy pool in muscles; and necessary for the folic acid metabolism and for the transformation of fatty acids into energy[3].

The most common causes of vitamin B_{12} deficiency are low dietary intake of the vitamin (e.g. a low intake of animal-source foods) and malabsorption[98]. It has been widely described that strict vegetarian individuals who consume diets completely free of animal-source foods are at high risk of vitamin B_{12} deficiency[98,100–105]; while lacto-ovo-vegetarians, who consume the vitamin in eggs, milk and other dairy products, are at lower risk of deficiency than vegans but still at greater risk of cobalamin depletion than omnivores[98,105–109]. Concerning infants, it has been stated that infants born to vitamin B_{12}-deficient mothers are at high risk of developing deficiency because of their low vitamin stores at birth[98,110]; furthermore, if they are breastfed, the risk increases giving the fact that mother's breast milk will be deficient in vitamin B_{12}[97,111,112]. In addition, the recent rise of exclusive breast feeding in developed countries may have thus spread the severely affected vitamin B_{12}-deficient infants worldwide[97].

Vitamin B_{12} malabsorption could happen due to pernicious anaemia, an autoimmune disease that prevents vitamin B_{12} absorption, unknown to occur before the age of 50; due to atrophic gastritis, which would prevent absorption of vitamin B_{12} due to a progressive reduction of hydrochloric acid secretion by the parietal cells that occurs with the age; or could appear as a result of Helicobacter pylori infection, which causes, over time, gastric atrophy[98].

Vitamin B_{12} deficiency is the direct cause of megaloblastic anaemia, peripheral neuropathy, fatigue, depression and cognitive impairment if deficiencies occur in early development stages[99,113]. Macrocytic anaemia caused by vitamin B_{12} deficiency is similar to that occasioned by folate deficiency, what makes it difficult to recognize[114]. In fact, contribution of vitamin B_{12} deficiency to the worldwide nutritional anaemia prevalence is perhaps misjudged because of the simultaneous deficiencies of iron, folate, and other vitamins, consequence of low-in-animal-protein diets. Furthermore, iron deficiency and thalassemia both mask macrocytosis, even when vitamin B_{12} deficiency is severe[97].

The other severe affectation derived from B_{12} deficiency, is the nerve degeneration, which could be fatal, produce sensitive alterations in legs, itching and numbness, and also concentration and memory loss, disorientation and dementia[133,13,114]. Vitamin B_{12} deficient infants and younger children may also suffer movement disorders. Symptoms in infants include irritability, abnormal reflexes, feeding difficulties, obnubilation progressing to coma, and permanent developmental disabilities if diagnosis is delayed and affects brain normal growth[97].

In Europe, Canada and United States, the estimated intake of vitamin B_{12} is higher than the recommendations fixed by the Food and Nutrition Board of the American Institute of Medicine (FNB-IOM) for all adults, older children and toddler[115]. Additionally, the use of supplemented food and/or supplements may reduce the risk of deficiency even for vegetarians. At any rate, it is necessary to monitor vegetarian individuals and no-vegetarian individuals in low-income countries where diets are low in animal-source foods because of their cost, lack of availability, and/or cultural and religious beliefs[105].

No toxicity or adverse effects have been described for an excessive intake of this vitamin, it has a low toxicity risk, because although when high oral doses of this vitamin are given, only a small amount can be absorved[13].

1.1.10. Vitamin B_9 (folate and folic acid)

Folate is the general term utilised for this water-soluble vitamin and makes reference to the different forms that are found naturally in foods. Instead, folic acid (pteroglutamic acid or PGA) is the term used to make reference to the synthetic form of folate found in supplements and fortified products[98,116].

The best alimentary sources of folate are dark green leafy vegetables, legumes, orange juice, nuts, asparagus and fortified cereals and cereal products. Except of liver, meat is not a good source of folate[98,116]. The bioavailability of folate is influenced by many factors, including the chemical

form of folate. Synthetic folic acid is about two fold more bioavailable (100-85%) than food folate (50%), due that folate must be hydrolysed to a more bioavailable form in the brush border of the intestine prior to absorption[98,115–118].

Folate functions as a coenzyme in the metabolism of nucleic and amino acids[13]. It is needed to produce and maintain new cells, being especially important during cellular division and rapid growth such as pregnancy and infancy, because it is essential in DNA replication[116].

The primary cause of folate deficiency is low intake of sources rich in the vitamin, such as legumes and green leafy vegetables[98]. Although few data about folate intakes is available, status is for sure poorer in populations who rely on unfortified cereals as a staple and low consumption of legumes and green leafy vegetables.

Folate deficiency hinders cellular synthesis and division, affecting spinal cord, a cell rapid turnover organ. Due RNA and protein synthesis are not completely impaired, big or amorphous blood cells appear (megaloblasts), resulting in megaloblastic anaemia. So, folate is needed by adults and children to produce normal blood cells and prevent from anaemia[13]. Furthermore, folate requirements are increased during pregnancy, due to the utilization for synthesis of DNA, RNA, amino acids and other compounds for foetal and maternal tissue growth. In fact, severe folate deficiency during pregnancy can result in megaloblastic anaemia in the mother[115,116].

Actually, the major clinical concern for folate deficiency is augmented incidence of foetal neural tube defects[116]. Hence, since early 90's the American Institute of Medicine (IOM) issued a recommendation that all women planning or capable of pregnancy consume a daily folic acid supplemental intake to reduce their likelihood of having a neural tube defects-effected pregnancy[119]. Later, in 2000's some European countries stated similar recommendations for periconceptional folic acid supplementation[120–123]. Accordingly, several epidemiologic studies have demonstrated that a periconceptional supplementation with folates can reduce the occurrence and recurrence of neural tube defects in the offspring[119,124,125].

In addition, there is some evolving evidence of the relationship between folic acid intake and/or supplementation with other health outcomes like reduced risk for offspring congenital heart defects[126], reduced risk of developing colorectal cancer[127] or reduced occurrence of mortality due to stroke[128].

Available literature regarding folate toxicity or adverse effects describes no evidence of toxic effects for an excessive intake of this vitamin[115]. Anyway, an excess of folic acid could mask the diagnose of vitamin B_{12} deficiency, and thus increase the risk of progressive unrecognised neurological damage[13].

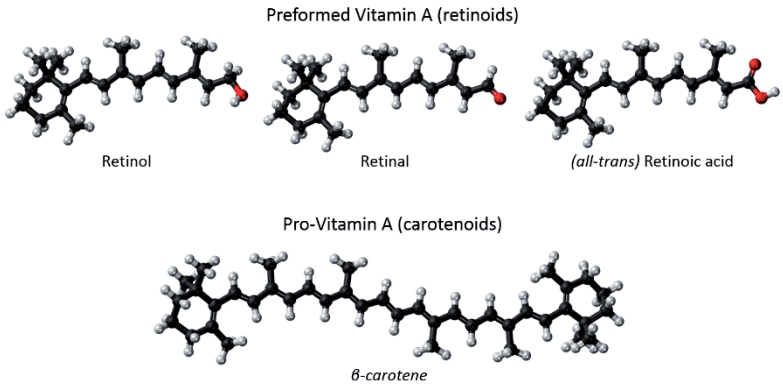
1.1.11. Vitamin A

The concept of vitamin A refers to a group of lipo-soluble molecules, which include preformed retinoid, such as retinol, retinal or retinoic acid; and several pro-vitamin A carotenoids among which β-carotene is the most important[38,129]. Figure 2 summarizes the main vitamin A forms.

Preformed vitamin A (retinoid) can be found almost exclusively in animal products, such as human milk, glandular meats, liver and fish oils (especially), caviar, egg yolk, whole milk, and other dairy products[129,130]. Pro-vitamin A carotenoids are found in green leafy vegetables (e.g. spinach, amaranth, ruff, chard, and young leaves of various sources), yellow vegetables (e.g. pumpkins, squash, sweet potato, and carrots), and yellow, orange or red non-citrus fruits (e.g. mangoes, apricots, loquats, tomato and papayas)[130]. Foods containing pro-vitamin A carotenoids tend to have less biologically available vitamin A (12μg β-carotene = 1μg retinol) but are more affordable than animal products. It is mainly for this reason that carotenoids provide most of the vitamin A activity in the diets of economically deprived populations[131,132]. In this direction, NANHES study stated that pro-vitamin A carotenoid provide ≤30% of daily vitamin A in United States; ≈80% in low-income populations in developing countries[133].

Besides the natural contribution from food, in many countries dairy products and margarines are enriched with retinol esters becoming an important source of the vitamin.

Figure 2. Vitamin A forms.



Adapted from Jynto[134].

Traditionally, vitamin A was considered fundamental due to the role it plays in the visual system, improving the night vision, preventing from visual diseases as cataracts, glaucoma, vision loss and crepuscular blindness[135]. Nowadays, vitamin A is considered essential for being systematically responsible for the formation, growth and differentiation of cells in all body tissues. It is needed for bones growth and development as well as it is essential for mucous, epithelia, skin, vision, nail, hair and tooth enamel cells' growth, maintenance and reparation. It also has to be with the stimulation of some immunological functions (what promotes the reparation of infected tissues and increases the resistance in front infections); in the reproduction process (intervenes in sperm production and in the normal development of the female reproductive cycle too); and in the correct foetal development[132,135,136]. Furthermore, it acts as an antioxidant that eliminates free radicals and protects DNA from mutagenic agents, prevents cellular aging and the occurrence of cancer[129].

Vitamin A deficiency can be occasioned by an insufficient consumption or as a consequence of other diseases. The primary consequence resulting from the deficiency of this vitamin is the night-blindness, which can become permanent blindness. Occasionally, vitamin A deficiency can produce xerophthalmia (ocular tissue keratinization). It is also related with several cutaneous problems and of immune-competence as well as with several birth defects on the offspring[129,136]. This deficiency has been classified as a public health problem in children in developing countries[135,137,138]. According to the World health Organization (WHO), vitamin A deficiency is the first cause of childhood blindness, and is associated with reduced immune function and increased mortality risk from common childhood infections and measles in developing countries[131,136,138].

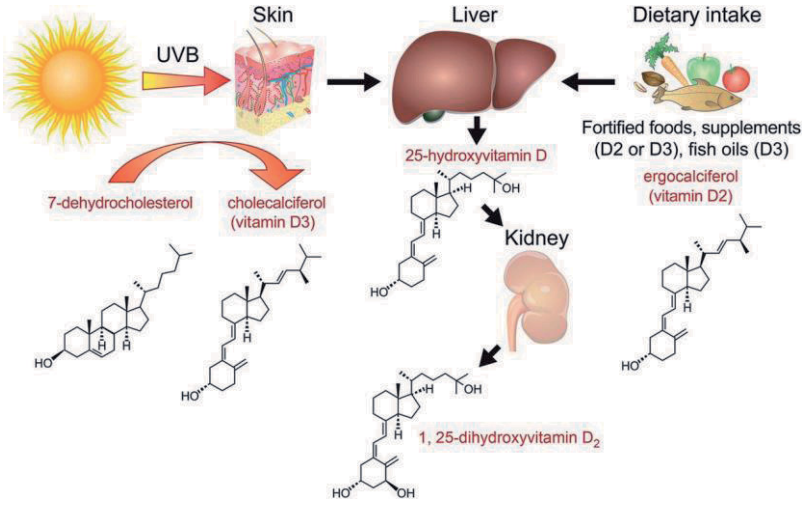
The excess of vitamin A is known as hypervitaminosis A. It can be acute (excessive intake), chronic (high intake along the time) or due to teratogenicity (excess of vitamin A in the mother during pregnancy that can occasion spontaneous abortion or defects in the offspring)[139]. The acute toxicity can produce gastrointestinal symptoms (acute enteritis), headache, blurred vision and muscular incoordination. In chronic affectation it is common the bone and muscular pain (hypercalcaemia: bone loss and soft tissue calcification), anorexia, skin disorders, headache, skin dryness, hair loss, liver damage, double vision, bleeding, vomiting, hip fracture and even coma[129,140]. This toxicity is only produced by the excessive consumption of preformed vitamin A (retinoid) due to overmedication, carotenoids do not produce this symptoms[129,141].

1.1.12. Vitamin D

Vitamin D is a lipophilic molecule that can either be synthesized in the epithelial cells from 7-dehydrocholesterol by exposure to sunlight[142], or can be provided by the diet already pre-formed. Vitamin D should not strictly be called a vitamin, in fact, some texts refer to it as prohormone D because its deficiency can be avoided with a sufficient exposure to sunlight[31].

The form synthesized in the skin from 7-dehydrocholesterol is called pre-vitamin D_3 or cholecalciferol while the dietary form can be pre-vitamin D_3 or a plant origin molecule known as pre-vitamin D_2 or ergocalciferol. Anyway, in humans, the two forms are hydroxylated into calcidiol [25-(OH)vitD] first, and into calcitriol [1,25-$(OH)_2$vitD] later, so can be considered equivalent and equal in potency[143]. Calcitriol is considered the biologically active form of vitamin D[144]. Figure 3 synthesises vitamin D sources and metabolism.

Figure 3. Vitamin D sources and metabolism.



UVB: ultraviolet radiation in B-wavelength region (320–290 nm).
From Al Mheid et al. 2013[145].

Skin exposure to the UVB radiation is the main source of vitamin D for human[146,147], it is known as the "sunshine vitamin"[143]. Its production is determined by several factors such as time of the day, residence latitude, year season, atmospheric pollution, skin colour and age; being darkly pigmented individuals at higher risk of vitamin D deficiency due to the competition of melanin with 7-dehydrocholesterol for UV absorption, or elderly due to age associated decreases in 7-dehydrocholesterol[144,147,148].
Vitamin D is found in reasonable amounts in cod liver oil, oily fish such as herring, mackerel, sardines, tuna or salmon, caviar, eggs, red meat, mushrooms, butter, fortified margarine and breakfast cereals[143,149,150]. Although the small amount of vitamin D found naturally in foods, there is a rising interest in vitamin D-fortified foods and supplements[144].

Vitamin D traditional function is the regulation of levels of calcium and phosphate in blood, which are also required for other functions like the normal mineralization of bones, the muscular contraction, the nerve conduction and general cellular function in all the cells of the body[3,31,147]. Additionally, vitamin D may also 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[147,151].
Vitamin D active form, calcitriol, regulates the transcription of several vitamin D-dependent genes which code for calcium transporting proteins and bone matrix proteins. Another function of vitamin D is to modulate the transcription of cell cycle proteins, which decrease cell proliferation and increase cell differentiation of a number of specialised cells of the body[31,151]. Emerging evidence states that this vitamin D anti-proliferative action may

play a role in reducing the risk of certain cancers as well as non-malignant conditions as type 1 diabetes, metabolic syndrome and autoimmune diseases like multiple sclerosis, hypertension and Chron's disease[151,152]; this vitamin also strengthens the immune system, preventing from infection[3].

Hypovitaminosis D is still a problem worldwide, particularly in developing countries, at high latitudes and in countries where skin exposure to sunlight is discouraged[147,153]. When sunlight exposure is insufficient, the dietary intake of vitamin becomes essential. Hence, if vitamin intake is inadequate, rickets can appear in children, producing curved pelvis and legs; whilst in adults we would see osteomalacia[142,143]. Vitamin D deficiency is as well related with an increased risk of osteoporosis and falls, additionally, vitamin D could play a role in reducing the risk of certain cancers, autoimmune diseases and hypertension[143,152].

Infants have higher vitamin D needs because of their high skeletal growth rate. Breastfed infants are particularly at risk of suffering vitamin D deficiency due to the low concentrations of it in human milk[154], this problem is further compounded when there is a lack of sun exposure[155,156] and if additionally they do not receive any vitamin D supplementation[150]; however infant formulas are supplemented with vitamin D in sufficient amount to prevent rickets. Anyway, some studies that analyzed breastmilk composition found an association of decreasing phosphorus content of breastmilk with a progressive increase in serum calcium, magnesium and protein concentrations, what may mean that a low phosphorus intake (thus serum concentration) stimulates vitamin D internal synthesis and thus improve calcium absorption in breastfeed infants [157].

Another period of the skeleton rapid growth is adolescence, but adolescents usually spend more time outdoors and therefore are exposed to levels of UV light sufficient for synthesizing vitamin D for their needs[158]. Excess production of vitamin D in summer is stored mainly in the adipose tissue[159] and available to sustain growth rates in winter months that follow, and if these stores are not enough, increased growth can lead to vitamin D insufficiency[160].

Surprisingly, the current interest in vitamin D has produced an increase in vitamin D serum determinations, showing a huge prevalence of biochemical vitamin D deficiency[147]. Reports from across the world indicate that hypovitaminosis D is widespread and is re-emerging as a major health problem globally[160].

Vitamin D is a quite toxic substance, but toxicity does not occur at the recommended intake levels. The excess of vitamin D intakes generates hypercalciuria and hypercalcaemia, thirst, anorexia, soft tissues calcification and urinary tract stones[31].

1.2. DIETARY RECOMMENDATIONS AND THEIR APPLICABILITY

1.2.1. Concept of nutritional requirements and dietary recommendations

The nutritional requirements are the minimum amount of a determined nutrient needed by an individual to maintain health; what includes maintaining body weight, avoiding nutrient body pools depletion and preventing from specific function failure or appearance of deficiency signs. In children and adolescents, nutritional requirements have to ensure a correct growth and development as well[161].

To determine an exact value for nutritional requirements is impossible even for an individual, because metabolism and activity conditions change every day. Anyway, it is reasonable to define a minimum theoretical nutritional requirement for similar groups of individuals[162] and after that, dietary recommendations may be calculated for those groups. Therefore, dietary recommendations are a theoretical concept utilised to describe those amounts that are safe intakes of nutrients for almost all the population in a specific group.

The concept of dietary recommendations was born due to the need of establishing some rules to guarantee a correct nutritional status of the population once that nutrition had been accepted by the scientific community to be an important factor influencing health[163]. Since early 20th Century, each country and its expert committees published their own dietary recommendations and during that process created their own terminology. Inevitably, this caused considerable confusion, due to many

terms and therefore many meanings (safe intakes of nutrients, daily recommended nutrient intakes, recommended dietary allowances, recommended dietary intakes, or dietary standards)[164].

In the European Union, dietary recommendations are known as **Dietary Reference Intakes (DRI)**.

1.2.2. Worldwide available Dietary Reference Intakes

There are different committees around the world, that use data from different populations to establish dietary recommendation addressed to those specific populations.
The most remarkable dietary recommendations are those from the Food and Agriculture Organization (FAO) in collaboration with the World Health Organization (WHO) and the United Nations University (UNU), from now onwards called FAO/WHO/UNU DRI. FAO/WHO/UNU DRI were established from data obtained from studies made with representative individuals of the entire world population; thus, they are useful for the utilisation all over the world.

The other recommendations to which it is mandatory to refer to are the American Dietary Reference Intakes, developed by the National Council of the United States and The Food and Nutrition Board of the American Institute of Medicine (FNB-IOM) for the North-American population; those recommendations are also used in other populations due to their scientific relevance and rigor in their calculations.

In Europe, there are dietary recommendations available in almost each country of the European Union. Those recommendations did not follow common criteria to establish recommendations when were developed; some countries based the DRI on studies and original data from their own country, others on data from FNB-IOM references or from the FAO/WHO/UNU DRI, others adapting existing data to their own country and others combining all methods together[163]. The Scientific Committee on Food of the European Union (EU-SCF) proposed nutrient and energy intakes for European population[165].

- Dietary Reference intakes from FAO/WHO/UNU (FAO/WHO/UNU DRI)

FAO has joint many groups of experts in nutrition from 1948 to periodically evaluate and interpret the updates of the scientific knowledge, being then able to define energy and nutrient requirements as the basis to establish adequate dietary recommendations and to design convenient nutritional policies. The World Health Organization started its collaboration with FAO in this issue during the fifties, and United Nations University did in 1981[166].

There have been many expert consultations made by WHO, FAO, FAO/WHO and FAO/WHO/UNU working on energy, protein, vitamin, mineral and trace elements requirements and studies about diet, nutrition and chronic diseases prevention. The conclusions from those meetings have been published elsewhere from 1950 to nowadays[31,167–169].

Dietary Reference Intakes set by the FAO/WHO/UNU comprise the following concepts[31]:

- Estimated Average Requirement (EAR): mean value of a nutrient estimated to cover the requirements of the 50% of the healthy population.
- Recommended Nutrient Intake (RNI): mean value of a nutrient estimated to cover the requirement of the 97.5% of the healthy population.
- Protective Nutrient Intake (PNI): supplementary amount of a nutrient to the RNI in a special period with increased needs.
- Tolerable upper intake level (UL): maximum level of intake of a nutrient at which there are few possibilities that adverse effects appear due to an excessive consumption, in healthy population of a specific age and gender group.

FAO/WHO/UNU established Dietary Reference Intakes for[31]:

- Macronutrients.
- Vitamins: thiamine, riboflavin, niacin, pantothenic acid, vitamin B_6, biotin, folic acid, vitamin B_{12}, vitamin C, vitamin A, vitamin D, vitamin E and vitamin K.
- Minerals: calcium, magnesium, iron, zinc, iodine and selenium.
- Fibre, alcohol and salt.

- Dietary Reference Intakes from the United States and Canada

The Food and Nutrition Board of the American Institute of Medicine (FNB-IOM) established in 1941 the first Recommended Dietary Allowances (RDA) for vitamins, minerals, proteins and energy. Those RDA have been the basis for nutritional policy, with ten updated publications between 1941 and 1989[170].

After the last review in 1989 and the publication of Recommended Nutrient Intakes in Canada in 1990[171], a cooperation started to review the RDA concepts, this time taking into account recent investigations about nutrients and their relation with health indicators and chronic diseases prevention in healthy population. Since 1997, the FNB-IOM has published several versions of Dietary Reference Intakes[13,34,115,172–177].

Dietary Reference Intakes set by the FNB-IOM comprise the following concepts[13]:

- Estimate Average Requirement (EAR): Amount of a nutrient which is adequate to satisfy the requirements of the 50% of the healthy individuals of a population group of a specific age range and gender.
- Recommended Dietary Allowances (RDA): Daily amount of a nutrient which is adequate to satisfy the nutritional requirements of almost all (97.5%) individuals of a population group of a specific age range and gender.
- Adequate Intake (AI): Mean daily recommended intake, based on healthy population mean intakes, determined through experimental

studies or extrapolation. Used when there is not enough scientific information to establish EAR or to calculate RDA.

- Tolerable Upper Intake Level (UL): Maximum intake level of a nutrient at which is almost impossible that health adverse effects appear in general population due to an excessive consumption.

FNB-IOM has established Dietary Reference Intakes for[13]:

- Macronutrients.
- Vitamins: thiamine, riboflavin, niacin, vitamin B_6, folic acid, vitamin B_{12}, vitamin C, vitamin A and vitamin E; adequate intakes for vitamin D, vitamin K, pantothenic acid, biotin and choline; and for all vitamins for infants.
- Minerals: copper, iodine, iron, magnesium, molybdenum, phosphor, selenium and zinc; adequate intakes for calcium, chrome, fluoride, manganese, potassium, sodium, chloride, and for all minerals in the case of infants.
- Fibre and water.

- Dietary Reference Intakes according to the Scientific Committee on Food of the European Union

The Scientific Committee on Food (EU-SCF) of the European Union was the responsible to create the Reference Values for the European Union and those were published by the European Community Commission in 1993[178].

Dietary Reference Intakes set by the EU-SCF comprise the following concepts[178]:

- Average Requirement (AR): Mean requirement of a population group that coincides with the amount that is adequate to satisfy the requirements of 50% of the healthy population, because of a symmetric distribution.
- Population Reference Intakes (PRI): Intake that is enough for virtually all healthy people in a group (97.5%).
- Acceptable Range of Intake (ARI). When there is not data available to set up Reference Intakes, a range of acceptable intake is established.
- Lowest Threshold Intake (LTI): Intake under which almost all the population (97.5%) does not maintain metabolic integrity.

EU-SCF has established Dietary Reference Intakes for[178]:

- Macronutrients.
- Vitamins: thiamine, riboflavin, niacin, vitamin B_6, folic acid, vitamin B_{12}, vitamin C, vitamin A and vitamin D.
- Minerals: calcium, phosphor, potassium, iron, zinc, iodine, selenium and copper.

Whatever they are called, all available DRI include similar set of concepts that are the dietary recommendations of nutrients that aim to be adequate to satisfy the requirements, to prevent from deficiency diseases, to reduce chronic diseases, to obtain an optimum health and to avoid toxicity due to overdose in a determined population; all these, exploiting the maximum potential of each nutrient. Table 1 summarizes the several set of concepts

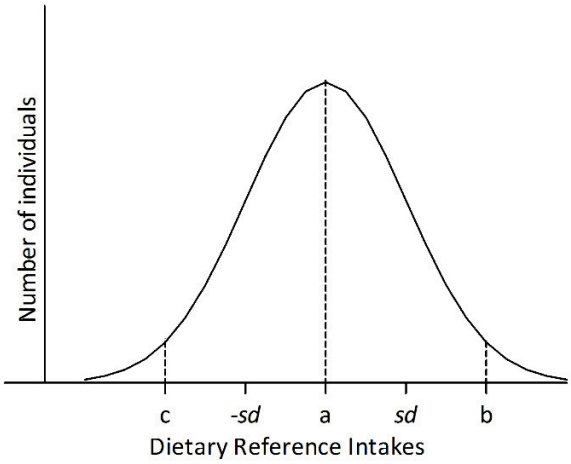
of different worldwide DRI tables and Figure 4 clarifies the distribution of the DRI concepts in population.

Table 1. Dietary Reference Intakes concepts.

FAO/WHO/ UNU 2004(1)	FNB-IOM 2006(2)	EU-SCF 1993(3)	Concept definition
EAR: Estimate Average Requirement	**EAR:** Estimate Average Requirement	**AR:** Average Requirement	Amount of a nutrient which is adequate to satisfy the requirements of the 50% of the healthy population
RNI: Recommended Nutrient Intake	**RDA:** Recommended Dietary Allowances	**PRI:** Population Reference Intake	Amount of a nutrient which is adequate to satisfy the requirements of the 97,5% of the healthy population
-	**AI:** Adequate Intake	**ARI:** Acceptable Range of Intake	Mean daily recommended intake, based on healthy population mean intakes. Used when EAR/AR and RDA/PRI are not available
PNI: Protective Nutrient Intake	-	-	Supplementary amount of a nutrient to the RNI/PRI/RDA in a special period with increased needs
-	-	**LTI:** Lowest Threshold Intake	Intake under which almost all the population does not maintain metabolic integrity
UL: Tolerable Upper Intake Levels	**UL:** Tolerable Upper Intake Levels	**UL:** Tolerable Upper Intake Levels	Maximum intake level at which is almost impossible that health adverse effects appear due to an excessive consumption

(1) Dietary Reference Intakes concepts established by the FAO/WHO/UNU cooperation[31]. (2) Dietary Reference Intake concepts described by The Food and Nutrition Board of the American Institute of Medicine (FNB-IOM) in a cooperation between United states and Canada[13]. (3) Dietary Reference Intake concepts according to the Scientific Committee on Food of the European Union (EU-SCF)[165,178].

Figure 4. Distribution of the Dietary Reference Intakes concepts in population.



a: EAR/EAR/AR, b: RNI/RDA/PRI, c: LTI according the Dietary Reference Intakes concepts provided by FAO/WHO/UNU, FNB-IOM and EU-SCF. Adapted from Cuervo M. et al., 2010[168].

All DRI are normally expressed as an amount of nutrient per person and day, however this does not mean that each nutrient need to be consumed every day in the exact portion indicated by the correspondent DRI; the total amount recommended per person and day should be interpreted as a mean over 5-10 days.

DRI values are set in accordance to the needs of healthy population with specific weight and height characteristics. Furthermore age, sexual development and women reproduction status also influence on some nutrient needs. As summarized in Table 2, all Committees provide DRI for several age ranges.

Table 2. Age ranges in the Dietary Reference Intakes tables.

Population group	FAO/WHO/UNU 2003[(1)]	FNB-IOM 2006[(2)]	EU-SCF 1993[(3)]
Infants (months)	[0-6], [7-11]	[0-6], [7-12]	[6-11]
Children (years)	[1-3], [4-6], [7-9]	[1-3], [4-8]	[1-3], [4-6], [7-10]
Men (years)	[10-18], [19-65], >65	[9-13], [14-18], [19-30], [31-50], [51-70], >70	[11-14], [15-17], ≥18
Women (years)	[10-18], [19-50], [51-65], >65 [pregnancy], [lactation]	[9-13], [14-18], [19-30], [31-50], [51-70], >70 [pregnancy], [lactation]	[11-14], [15-17], ≥18 [pregnancy], [lactation]

Adapted from Cuervo M. et al., 2010[168].

1.2.3. Dietary reference intakes utilities and limitations

Since the first dietary recommendations (RDA) arose to ensure an adequate diet for the United States soldiers during the Second World War in 1941, the utility of Dietary Reference Intakes has been widely diversified[163].

Dietary Reference Intakes tables can be used[13,31,163,173]:

- For planning diets for homogeneous groups of population (therapeutic diets, collective diets, institutional dietary planning).
- For the evaluation of dietary intake, as a reference for the calculation of the adequacy of intake (taking into account that DRI are a 20% higher than real physiological requirements). Assessment of disease risk.

- For Nutrition education or divulgation purposes and for guidance for food selection (public health and disease prevention policies or development of dietary guidelines)
- To define the objectives for food fortification and development of new industrialised or modified food products.
- For food labelling and nutritional marketing.
- For food safety considerations

It must be taken into account that not all the DRI tables are designed to be used for all the utilities described above.

DRI tables have some limitations that should be considered[13,31,163]:

- DRI tables do not cover 100% of the population, due that recommended amounts are statistically calculated taking the 97.5% of the population as a reference.
- There are some nutrients for which DRI are not available.
- DRI tables are developed for healthy population without taking into account special needs. It is worth noting that it would be needed to split DRI tables in more population groups considering increased longevity and increasing scientific knowledge on infants' nutrition.
- DRI tables are something veering with scientific emerging knowledge, like in example could be nutrigenomics.

1.2.4. Use of the Dietary Reference Intakes for the evaluation of dietary intake adequacy

The endpoint after evaluating an individual or population dietary intake is to assess if that intake satisfies the needs established through the DRI for that individual or population. Unluckily, neither the exact nutrient intake nor the nutrient requirements are known for every single individual, hence the nutrient intake adequacy needs statistical approaches to be calculated[179].

In 2000, the American Institute of Medicine (IOM) published a guideline describing how DRI must be applied both, for the planning of dietary interventions, and for nutritional assessment (including evaluation of adequacy) at individual and population levels[173]. This methodology proposed by the IOM can be applied in Europe, anyway it would be necessary to develop a protocol describing how European countries should proceed when assessing nutrient intake adequacy[179].

From the literature reviewed, the IOM methodology was the most used to evaluate nutrient intake adequacy[179–185]. Anyway, many researchers do not follow IOM guideline and keep on assessing adequacy by different methods[179].

It should be highlighted that the methodology to assess nutrient intake adequacy varies when referring to individuals or to populations. Although EAR, AI, and UL can be utilised to assess intakes of individuals and groups, RDA should never be utilised for groups. The choice of a particular DRI for

the nutritional assessment will provide different outcomes, as detailed in Table 3.

Table 3. Uses of the Dietary Reference Intakes for assessing adequacy of intake.

DRI	At individual level	At group level
EAR	to examine the possibility of inadequacy	to estimate the prevalence of inadequate intakes within a group
RDA	usual intake at or above this level has a low probability of inadequacy	do not use to assess intakes of groups
AI	usual intake at or above this level has a low probability of inadequacy	mean usual intake at or above this level implies a low prevalence of inadequate intakes
UL	usual intake above this level places an individual at risk of adverse effects from excessive nutrient intake	to estimate the % of the population at risk of adverse effects from excessive nutrient intake

DRI: Dietary Reference Intakes. EAR: Estimate Average Requirement, RDA: Recommended Dietary Allowance, AI: Adequate Intake, UL: Tolerable Upper Intake Level. Adapted from Institute of Medicine, 2000[173].

In the case of group adequacy assessment, a review performed by Román-Viñas et al. in 2009[179] did not find any publication dealing with a methodology to assess nutrient intake adequacy at individual level, except those explaining the procedure proposed by the IOM[173]. Instead, many nutrient intake adequacy assessment methods at population level were described in many population-based surveys, although scientific basis to support those procedures were not explained[179,180].

Either one way or another, whenever possible, the assessment of dietary adequacy should combine other biological parameters such as anthropometry, biochemical indices and clinical status. Dietary intake adequacy should be assessed on the totality of the evidence, not on dietary intake data alone.

1.3. MICRONUTRIENT STATUS IN EUROPEAN CHILDREN

Although vitamin and mineral deficiencies adversely affect a third of the world's population[186], Europe is considered a developed region in which nutrition is assumed to be adequate to satisfy population requirements. Consequently, public health and nutrition policies are focused on addressing problems of over-consumption such as obesity, hypertension or cardiovascular diseases[187].

However, several national dietary surveys reveal some prevalence of suboptimal intakes for a number of micronutrients across the region as iron, calcium, zinc, vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6 (niacin), vitamin D and folate (vitamin B_9)[188–193]. A review performed by the EURRECA[a] project in 2011, revealed high prevalence of inadequate micronutrient intakes within adults in Europe, being between 11 to 30% for copper, folate, selenium, iodine, vitamin B_{12} and vitamin C[184].

Few national nutrition surveys focusing in children have been carried out through Europe, in 2001, Serra-Majem[194] compiled the conclusions from some reviews of studies performed in children from 6 to 18 years of age: in Nordic countries children and adolescents perfectly met requirements for calcium, but vitamin D, zinc, selenium and iron were below the

[a] EURRECA: EURopean micronutrient RECommendations Aligned is a network of excellence funded by the European Commission and established to identify and address the problem of differences between countries in micronutrient recommendations. It is made up of 34 partners from 17 countries, drawn from nutrition science, industry, consumer groups, national nutrition societies and health professions. Further details of thenetwork can be found in EURRECA website: http://www.eurreca.org.

recommended nutrient intakes[195]. In Southern Europe the information was scarce and very local; anyway, from data available, a risk of deficiency for calcium, iron, zinc and vitamins A and E was detected[196]. In central and Eastern Europe calcium and vitamin C intake seemed to be not enough[197]. And, in Western Europe (United Kingdom, Ireland, France and the Netherlands), calcium and iron were the problematic nutrients[198].

In 2004, the European Nutrition and Health Report of the European Academy of Nutritional Sciences[199,200] grouped together the results of several national nutrition studies and concluded that, in children from 1 to 14 years of age, vitamin D and folate were low in all countries assessed (Austria, Belgium, Denmark, Finland, Germany, Greece, Hungary, Italy, Norway, Spain and United Kingdom). Insufficient potassium intakes were found in Austrian, German, Hungarian and Italian children. In all countries, calcium intake of younger children was on average higher and more sufficient than that of older children, being insufficient in Austrian, German, Hungarian, Italian and Norwegian children. Iron was low in girls at fertile age. Children and adolescents are a population group likely to have higher risks of micronutrient deficiencies.

Assessing the degree of micronutrient adequacy of intake to the recommendations, not at national level, but across Europe, is a challenge due to the disparity of methods utilised to assess dietary intake, the variability of methods utilised to assess micronutrient intake adequacy to the recommendations and though to the different reference values utilised by each single study[187,201]. Nevertheless, a very recent publication deals, for the first time, with multiple countries published national survey data to

assess the adequacy of micronutrient intakes. This article shows the results obtained re-analysing raw data from different studies all together, using a single set of dietary recommended intakes and using a single method to determine adequacy[187]. The results of this European analysis are given as the mean amount of each micronutrient consumed by every age group, and a % of individuals under the estimate average requirement (EAR) plus a % of individuals under the lowest threshold intake as well. Almost for all the nutrients, the proportion of children with daily intakes below the EAR was measurable in at least one country, as in the case of iodine and iron for which in German children from 4 to 10 years the 55% of boys and 61% of girls, and the 94% of girls, respectively showed intakes below the EAR. Vitamin D intakes were below the EAR in all ages children in every country.

Currently, there are not studies available which were specially designed to collect micronutrients intake data from healthy children from different European countries using the same standardised methodology and analysing intake adequacy all together. Nevertheless this initiative was performed for adolescents from 13 to 16 years, by the HELENA study[b].

[b] HELENA study was a comprehensive three year research programme under the European Union 6th Framework project and was officially concluded in April 2009.
It was designed to understand and effectively enhance nutritional and lifestyle habits of adolescents in Europe. One of the strengths and innovative aspects of the project was the use of common methodology that would result in reliable and comparable data between the countries. The project involved 26 partners from academia and industry areas in 10 countries (Austria, Belgium, France, Germany, Greece, Hungary, Italy, Spain, Sweden and UK).
During the HELENA study, three main objectives were attained. In the first place, the nutritional status of more than 3000 European adolescents between 13 and 17 years-old was assessed through surveys, cross-country and cross-sectional studies in the different countries. The following aspects were included: Dietary intake, nutrition knowledge and eating attitudes; Food choices and preferences, Body composition, Plasma lipids and metabolic profile, Vitamin status, Immune function related to nutritional status, Physical activity and fitness and Genotype (to analyze gene-nutrient and gene-environment interactions).

1.4. BONE MINERAL DENSITY AND NUTRITIONAL INTAKE

Childhood is a period characterized by growth, development and maturation of the different body systems, including the skeletal tissue. Bone mineral density (BMD) varies with the age of the individual. Mineralization increases during the first years of life, very fast from birth to three years of age and progressively slower later. This mineralization process will end at 18-20 years of age[3,33]. In fact, 90-95% of total body bone mass is achieved by the end of the second decade of life, and thereafter mineral density will start decreasing[202,203].

Several factors can influence bone mineralization process, so bone mineral density, either endogenous like genetic factors (race, heredity) or hormonal status; either exogenous such as nutrition (energy, protein, calcium, phosphorus and vitamin D), physical activity and body composition[204,205].

1.4.1. Nutritional factors influencing bone mineral density

- Caloric intake and body composition

Caloric intake is a factor influencing bone health through its influence on body composition, so on body weight and body mass index (BMI).

It has been shown that body weigh influences bone mineral density, being that obese children have higher mineral density that normal-weight children[206]. Anyway, height and lean mass have been set as the strongest determinants of bone mineral content when assessing obese and normal-

weight groups of children[207]. Although obese children show higher fat mass index (FMI), what might be really influencing bone mineral density might be the body size, through the increased mechanical burden of overweight and obese individuals and muscle strength that these subjects must endure during locomotion[208,209].

Contrarily, undernutrition or an excessive decrease of caloric intake has been related with low bone mineral density, as showed in a study with girls diagnosed with anorexia nervosa[210]. In the same study, the authors found an equation to correctly predict whole body bone mineral density from BMI.

- Protein intake

Dietary proteins are considered a key nutrient for the bone health. The IGF-1 increase due to protein intake has an anabolic effect on bone, as described long time ago by Clemmons[211].

Protein intake has been related with bone mineral content and bone mineral density. Many studies indicate that protein deficient intake affects bone negatively deteriorating bone mass, bone matrix structure and strength[212]. High protein intakes, including those from animal sources, have been associated with increased bone mineral mass and reduced incidence of osteoporotic fractures in several cross-sectional and longitudinal observations[110,212–217].

Nevertheless, there is some controversy about the fact that protein excess induces chronic metabolic acidosis, which at the time would increase

calciuria and enhance mineral looseness; instead, increased gut absorption of calcium have been shown[212,217–219]. Protein DRI from the Institute of Medicine, 2002 seem to disagree with this hypothesis because they recommend to increase protein intake up to 35% of calories[175].

Anyway, protein deficiency is not an issue for infants and children in our geographical area giving that recommendations are widely satisfied[33].

- Calcium intake

The association between calcium and bone is widely known, as almost all body calcium (99%) is located in the skeleton[31]. Many studies demonstrate that higher calcium intake or calcium supplementation may lead to better bone mineral density[220–223]. However, there is some controversy as to roundly ensure that association[224–228]. It should be taken into account that bone mineralization is a multifactorial issue.

It also has to be clarified if the beneficial effect of calcium appropriate intake or of calcium supplementation is maintained or not over the time[221,229,230]. Thus, it will be more important to maintain a good calcium intake from birth rather than a punctual supplementation action in a determined period[33].

What is clear is that infancy is a critical period in which requirements are increased due to rapid growth; furthermore in the adolescence peak bone mass is reached and calcium adequate intake may be assured[13].

- Phosphorus intake

Together with the calcium, an 80% of phosphorus can be found in the mineral structure of the bones[32].

Diets high in phosphorus and low in calcium could drive to sustained PTH increase and interfere with calcium absorption. This adjustments in calcium regulating hormones (associated with elevated P) could have adverse effects on the skeleton, by stimulating bone resorption, and thus, producing a decrease in BMD[33].

It is known that high dietary phosphorus lead to slight drops of calcium and to correspondingly increased PTH concentrations[231]. High phosphorus intake produces an increased level of plasma phosphorus that reduces urine calcium loss, reduces renal synthesis of 1.25-$(OH)_2$vitD, reduces serum ionized calcium and leads to an increase in PTH release[232,233]. This reduction in 1.25-$(OH)_2$vitD synthesis may mitigate the hyperphosphatemia by a reduction of P absorption. And the increase in PTH stimulates bone resorption[34].

- Vitamin D intake and synthesis

Vitamin D, as well as PTH, plays an important role in calcium and phosphorus absorption and metabolism, being that a vitamin D deficiency could drive to a reduced calcium and phosphorus absorption, and thus, worst mineralization[147].

Vitamin D availability mainly depends on solar exposure due that few foods are vitamin D-rich[13].

In addition to those mentioned above, many other nutrients have been related to bone health, such as magnesium (which is deposited in the bone together with Ca and P), zinc, vitamin K (as cofactor of osteocalcin synthesis), vitamin C, retinol, B vitamins, Na/K ratio, and so on[33].

1.4.2. Other factors influencing bone mineral density

- Physical activity

One of the main functions of the skeleton is the locomotion. Bone mass and geometry are adapted to the tension of mechanical load and muscular activity[230]. Thus, muscle mass and strength are predictors of bone strength[234].

Weight-bearing physical activity (such as walking, running or weight training, but not cycling or swimming) undertaken in a regular manner favours bone resistance and improves bone mineral content, as well as inactivity drives to bone loss[235]. Several studies with children and adolescent demonstrated an increase in bone mineral content in those who were included in a jumping programme against the controls[235–240]. Furthermore, a meta-analysis performed in 2007 by Hind, reported effects of physical activity on bone, affirming that weight-bearing exercise could

augment bone mineral gain in children, especially during early adolescence[241].

Although some of the studies showed the increase of bone mineral content to be sustained for up to 8 years after the intervention[237,242,243], it is difficult to demonstrate if the increase of bone mineral density that sport causes in children and adolescent would be maintained along the time[33].

Recommendations of physical activity for children and adolescents aged 5 to 17y are of about 60 minutes per day, and vigorous-intensity activities should be included 2-3 times per week, according to WHO[244]. Exceeding these recommendations for children, both in duration or in intensity may drive to adverse effects such as puberty delay and amenorrhea which can compromise bone mass gain[245,246].

1.4.3. Bone mineral density insufficiency

Bone mineral mass results from the difference of the amount of bone formed and lost through modelling during the first two decades of life and the constant remodelling during life. When the amount of bone resorbed exceeds the amount of bone formed, there is a net loss of bone mineral mass[230].

- Osteoporosis and osteopenia

World Health Organization defines osteoporosis as a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture[247,cited in 248]. In osteopenia there is also reduced bone mass, although without microarchitecture affectation[245].

According the WHO, osteoporosis is defined as a bone mineral density 2.5 times below the average value for young healthy women (a T-score <-2.5 SD). A second, higher threshold more appropriate for epidemiological studies describes 'low bone mass' or osteopenia as a T-score that lies between –1 and –2.5 SD[249]. The criterion used for children are generally the same mentioned above but applied to the z-scores of the bone mineral density. Gordon et al. proposed to consider children with a BMD under -2 SD as children with low mineral density for their chronological age[250].

Osteoporosis is an important health problem throughout the world. About 33% of women and 20% of men from the age of 50 onwards undergo some kind of fracture[251]. Given that the BMD along life depends upon the peak bone mass reached by the end of the second decade of life, osteoporosis is not only an adulthood and elderly concern; it might be prevented from childhood, guaranteeing the adequate conditions to ensure the development of the best bone mass possible[252].

Hypothesis and Objectives

2. HYPOTHESIS AND OBJECTIVES

2.1. HYPOTHESIS

Current eating habits evolution could drive to an inadequate consumption of vitamins and minerals with regard to nutritional requirements and dietary recommendations, during the first eight years of life, in children/infants from 5 European countries.

2.2. OBJECTIVES

Main objectives

- To describe dietary intake of micronutrients during the eight first years of life, in children from 5 European countries.

- To determine the micronutrient intake adequacy during the first eight years of life, in children from 5 European countries, with the dietary recommendations established for each age period.

Secondary objectives

- To analyse the relation between calcium intake and bone mineral density.

Methods

3. METHODS

3.1. DESIGN

This study was performed as a secondary analysis of the European Childhood Obesity Project (EU CHOP). The design of the present dissertation is a prospective observational study to analyse the micronutrients intake and its adequacy to the dietary recommendations in a multicentre European sample of children and its relation with different health outcomes in a subsample.

The EU CHOP was a double-blind, randomized and controlled clinical trial that compared two groups of healthy infants, each fed with cow milk-based formula during the first year of life with either a higher (HP) or lower protein content (LP). Additionally, a group of exclusively breastfed infants (BF) was followed up in parallel.

The composition of the study formulas complied with the 1991 EU Directive on Infant and Follow-on Formulas[253]. HP and LP formulas had the same energy content; the amount of protein of the formulas was close to the highest and lowest levels, respectively, of the range accepted by the EU Directive mentioned above. Concerning micronutrients composition, on the one hand the minerals content differed slightly between the two formulas, however, on the other; vitamin concentration was exactly the same in both. Complete nutritional composition of the formulas is detailed in Table 4.

Table 4. Nutritional composition of the European Childhood Obesity Project study formulas.

	Infant formulas		Follow-on formulas	
	Lower protein	Higher protein	Lower protein	Higher protein
Energy and macronutrients (Kcal/100ml and g/100ml)				
Energy	69.9	69.8	72.7	72.5
Protein	1.25	2.05	1.6	3.2
Lipids	3.9	3.5	4.0	3.27
Carbohydrates	7.5	7.5	7.6	7.6
Minerals (mg/100ml)				
Sodium	27	25	40	47
Potassium	105	99	158	163
Calcium	84	78	117	120
Phosphorus	65	70	87	95
Iron	0.8	0.9	1.3	1.4
Zinc	0.5	0.5	0.6	0.7
Magnesium	7	7	11	12
Iodine	7	7	11	11
Vitamins (µg/100ml)				
A	55	55	64	64
D	1	1	1.5	1.5
E	0.7	0.7	0.8	0.8
B-6	0.05	0.05	0.12	0.12
B-12	0.26	0.26	0.3	0.3
Folic acid	9	9	17	17

Adapted from Koletzko B et all. Am J Clin Nutr2009.[254]

The EU CHOP was a multicentre project, with representation in 5 European countries: Germany (Munich and Nuremberg), Belgium (Brussels and Liege), Italy (Milano), Poland (Warsaw) and Spain (Reus and Tarragona).

After the first year of intervention, participants were followed-up until 11 years of age; during that period, and considering the inestimable value of this sample of healthy children, many health parameters were assessed: growth and body composition, obesity risk parameters, cardiovascular risk parameters, mental performance development, renal health, epigenetics, metabolomics and general development.

The EU CHOP trial was funded by the 5th Framework Programme from the European Union and was registered at clinicaltrials.gov as NCT00338689. The follow up of the participants was funded by the 6th Framework Programme (FOOD-CT-2005-007036) and also by the 7th Framework Programme (FP7-KBBE-2007-1, ref. nº 212652; and FP7-289346-EarlyNutrition).

3.2. STUDY POPULATION

The recruitment of participants for the EU CHOP study was conducted at the maternities of each study centre or families were contacted through their paediatricians during the first 8 weeks of life.

Participants were eligible if they fulfilled the indicated inclusion criteria:

- To be a healthy infant born between 1st October 2002 and 31st July 2004.
- To be a singleton borned at term (>37 weeks).
- To be born with appropriate weight for gestational age: above 10th percentile and below or equal 90th percentile of Lubchenco intrauterine growth charts from 1963[255].
- Infant should present major malformations which interfere with nutrition or growth.
- Mother should be at least 18y, with no hormonal or metabolic disease, no gestational diabetes and no drug addiction during pregnancy.
- Maternal command of the local language and residence in the study area were required.
- No participation in other clinical trials.

To include an infant to the breastfed group, it had to be exclusively breastfed since birth (≥90% human milk) and it was considered noncompliance to be fed with >10% of feedings or >3 bottles of formula/week for the first 3 months of life. And to be randomized to one of the study formula groups, formula-fed infants had to be exclusively

formula-fed at the end of the eighth week of life (≥90% cow milk-based formula). Exclusion criteria for the intervention groups were to feed nonstudy formula or breastfeeding for >10% of feedings or >3 nonstudy formula bottles/week over ≥1week in the first 9 months of life.

The initial recruitment was of 1679 healthy infants, from whom 653 were still participating in the study at eight years of age.

For the description of the micronutrient intake, its adequacy to the recommendations, all the EU CHOP study participants from whom some dietary intake information was obtained between the third and the 96th month of life, were included.

For the analisys of the influence of calcium intake on bone mineral density, only a Spanish subsample of the EU CHOP study participants, that were evaluated by Dual-energy X-ray absorptiometry (DXA) and also had dietary intake information avaliable, were elegible.

3.3. MEASURES

The following table (Table 5) shows the list of outcomes of the present study, the collection method and the timepoint at which each outcome was assessed.

Table 5. List of outcomes by collection method and timepoints of collection.

Outcomes	Method	Timepoint (months)
Basline data		
Initials, date of birth, gender, country	Medical records	At recruitment
Dietary Intake Assessment		
Energy, macro and micronutrients intake	3-days food records	3, 6, 12, 24, 36, 48, 60, 72, 96
Dietary Intake Adequacy to Recommendations		
Adequacy at individual level	IOM EAR method	3, 6, 12, 24, 36, 48, 60, 72, 96
Adequacy at group level	IOM EAR cut-point method	3, 6, 12, 24, 36, 48, 60, 72, 96
Body composition assessment		
Weight, lenght	Anthropometry	3, 6, 12, 24, 36, 48, T60, 72, 84, 96
Bone health	DXA	84
Physical activity assessment		
Physical activity	PAQ-C questionnaire	84

IOM: American Institute of Medicine, EAR: Estimated Average Requirements, DXA: Dual-energy X-ray Absorptiometry. PAQ-C: Physical Activity Questionnaire for children.

3.3.1. Dietary Intake Assessment

Dietary intake was recorded by parents or carers with 3-day weighed/estimated food records (2 week days + 1 weekend day, consecutive), that were completed at 3, 6, 12, 24, 36, 48, 60, 72 and 96 months of life, using food scales (Unica 66006; Soehnle, Murrhardt, Germany) and/or a validated food picture atlas (self-designed by the EU CHOP study nutritionists) to estimate serving portions. To ensure as higher quality of data as possible, food records were especially designed for each age range (from 1st to 3rd month, from 4th to 9th, from 12th to 24 month, and from 36th month onwards) and, once completed, they were reviewed by the dieticians-nutritionists along with the families during the follow up visits. Annex I shows the food records design.

Dietary intake of breastfed infants was not assessed while they were fed exclusively with human milk, but it was assessed after that period with the introduction of complementary feeding.

A Standard Operation Procedures (SOP) protocol was developed to ensure an harmonised interpretation and quantification of food records information. Furthermore, a software to convert food amount into nutrients was specifically developed for the study (NutrCalc®). Initially, this tool contained all the food items from the German BLS II.3 food composition table[256], and subsequently, study nutritionists added the nutritional composition of more than 2200 local food items, according to information from the manufacturers, local nutrition composition tables[257–262] or ingredient details avaliable in the product label.

The German BLS II.3 food composition database sorts food items in Groups and Subgroups. With the objective of determining through which food sources was each micronutrient obtained in the EU CHOP sample, local food items were also classified into BLS food groups and subgroups, if possible; or into new groups specially adapted or created following the Standard Operating Procedure Manual for NutrCalc Food Classification.

The aims of this classification process were:

- To standardize food description and classification in the multicentre setting.
- To properly identify food items characteristics by their group and subgroup.
- To obtain the best classification possible, mouldable according to different food intake analysis needs. Having subgroups, would allow to group and ungroup food items according to different analysis' objectives.

Energy and macronutrients daily intake were measured and expressed as absolute value:

- Energy (Kcal/day).
- Protein (g/day)
- Carbohydrates (g/day).
- Fats (g/day).

Dietary intake data from the following micronutrients were measured and expressed as absolute value, relative to body weight and relative to total energy intake:

- Vitamins: vitamin A, vitamin D, vitamin B_{12} and folate (µg/day, µg/Kcal/day).
- Minerals: sodium, potassium, calcium, phosphor, iron, zinc and magnesium (mg/day, mg/kcal/day and mg/Kg/day in the case of zinc); and iodine (µg/day, µg/Kcal/day).

Prevalence of underreporters was explored using the Goldberg[263] cut-offs and considering physical activity level recommended for universal application at 1.55. Basal metabolic rate was calculated using Schofield equations[264].

Further details on EU CHOP study methodology on nutritional assessment have been published elsewhere[265].

3.3.2. Dietary intake adequacy to the recommendations

Due to the multicentre particularities of our study sample, the best appropiate dietary reference intakes to assess dietary intake adequacy were the FAO/WHO/UNU DRIs[31]. And where reference values were not available from FAO/WHO/UNU, they were drawn from the FNB_IOM (this happened for phosphorus, magnesium, iodine and vitamin D)[34,172,174].

- Assessment of micronutrient intake adequacy at individual level

To assess dietary intake adequacy to the recommendations at individual level we performed a quantitative evaluation, according to the IOM methodology[173], using Estimated Average Requirements (EAR).

We applied the IOM formula shown in Figure 5 to our data. Giving that the result of this formula is like a z-score of the intake, we converted the value (ratio) obtained into a probability of inadequate intake (PII) using the equivalence given by the normal distribution tables of z-scores. Afterwards we transformed the PII into probability of adequate intake (PAI), by subtracting PII to 100, as detailed in Figure 6.

Figure 5. Use of estimated average requirement for individual adequacy assessment.

$$D = \tilde{y} - r$$

$$SD_D = \sqrt{(SD_r^2 + SD_{within}^2 / n)}$$

$$ratio = D/SD_D$$

D is difference between the mean observed intake of an individual ($\tilde{\mathbf{y}}$) and the median requirement (**r**) for the life stage and gender group (EAR). $\mathbf{SD_D}$ is standard deviation of D. $\mathbf{SD_r}$ is SD of the EAR (estimated to be 10 of the EAR for most nutrients), $\mathbf{SD_{within}}$ is SD of the nutrient intake (normally estimated from large surveys as CSFII, we used ow own sample SD), and, **n** is number of days of intake available.
Adapted from IOM. Dietary Reference Intakes: Applications in Dietary Assessment, 2000[173].

Figure 6. Transformation of Probability of Inadequate Intake into Probability of Adequate Intake.

$$PAI = 100 - PII$$

PAI: Probability of Adequate Intake, PII: Probability of Inadequate Intake.

Finally, we categorized this PAI into five groups to show results: >95%, 75-95%, 50-75%, 25-50% and <25% of PAI. Descriptive results are expressed as N(%) of participants in each PAI category >95%, 75-95%, 50-75%, 25-50% and <25%.

Another simplified categorization was also done for the analysis of the relations of calcium intake adequacy with bone mineral health, being the children with a very high PAI (PAI >95%) compared versus all the others.

An important consideration about this methodology is that it is only applicable to normally distributed intake data. The method is only valid when the coeficient of variation of intake calculated as detailed in Figure 7 is under 70.

Figure 7. Calculation of coeficient of variation of dietary intake.

$$CV = (SD_{intake}/mean_{intake}) * 100$$

CV: coeficient of variation. SD_{intake}: standard desviation of micronutient intake. $mean_{intake}$: mean intake of the micronutrient.

- Assessment of micronutrient intake adequacy at group level

Group adequacy was calculated with the EAR cut-point method described by the IOM[173].

Results are expressed as prevalence of adequacy, N (%) of participants with intakes over the EAR.

3.3.3. Body composition assessment

- Anthropometry

Nude weight and length were determined twice at each timepoint (3, 6, 12, 24, 36, 48, 60, 72, 84 and 96 months of life) by trained personnel, and following the WHO recommendations based on the Lohman reference manual[266].

Measurement procedures were stanardized to avoid inter-observer differences. The same equipment, periodically calibrated, was used in all the centres for measuring weight (Seca 336 baby scales [precision ±10g] at ages ≤24 months, and Seca 702 scales from >24 months; Seca, Hamburg, Germany); recumbent length (Seca 232 stadiometre [precision ±1mm] until the age of 6 months, and PED LB 35-107 X scales after 6 months; Seca, Hamburg, Germany and Ellard Instruments, Monroe, WA; respectively); and standing height (Seca 242 stadiometre [precision ±1mm]).

Weight was expressed in kg and length and height in cm. Body mass index (BMI) was calculated as weight (Kg)/height $(m)^2$. Z-scores of BMI were calculated using the growth reference values from the United States

National Center for health Statistics, recommended by WHO for children from 5 to 19 years old[267].

- Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) was performed to the Spanish EU CHOP study subsample at 7 years of age (84 months), using a Lunar Prodigy Primo device, to mesaure fat mass and bone densitometry. Radiation exposure was 0.4 mGy and 0.9 mGy for the whole body and for the lumbar spine, respectively. A 76-KeV X-ray source of energy was used and the precision error of the test was 1%. The same technician performed all measurements to avoid inter-individual variations.

Whole body (WB) and lumbar spine at L1-L4 level (LS) bone mineral content (BMC) (g) and bone mineral area (BMA) (cm^2) were measured. Total and lumbar bone mineral density (BMD) (g/cm^2) were directly obtained from the Lunar device software. Internal z-scores were calculated for the WB and LS BMD. Children with values of bone mineral density below -1 z-score were classified as children with osteopenia according to WHO recommendations[31]. As the term “osteoporosis” is controversial in children and adolescents[250], we will use the recommended term “low bone mineral density for age” to classify those children with a BMD z-score below −2, rather than osteoporosis.

Fat mass index (FMI) was calculated as fat mass (kg)/ height (m^2). Fat mass index gender z-scores were calculated using reference values published by Wells et al., 2012[268].

3.3.4. Physical activity

Physical Activity was assessed with the Physical Activity Questionnaire for children (PAQ-C) completed by children with the help of parents or careers at 7 years of age. PAQ-C was designed to assess general physical activity levels. It includes questions about activities practiced during the last 7 days, asking about regulated sports and physical education at school, extra scholar activities and games that involved movement. PAQ-C total score was calculated following its guidelines[269,270].

3.4. DATA TREATMENT AND STATISTICS

3.4.1. Statistics

Descriptive results are expressed as mean ± standard deviation (SD) and median and percentiles 25 and 75 (Pctl 25-75) according to distribution of variables, tested with a Kolmogorov-Smirnov test & Q-Q graphics of normal distribution. Frequency of categorical variables is presented as N (%).

T-tests were used for statistical cross-sectional comparisons between two groups, and One-way ANOVA and Bonferroni post-hoc analysis for comparisons between more than two groups. Pearson or Spearman correlations were used to determine linear relationships between pairs of quantitative measures, according to distribution. Chi squared was used to compare the frequency of osteopenia according to calcium intake adequacy.

Linear Regression models were performed to quantify the effect of calcium intake and calcium high probability of adequate intake (PAI>95%) during childhood on bone mineral density at 7 years, adjusting by anthropometry (BMI at 7 years), physical activity at 7 years and dietary factors such as protein, phosphorus and vitamin D during childhood. Collinearity of variables was considered. Binary logistic regressions were performed to determine the risk of having osteopenia or low bone mineralization for age at 7 years by not having a high probability of adequate calcium dietary intake (PAI<95%) at different ages. Analyses were adjusted by the same factors than linear regression analyses.

Statistical significance was accepted at level $p<0.05$. For the statistic treatment of the data the 22.0 version of the IBM SPSS Statistics software was used (IBM Corp., Armonk, NY, USA).

3.4.2. Variables list

Table 6. Variables list.

Variables	Units or Categories
Dietary Intake Assessment	
Energy	Kcal/day
Protein	g/day, g/kcal/day
Carbohydrates	g/day, g/kcal/day
Fats	g/day, g/kcal/day
Vitamin A	µg/day, µg/Kcal/day
Vitamin D	µg/day, µg/Kcal/day
Vitamin B_{12} (cobalamin)	µg/day, µg/Kcal/day
Vitamin B_9 (folate)	µg/day, µg/Kcal/day
Sodium	mg/day, g/Kcal/day
Potassium	mg/day, g/Kcal/day
Calcium	mg/day, g/Kcal/day
Phosphor	mg/day, g/Kcal/day
Iron	mg/day, g/Kcal/day
Zinc	mg/day, g/Kcal/day, mg/Kg/day
Magnesium	mg/day, g/Kcal/day
Iodine	µg/day, µg/Kcal/day

(Continued on next page)

*(**Table 6.** Continued)*

Variables	Units or Categories
Dietary adequacy to recommendations	
Probability of adequate intake (continuous)	%
Probability of adequate intake (categorical)	>95%, 75-95%, 50-75%, 25-50% and <25%
High probability of adequate intake (PAI>95%)	Yes, No
High probability of adequate intake at 6 & 5 years	Yes, No
High probability of adequate intake at 6, 5 & 4 years	Yes, No
Prevalence of adequacy at group level	%
Body composition assessment: Anthropometry	
Weight	g
Height	cm
BMI	Kg/m^2
BMI z-score	
Fat mass index	Kg/m^2
Body composition assessment: DXA	
Bone mineral content (BMC), whole body and lumbar spine	g
Bone mineral density (BMD), whole body and lumbar spine	g/cm^2
Bone mineral density z-score, whole body and lumbar spine	-
Osteopenia	Yes/No
Low bone mineral density for age	Yes/No

BMI: Body Mass Index. DXA: Dual-energy X-ray Absorptiometry.

3.5. ETHICS

The study was performed according to the Helsinki II declaration[271] and following the guidelines for ethical conduct of medical research involving children[272].

The EU CHOP study protocol and all its amendments were designed following the CONSORT Statement (guidelines for clinical trials)[273]. The study protocol and all its amendments were submitted and approved by the Ethics Committees of all the study centres where the study was conducted.

Written informed consent was obtained from all parents or legal representatives of the participant infants before any data was obtained. Consecutive informed consents were obtained after every new amendment to the original protocol. A specific informed consent was obtained in the Spanish subsample before the DXA evaluation.

The EU CHOP trial registered at clinicaltrials.gov as NCT00338689.

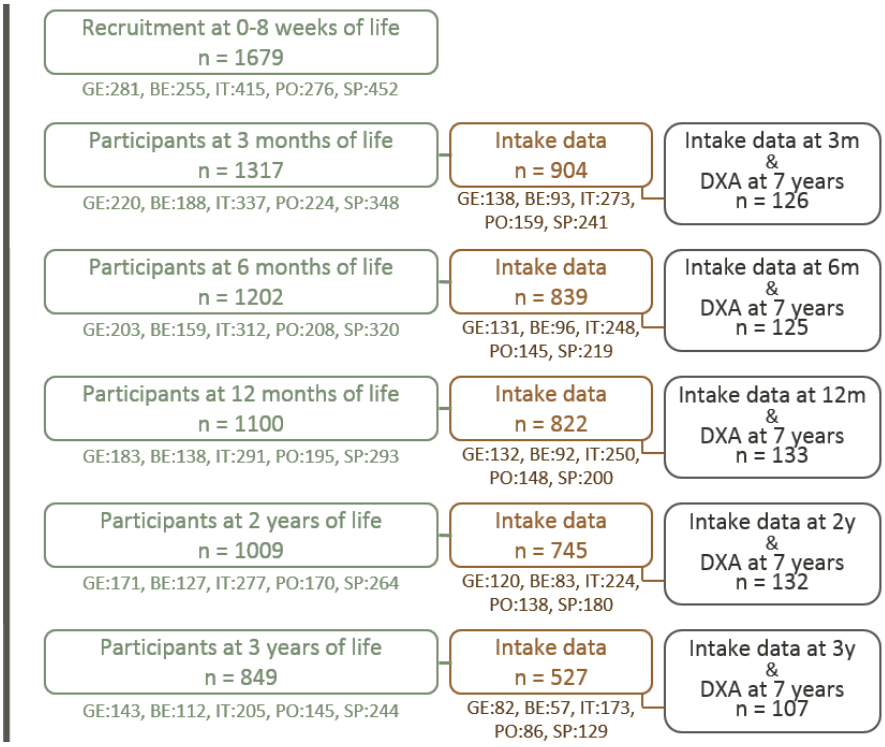
Results

4. RESULTS

4.1. DESCRIPTION OF THE STUDY SAMPLE

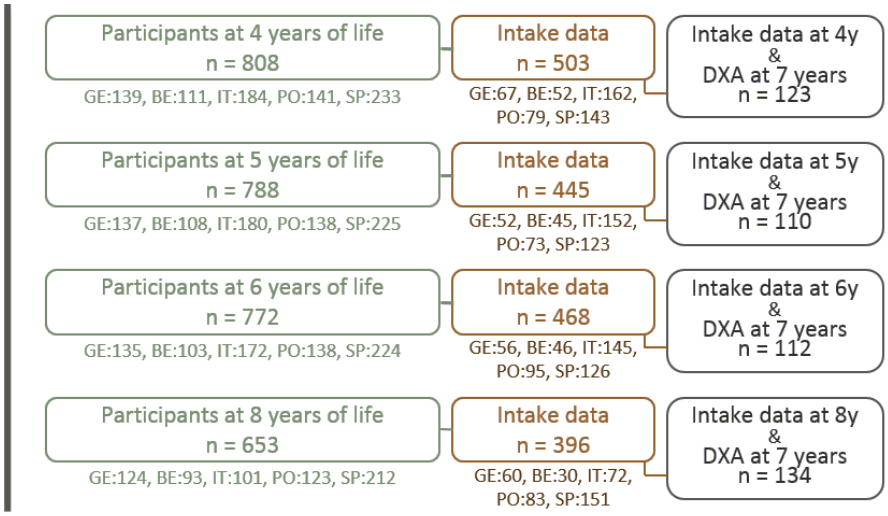
Figure 8 shows the evolution of the participation of children in the study from birth to 8 years.

Figure 8. Flowchart of participants in the study.



(Continued on next page)

*(**Figure 8.** Continued)*



The study flowchart shows, on the left column, the subjects taking part in the study at each timepoint, from birth to 8 years of age. In the middle, the children taking part in the study from which intake data was available at the corresponding timepoint. And on the right side, the children for whom Dual-energy X-ray Absorptiometry (DXA) evaluation results and intake data were available. GE: Germany, BE: Belgium, IT: Italy, PO: Poland, SP: Spain. DXA: Dual-energy X-ray Absorptiometry. m: months (3m = 3 months, 6m= 6 months, and so on).

4.2. DESCRIPTIVE ANALISIS OF MICRONUTRIENTS INTAKE

4.2.1. Description of micronutrients intake

Micronutrients intakes at each age are showed as mean ±SD and as median [Pctl 25-75]; one table for each micronutrient is provided (Tables 7 to 18). In the same tables, FAO/WHO/UNU and FNB-IOM estimated average requirements (EAR) are detailed for further information, when available.

EU CHOP underreporting prevalence was determined to be 26.2% at 6 months, 35.3% at 12 months, 47.1% at 2 years, 57.1% at 3 years, 48.8% at 4 years, 45.9% at 5 years, 49.5% at 6 years and 59.5% at 8 years. However, underestimation of energy intake was estimated to be only around 13%.

Table 7. Sodium intake per day.

Age	n	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]
3m	904	204.3 ±50.6	206.7 [181.1-233.0]
6m	839	310.0 ±109.6	311.3 [234.5-371.2]
12m	822	558.8 ±281.1	495.8 [360.8-662.8]
24m	745	961.6 ±419.8	903.0 [652.5-1198.2]
36m	527	1132.0 ±425.4	1083.6 [833.9-1385.1]
48m	503	1244.6 ±441.1	1213.1 [937.0-1504.6]
60m	445	1349.0 ±446.2	1308.3 [1024.7-1623.8]
72m	468	1459.1 ±466.9	1410.7 [1139.3-1713.6]
96m	396	1692.9 ±530.6	1639.3 [1319.8-2011.5]

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. There are not estimated average requirements (EAR) established for sodium by the FAO/WHO/UNU[274], nor by the Food and Nutrition Board of the Institute of Medicine[176].

Table 8. Potassium intake per day.

Age	n	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]
3m	904	797.6 ±200.7	812.0 [710.5-911.0]
6m	839	1209.5 ±412.3	1202.7 [944.8-1478.4]
12m	822	1380.8 ±521.3	1343.7 [1001.3-1685.8]
24m	745	1571.6 ±505.7	1526.4 [1216.7-1856.0]
36m	527	1635.8 ±425.0	1604.3 [1322.5-1904.5]
48m	503	1745.1 ±463.1	1713.6 [1439.1-2023.3]
60m	445	1802.5 ±468.7	1795.1 [1463.5-2087.1]
72m	468	1892.0 ±464.2	1877.1 [1570.2-2172.2]
96m	396	2004.7 ±521.5	1959.9 [1653.3-2314.8]

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. There are not estimated average requirements (EAR) established for potassium by the FAO/WHO/UNU[274]. There are not EAR established for potassium by the Food and Nutrition Board of the Institute of Medicine[176].

Table 9. Calcium intake per day.

Age	n	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]	EAR FAO/WHO/UNU (mg)[(1)]
3m	904	627.5 ±169.1	645.0 [564.2-722.6]	240[a]-300[b]
6m	839	770.8 ±244.6	776.9 [624.6-917.2]	240[a]-300[b]
12m	822	713.3 ±258.7	707.7 [560.8-842.1]	440
24m	745	698.9 ±280.8	678.4 [515.7-861.4]	440
36m	527	662.1 ±255.1	624.6 [485.8-816.3]	440
48m	503	656.4 ±253.9	648.1 [468.1-813.0]	440
60m	445	655.9 ±257.3	624.5 [468.7-815.5]	440
72m	468	647.7 ±251.5	600.7 [464.3-799.7]	440
96m	396	683.9 ±251.7	669.6 [500.1-830.1]	440

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. [(1)]:Estimated average requirements (EAR) described by the FAO/WHO/UNU FAO/WHO/UNU[31]. [a]: for breastfed, [b]: for formula fed.

Table 10. Phosphorus intake per day.

Age	n	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]	EAR FNB-IOM (mg)[(1)]
3m	904	514.9 ±157.6	536.8 [466.5-604.5]	-
6m	839	611.6 ±186.7	620.5 [515.4-721.3]	-
12m	822	656.0 ±216.5	645.2 [523.7-782.1]	380
24m	745	771.8 ±246.1	748.3 [613.5-910.9]	380
36m	527	791.1 ±226.3	774.7 [637.6-918.7]	380
48m	503	823.8 ±232.0	808.5 [647.9-961.6]	405
60m	445	838.6 ±229.1	810.2 [662.5-984.9]	405
72m	468	859.6 ±210.8	842.9 [704.2-975.2]	405
96m	396	934.6 ±236.0	909.0 [765.7-1065.5]	405

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. There are not estimated average requirements (EAR) established for phosphorus by the FAO/WHO/UNU[274]. [(1)]: EAR established for phosphorus by the Food and Nutrition Board of the Institute of Medicine[34].

Table 11. Iron intake per day.

Age	n	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]	EAR FAO/WHO/UNU (mg)[(1)]
3m	904	6.45 ±2.27	6.75 [5.83-7.69]	-
6m	839	9.61 ±3.66	9.57 [7.50-11.63]	7.2
12m	822	8.50 ±4.78	7.94 [5.37-10.54]	4.6
24m	745	6.61 ±3.89	5.55 [4.26-8.14]	4.6
36m	527	6.51 ±2.76	5.92 [4.57-7.99]	4.6
48m	503	6.87 ±2.83	6.29 [5.00-7.85]	5.0
60m	445	7.22 ±2.69	6.65 [5.45-8.35]	5.0
72m	468	7.67 ±2.81	7.17 [5.80-8.96]	5.0
96m	396	9.01 ±3.10	8.45 [6.75-10.41]	7.1[a]-12.0[b]

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. [(1)]: Estimated average requirements (EAR) described by the FAO/WHO/UNU Joint expert consultation[31]. [a]: EAR for male. [b]: EAR for female.

Table 12. Zinc intake per day.

Age	n[1]	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]	Intake (mg/day/Kg) mean ± SD	Intake (mg/day/Kg) median [Pctl 25-75]	EAR FAO/WHO/UNU (mg/Kg)(2)
3m	904/899	3.85 ±1.16	4.00 [3.48-4.48]	0.65 ±0.20	0.67 [0.59-0.76]	0.64[a]-0.57[b]
6m	839/829	4.50 ±1.93	4.42 [3.70-5.22]	0.58 ±0.23	0.56 [0.48-0.67]	0.25
12m	822/812	5.81 ±11.58	4.55 [3.48-5.84]	0.60 ±1.12	0.46 [0.36-0.59]	0.28
24m	745/733	5.88 ±3.09	5.25 [4.12-6.66]	0.48 ±0.26	0.42 [0.33-0.54]	0.28
36m	527/512	5.94 ±2.52	5.57 [4.57-6.86]	0.41 ±0.19	0.38 [0.31-0.47]	0.28
48m	503/491	6.25 ±2.48	5.80 [4.77-7.10]	0.38 ±0.17	0.35 [0.29-0.42]	0.23
60m	445/439	6.51 ±2.33	6.20 [5.16-7.34]	0.34 ±0.13	0.33 [0.26-0.39]	0.23
72m	468/462	6.82 ±2.34	6.47 [5.43-7.69]	0.32 ±0.12	0.30 [0.25-0.35]	0.23
96m	396/394	7.57 ±2.64	7.181 [5.95-8.57]	0.27 ±0.10	0.25 [0.21-0.32]	0.18

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. [1]: number of children with intake data/number of children with intake data and anthropometry data. (2): Estimated average requirements (EAR) described by the FAO/WHO/UNU Joint expert consultation[31]. [a]: EAR for male. [b]: EAR for female.

Table 13. Magnesium intake per day.

Age	n	Intake (mg/day) mean ± SD	Intake (mg/day) median [Pctl 25-75]	EAR FNB-IOM (mg)[(1)]
3m	904	52.9 ±19.0	56.2 [48.8-63.0]	-
6m	839	82.9 ±32.6	82.4 [64.8-101.1]	-
12m	822	107.2 ±45.7	102.6 [75.6-130.8]	65
24m	745	140.3 ±46.6	135.5 [108.4-164.9]	65
36m	527	151.3 ±40.4	146.3 [124.1-175.3]	65
48m	503	164.2 ±45.0	159.6 [133.5-190.9]	110
60m	445	175.4 ±48.6	169.1 [143.4-199.7]	110
72m	468	183.5 ±47.8	176.7 [149.8-211.7]	110
96m	396	203.3 ±49.9	198.2 [168.0-233.4]	110

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. There are not estimated average requirements (EAR) established for magnesium by the FAO/WHO/UNU[274]. [(1)]:EAR established by the Food and Nutrition Board of the Institute of Medicine[34].

Table 14. Iodine intake per day.

Age	n	Intake (µg) mean ± SD	Intake (mg) median [Pctl 25-75]	EAR FNB-IOM (µg)[(1)]
3m	904	52.8 ±19.0	56.1 [49.0-62.8]	-
6m	839	69.0 ±28.8	69.9 [55.8-84.3]	-
12m	822	62.0 ±30.7	60.5 [45.7-74.5]	65
24m	745	58.2 ±48.4	47.9 [32.8-69.8]	65
36m	527	58.8 ±75.0	48.8 [37.2-67.9]	65
48m	503	58.0 ±43.2	47.9 [36.7-68.5]	65
60m	445	60.6 ±39.0	53.2 [40.6-69.4]	65
72m	468	62.1 ±44.2	53.7 [43.3-68.6]	65
96m	396	63.4 ±42.3	53.0 [43.5-68.4]	65

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. There are not estimated average requirements (EAR) for iodine by the FAO/WHO/UNU[274]. [(1)]: EAR established by the Food and Nutrition Board of the Institute of Medicine[174].

Table 15. Vitamin B_{12} (cobalamin) intake per day.

Age	n	Intake (µg/day) mean ± SD	Intake (µg/day) median [Pctl 25-75]	EAR FAO/WHO/UNU (µg)[(1)]
3m	904	1.94 ±0.70	2.08 [1.80-2.33]	0.3
6m	839	1.85 ±0.82	1.88 [1.50-2.22]	0.3
12m	822	2.17 ±3.59	1.76 [1.22-2.45]	0.7
24m	745	2.67 ±1.89	1.63 [2.40-3.31]	0.7
36m	527	2.80 ±2.01	2.51 [1.72-3.34]	0.7
48m	503	2.85 ±1.84	2.55 [1.73-3.56]	1
60m	445	3.18 ±1.71	2.85 [2.01-3.90]	1
72m	468	3.42 ±2.94	2.83 [2.05-3.91]	1
96m	396	3.80 ±2.39	3.38 [2.40-4.54]	1.5

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. [(1)]: Estimated average requirements (EAR) described by the FAO/WHO/UNU Joint expert consultation[31]

Table 16. Vitamin B_9 (folate) intake per day.

Age	n	Intake (µg/day) mean ± SD	Intake (µg/day) median [Pctl 25-75]	EAR FAO/WHO/UNU (µg) [(1)]
3m	90	67.8 ±24.3	72.0 [62.7-81.0]	65
6m	83	110.8 ±46.6	110.8 [85.0-137.4]	65
12m	82	111.4 ±54.0	106.4 [74.0-142.3]	120
24m	74	110.0 ±48.1	101.9 [79.1-130.7]	120
36m	52	115.6 ±43.1	110.7 [84.2-136.9]	120
48m	50	123.5 ±45.8	117.5 [93.3-145.3]	160
60m	44	132.1 ±46.9	127.7 [98.4-161.3]	160
72m	46	144.0 ±51.3	137.3 [105.7-175.0]	160
96m	39	159.7 ±56.3	154.8 [121.1-194.9]	250

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. [(1)]: Estimated average requirements (EAR) described by the FAO/WHO/UNU Joint expert consultation[31].

Table 17. Vitamin A intake per day.

Age	n	Intake (µg) mean ± SD	Intake (µg) median [Pctl 25-75]	EAR FAO/WHO/UNU (µg) (1)
3m	90	463.3 ±87.4	453.8 [408.8-512.6]	180
6m	83	860.0 ±827.0	603.6 [481.5-837.5]	180
12m	82	998.4 ±1314.3	775.3 [520.7-1118.0]	200
24m	74	817.3 ±1281.0	613.9 [416.3-873.0]	200
36m	52	765.0 ±1239.2	5551.9 [381.1-805.3]	200
48m	50	787.0 ±672.5	622.9 [403.1-934.4]	200
60m	44	821.0 ±882.3	605.0 [398.4-879.6]	200
72m	46	897.6 ±954.7	611.3 [414.2-938.9]	200
96m	39	905.3 ±933.2	679.6 [453.9-993.7]	250

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. (1): Estimated average requirements (EAR) described by the FAO/WHO/UNU Joint expert consultation[31].

Table 18. Vitamin D intake per day.

Age	n	Intake (µg/day) mean ± SD	Intake (µg/day) median [Pctl 25-75]	EAR FNB-IOM (µg)(1)
3m	904	7.52 ±2.71	8.00 [6.97-8.97]	-
6m	839	9.33 ±3.92	9.36 [7.45-11.50]	-
12m	822	6.60 ±5.07	6.37 [3.75-8.84]	10
24m	745	2.85 ±3.47	1.55 [0.72-3.81]	10
36m	527	1.97 ±2.00	1.24 [0.67-2.42]	10
48m	503	1.64 ±1.63	1.20 [0.62-1.99]	10
60m	445	1.62 ±1.70	1.21 [0.67-1.97]	10
72m	468	1.67 ±1.56	1.29 [0.77-2.03]	10
96m	396	2.07 ±1.83	1.67 [1.06-2.62]	10

[Pctl 25-75]: Percentile 25 and percentile 75. m: Months. There are not estimated average requirements (EAR) established for sodium by the FAO/WHO/UNU[274]. (1): EAR established by the Food and Nutrition Board of the Institute of Medicine[172].

4.2.2. Description of micronutrients intake by gender

Differences in the intake of micronutrients between genders were analysed. In most of the cases, when differences were statistically significant, the consumption of concerned micronutrient was higher in males. Table 19(A) shows significant differences found between genders for each micronutrient and Annex III shows mean micronutrients intake and gender differences complete data.

Given that energy intake was significantly higher in boys at almost all timepoints (see macronutrient intake data on Annex IV), it was meaningful to adjust micronutrients intake by energy. The objective was to check if the differences in micronutrients intake found between genders were due to the higher energy intake of boys compared with girls; or if the differences were due to a qualitative variation of the diet.

After adjusting by energy intake, all gender differences in the intake of micronutrients disappeared after the age of 3 years, when it seems that energy intake starts to be very different between genders. And, on the contrary to what happened before adjusting, the micronutrients that showed significant gender differences were consumed in greater amounts by girls. Furthermore, the only differences that were preserved after adjusting by energy were those found for zinc and vitamin D at 24 months and for iodine at 36 months, and, curiously, those were the few cases in which micronutrient intakes had been higher in girls, so for sure, in those cases, differences were not due to energy intake differences. In addition, 24 months was the only age at which there were not energy intake differences.

Table 19 shows statistically significant differences found in the micronutrient intakes between genders, before (A) and after (B) adjusting by energy intake. Annex III shows mean micronutrient intake and differences by genders; information is also available adjusted by energy intake.

Table 19. Comparison of micronutrients intake between genders.

	(A) Micronutrients intake per day									(B) Micronutrients intake/Kcal and day								
	3	6	12	24	36	48	60	72	96	3	6	12	24	36	48	60	72	96
Na	***								***			*	*					
k	***				*	*	*		***			*						
Ca	***								**									
P	**					*			***									
Fe	*								***									
Zn	**		*	*					***				**					
Mg	*								**									
I	*				*									**				
Vit B_{12}	*													*				
Vit B_9	**								**				**					
Vit A	***																	
Vit D	*			**					*				**					

*: p<0.05 male vs female, **: p<0.01 male vs female and ***: P<0.001 male vs female according to T-Test between genders. Highlighted in blue when mean intake was higher in males and in pink when mean intake was higher in females.

4.2.3. Description of micronutrients intake by country

Country differences were analysed and were found in the intake of sodium, potassium, calcium, phosphorus, iron, zinc, magnesium and iodine ($p<0.001$). Intake of vitamins B_{12}, B_9, A and D were also different between countries ($p<0.001$).

It must be considered that there were also statistical significant energy intake differences between countries at all timepoints ($p<0.001$). Summarizing, Spanish and Polish children were those with highest energy intake at almost all ages. Spanish children had higher energy intake than Polish at 3 and at 12 months; and Polish children had higher energy intake than Spanish at 3 years. Italians had similar energy intakes to Spanish and Polish at 5 and 6 years of age. Finally, at 8 years, energy intake became very similar in all countries. All details about energy and macronutrient intakes and all country differences can be found in Annex V.

Figures 9 to 20 show micronutrients intake by country. Figures (A) show raw micronutrient intakes and Figures (B) show intakes adjusted by energy. Some details about specific country differences are given below for each micronutrient, and complete information about micronutrient intakes and all country differences can be found in Annex VI.

Figure 9. Daily sodium intake by country.

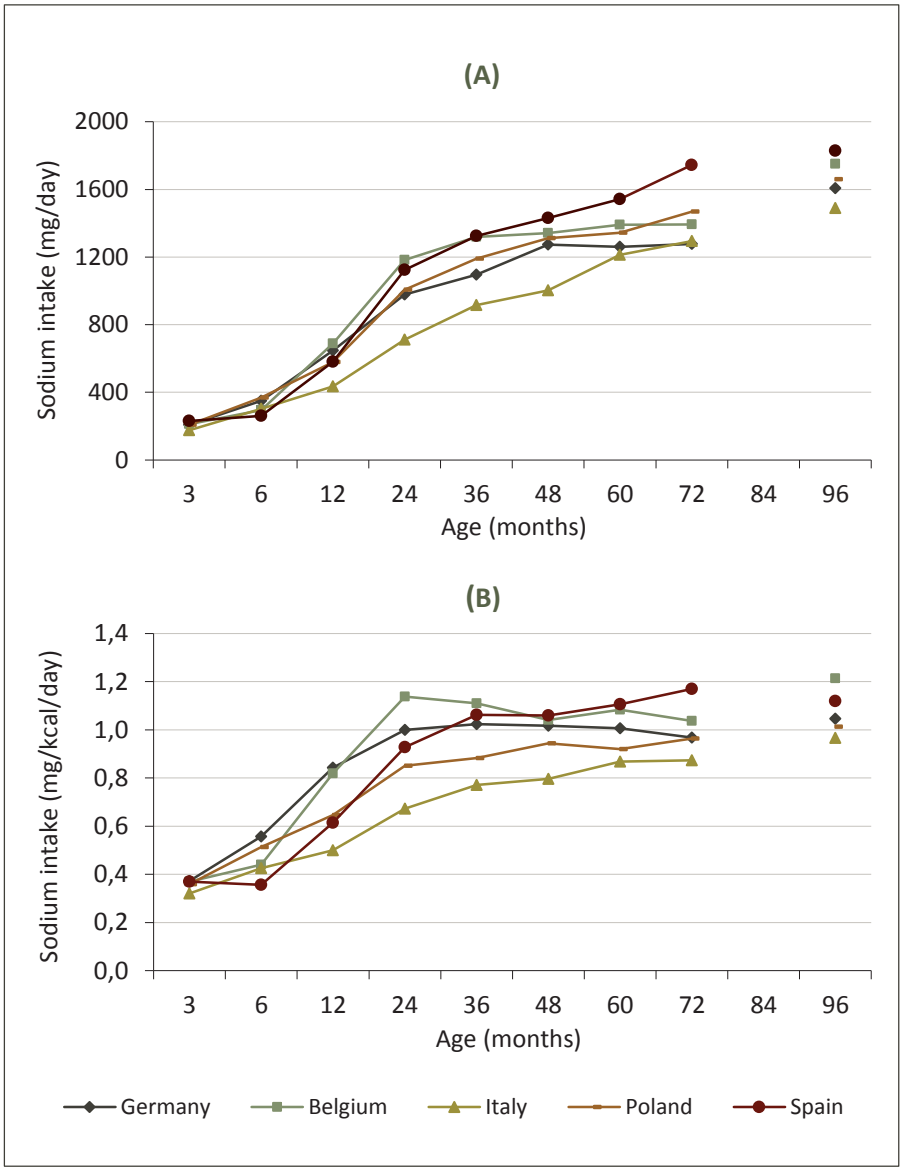


Figure A: Daily sodium intake by country (mg/day). Figure B: Daily sodium intake by country, adjusted by energy (mg/Kcal/day).

Sodium raw intake of the Spanish sample was higher than it was in the other countries, with the exception of the second semester of life; being these differences statistically significant in comparison with Italians ($p<0.001$), who had the lowest sodium intake during almost all the study period. After adjusting sodium consumption by energy, Germany and Belgium were the countries with higher sodium intake by calorie until 36 months of life, when Spain became the greater sodium consumer again. Italy kept being the country with less sodium consume, either raw or adjusting by energy ($p>0.001$ vs all countries) (Figure 9).

The greater consumers of potassium were Spanish children from the 12th month of life, and from 36th month onwards so it were Polish children. Italians and Germans were those with the lowest consumption of potassium at almost all timepoints. Belgian children were in the middle way between higher and lower consumers. After adjusting potassium intake by energy, Spanish children were still those with the highest consume of potassium and Italians those with the lowest (Figure 10).

Calcium consumption of Spanish children was the highest compared with the other countries ($p<0.05$ at 3, 36 and 48; $p<0.001$ at 12, 24, 60, 72 and 96 months), with exception of 6 months when Polish children had the highest consume ($p<0.05$ vs all countries). After adjusting by energy, a decrease of calcium density of the diet was highlighted. Spanish children were those with the highest calcium intake by calorie ($p<0.001$ vs GE and PO from T12 onwards), Germans and Polish showed the lowest intake of calcium by kcal at almost all timepoints (Figure 11).

Figure 10. Daily potassium intake by country.

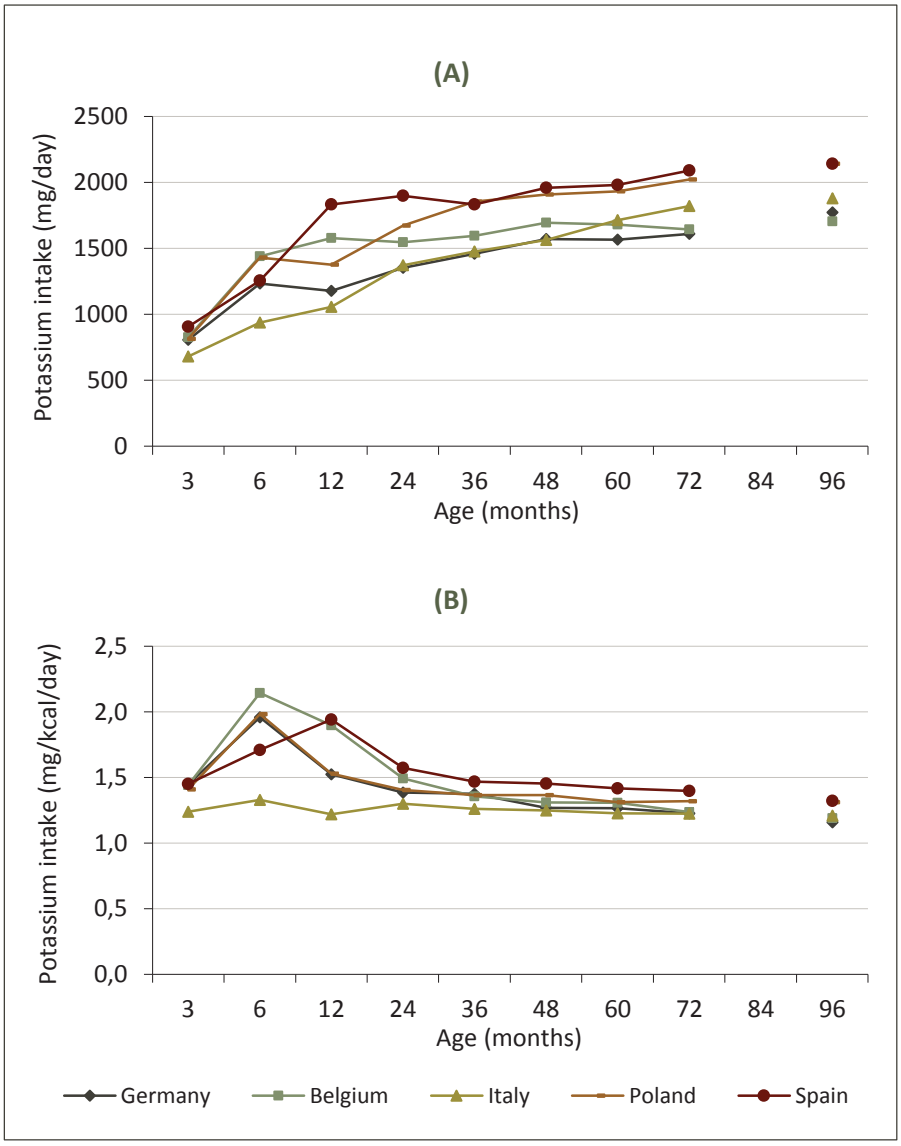


Figure A: Daily potassium intake by country (mg/day). Figure B: Daily potassium intake by country, adjusted by energy (mg/Kcal/day).

Figure 11. Daily calcium intake by country.

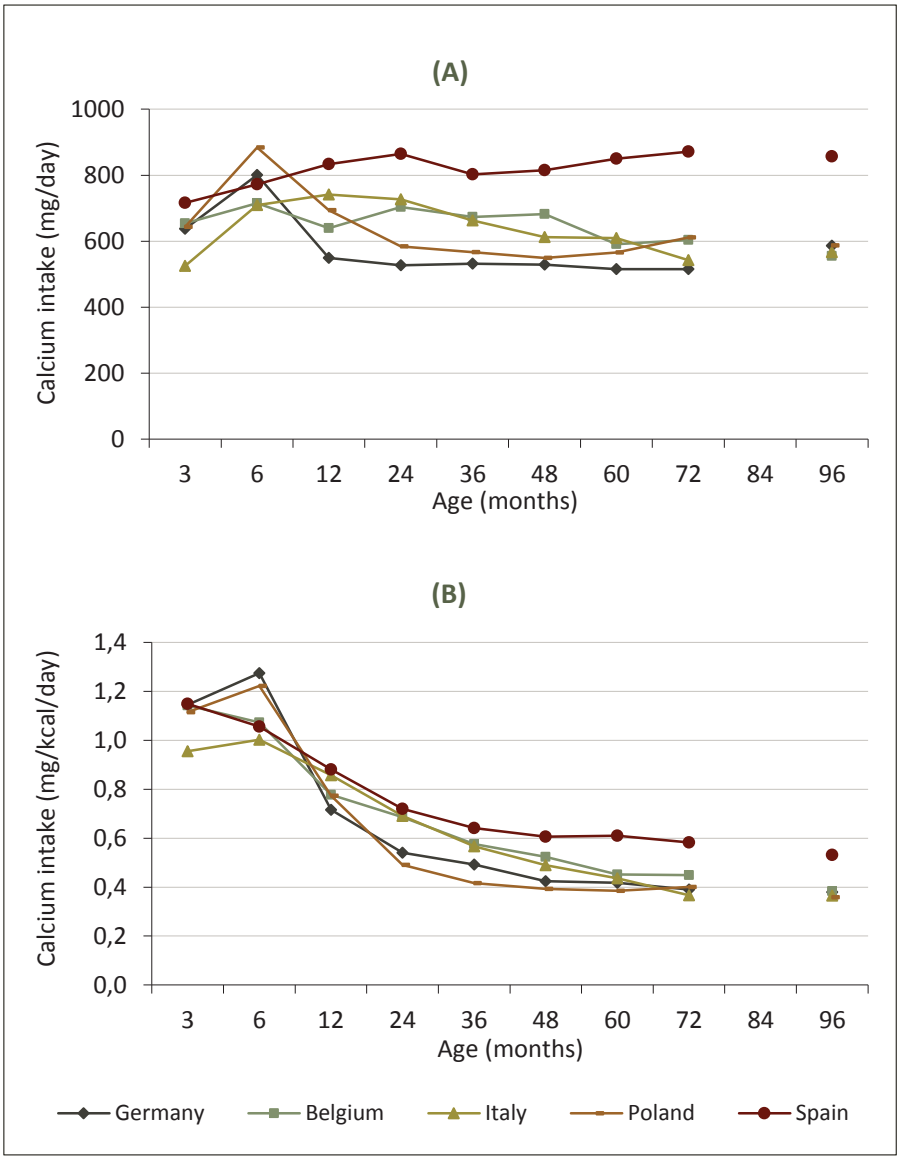


Figure A: Daily calcium intake by country (mg/day). Figure B: Daily calcium intake by country, adjusted by energy (mg/Kcal/day).

Figure 12. Daily phosphorus intake by country.

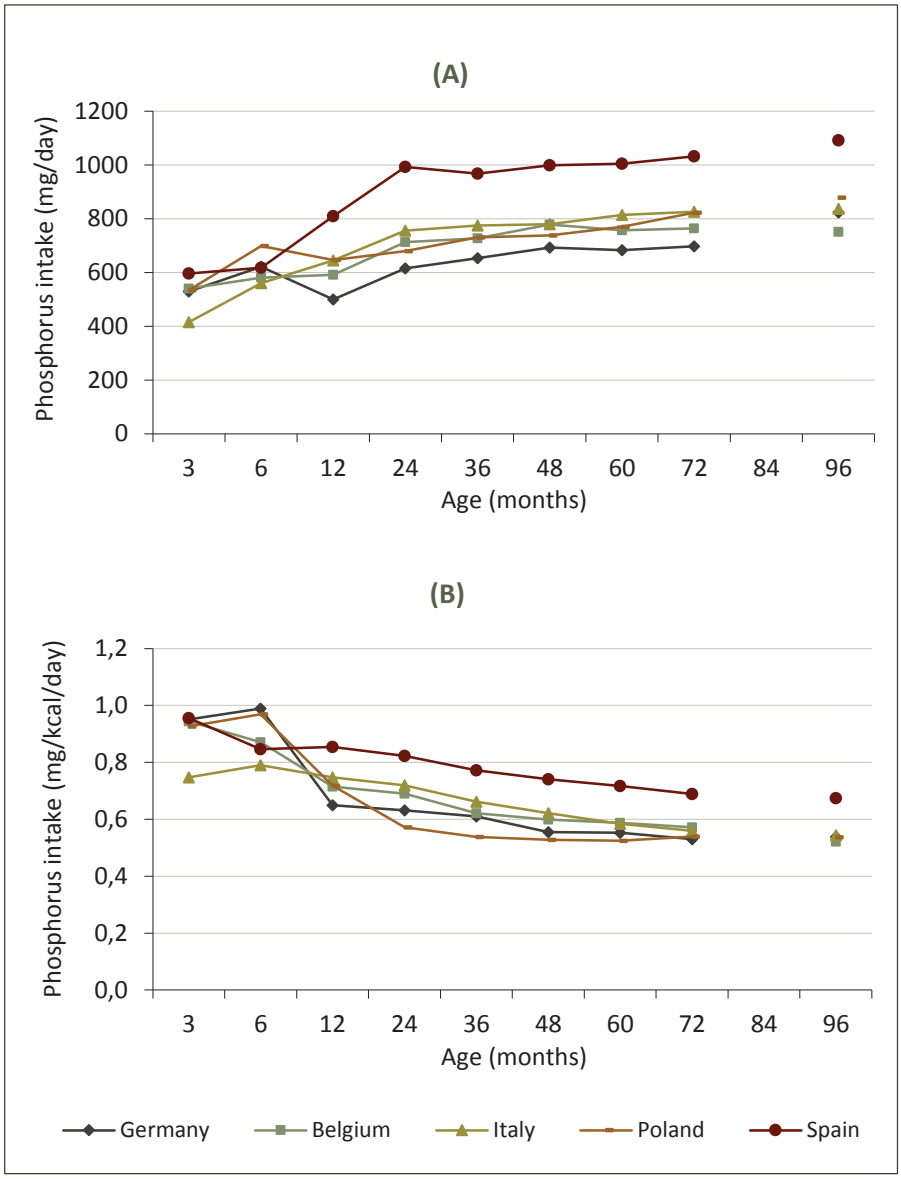


Figure A: Daily phosphorus intake by country (mg/day). Figure B: Daily phosphorus intake by country, adjusted by energy (mg/Kcal/day).

Phosphorus was highly consumed by Spanish children in comparison with all the other children ($p<0.001$), with exception at 6 months of life when Polish children were those with the highest intake ($p<0.001$). German children were, from 12 months of age, those with the lowest intake, and these differences were statistically significant in comparison with Spanish children ($p<0.001$). After adjusting by energy intake, Spanish children showed still the highest consume ($p<0.001$ vs all countries from 12^{th} month) (Figure 12).

Iron intake was significantly higher in Spain at all ages compared with the other countries ($p<0.001$). Contrarily, Italians and Germans were those with lower iron consumption, this difference was statistically significant almost at all timepoints versus the Spanish, and during first years of life also versus Belgians and Polish. After adjusting by energy, Spanish were the children with highest iron intake by kcal ($p<0.001$ vs. all countries, except Belgium at T24). Italians were the ones with lowest consume from birth to 3 years ($p<0.001$) (Figure 13).

Spanish children showed the highest raw intake of zinc during all the study period, being near to the other countries during the first 6 months of life, but getting especially different from T24 onwards ($p<0.001$ vs all countries, $p<0.005$ vs Belgium at 36months). Germany was the country where zinc was consumed in smaller amounts during all the period. The highest zinc intake by kcal was also in Spain ($p<0.001$ vs all countries from 24 to 72 months), Belgium had similar zinc consume as Spain at 24 and 36 months (Figure 14).

Figure 13. Daily iron intake by country.

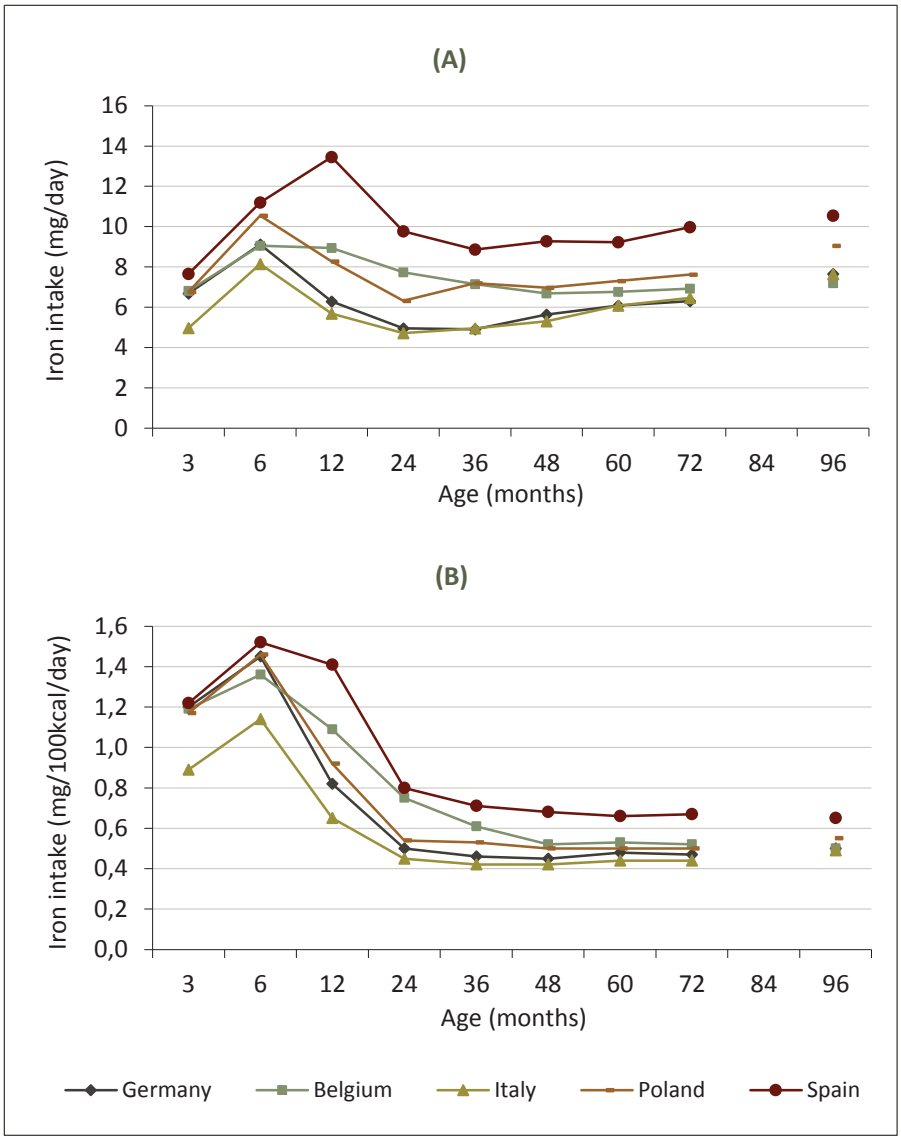


Figure A: Daily iron intake by country (mg/day). Figure B: Daily iron intake by country, adjusted by energy (mg/100Kcal/day).

Figure 14. Daily zinc intake by country.

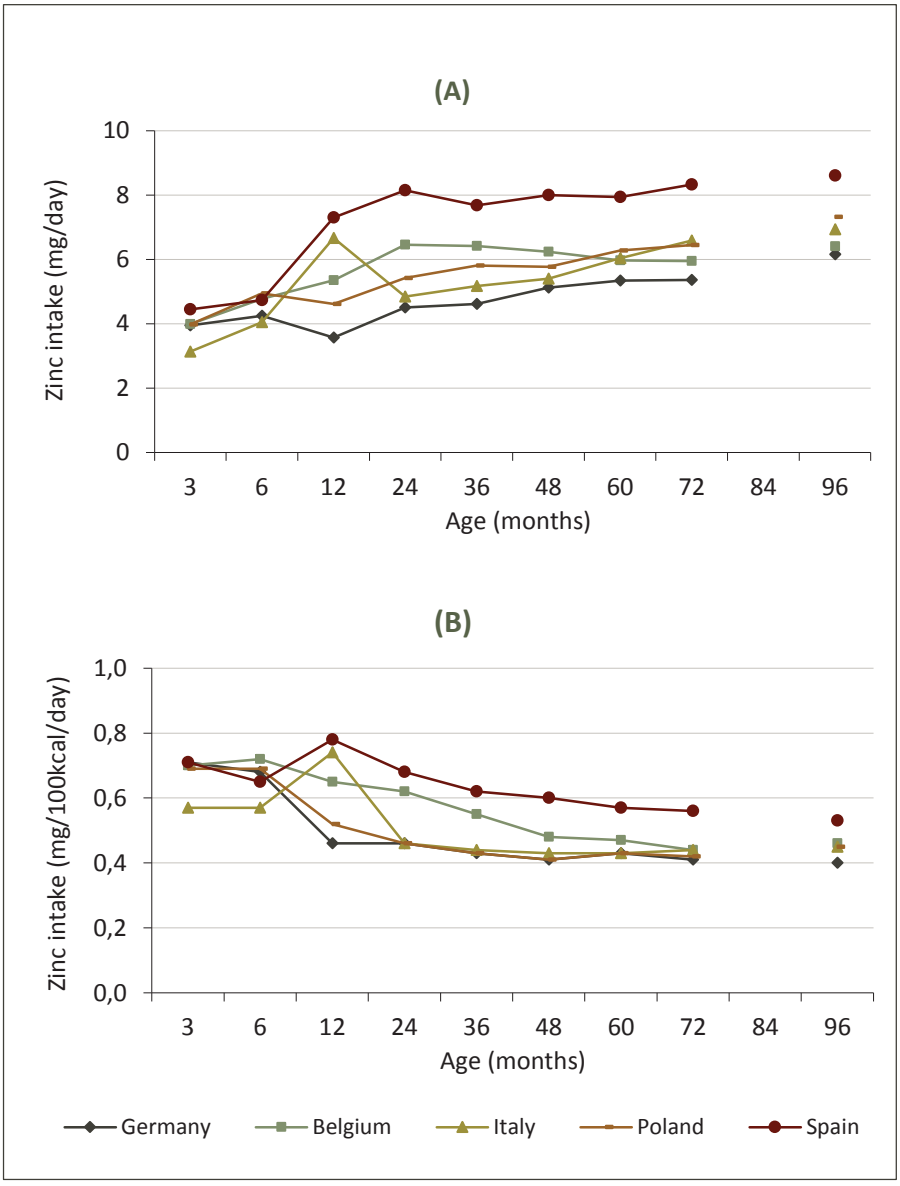


Figure A: Daily zinc intake by country (mg/day). Figure B: Daily zinc intake by country, adjusted by energy (mg/100Kcal/day).

Figure 15. Daily magnesium intake by country.

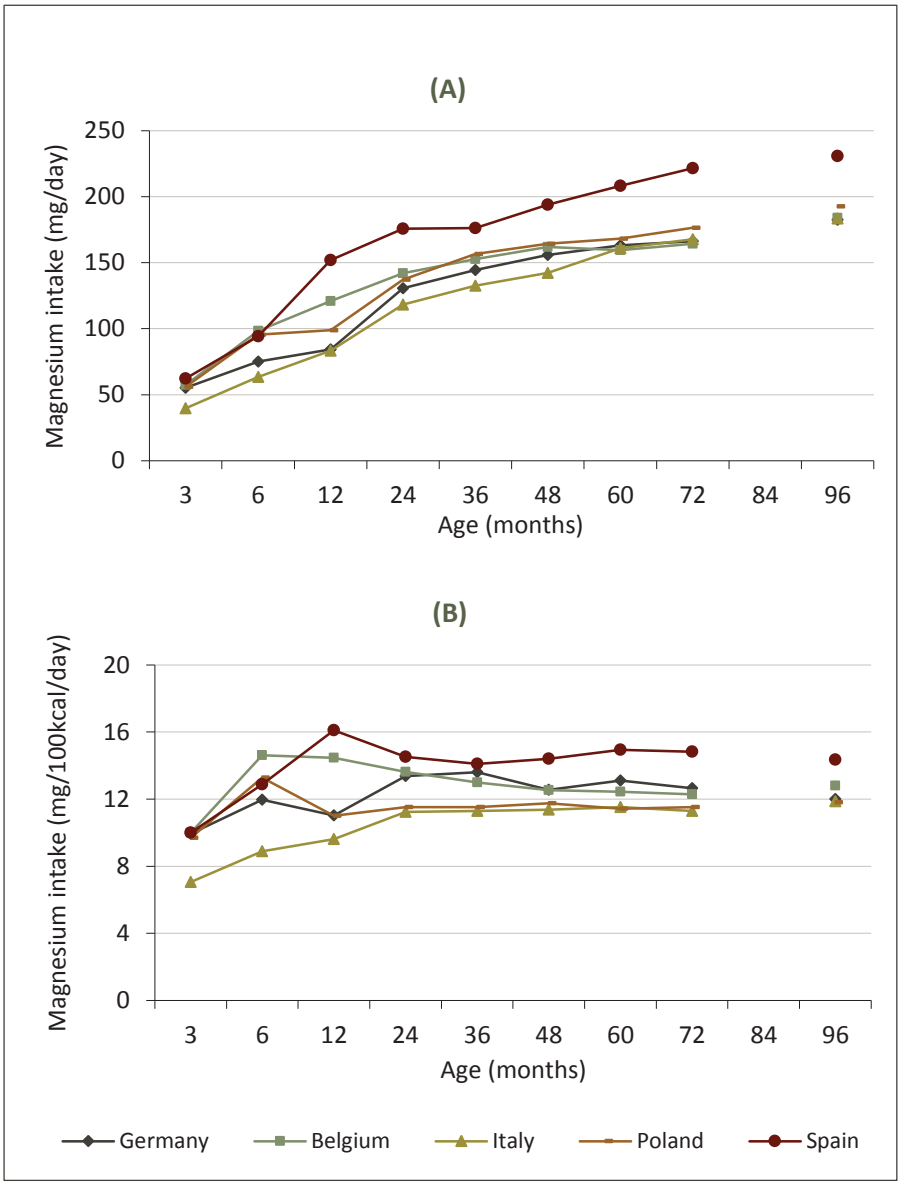


Figure A: Daily magnesium intake by country (mg/day). Figure B: Daily magnesium intake by country, adjusted by energy (mg/100Kcal/day).

From 12th months of age, Spanish children took the highest amount of magnesium in comparison with children from the other countries ($p<0.001$). Over all the study period, Italian children were those with the lowest intake of magnesium, significantly lower than the others ($p<0.001$) until 36 months of life, and still lower at later timepoints in comparison with Spanish ($p<0.001$). After adjusting magnesium intake by energy, Spanish children were still those with the highest consumption of magnesium, and Italians those with the lowest, being significant the differences between them ($p<0.001$) (Figure 15).

Italians had the lower iodine intake from birth to 12 months ($p<0.05$). From 24 months of life onwards, Polish were those with lowest iodine intake ($p<0.05$ vs. almost all countries). All the other countries had similar intakes of iodine throughout the entire period. After adjusting iodine intake by energy, the consumption profile did not change too much, Italy was still the country with lowest intake during the first year, and Poland was still the smaller consumer of iodine from 24month onwards (Figure 16).

The first 6 months of life, Italy had the lowest consume of vitamin B_{12} ($p<0.001$), and Spain had the highest ($p<0.05$ versus all countries). From the 24th month of life, Belgium reached the Spain levels of vitamin B_{12} intakes and both were higher than those in Germany and Italy ($p<0.05$). Poland consumption that had been in between the highest and the lowest intakes, became similar to the German and Italian ones from T48 onwards. When vitamin B_{12} was adjusted by energy, Spain and Belgium were still those with higher intakes compared with Italy, Germany and Poland that were those with lower intakes ($p<0.01$) (Figure 17).

Figure 16. Daily iodine intake by country.

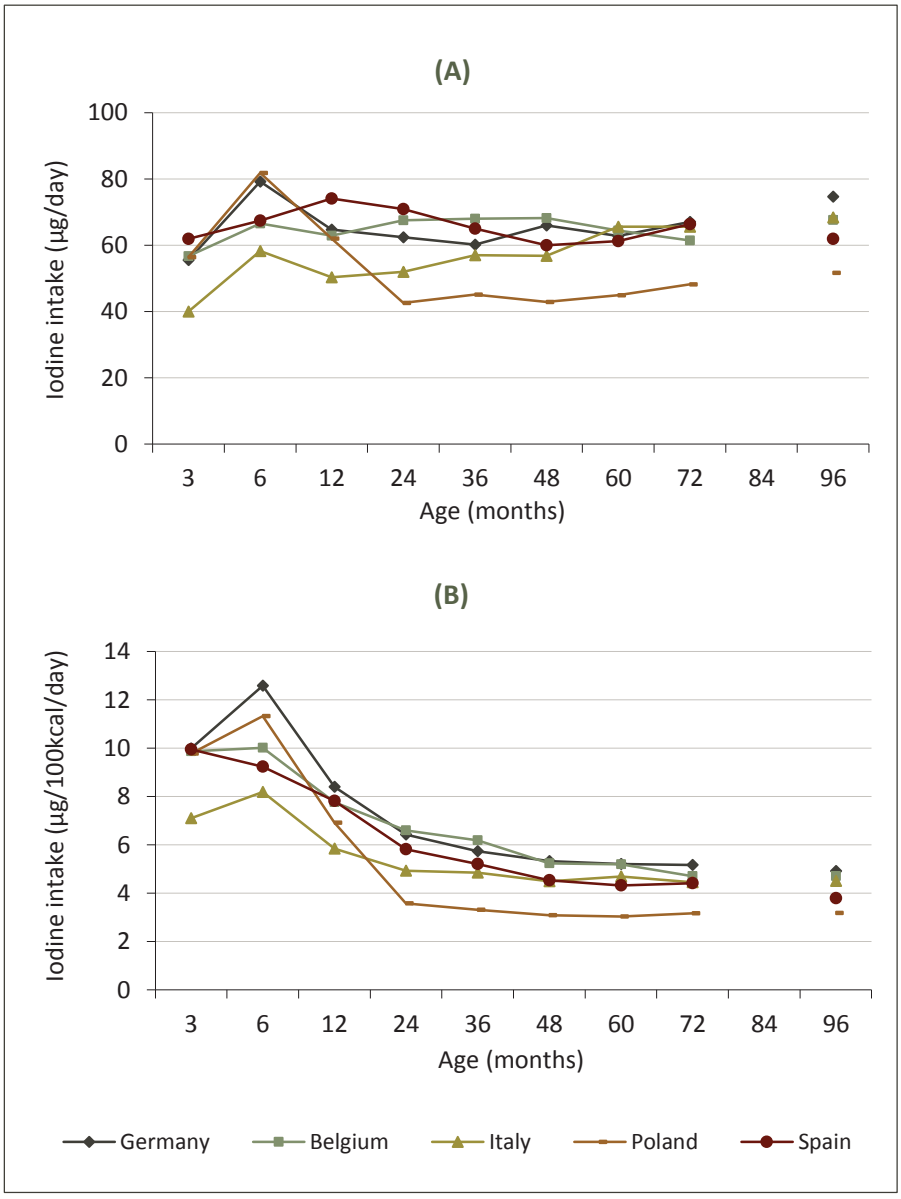


Figure A: Daily iodine intake by country (µg/day). Figure B: Daily iodine intake by country, adjusted by energy (µg/100Kcal/day).

Figure 17. Daily vitamin B_{12} (cobalamin) intake by country.

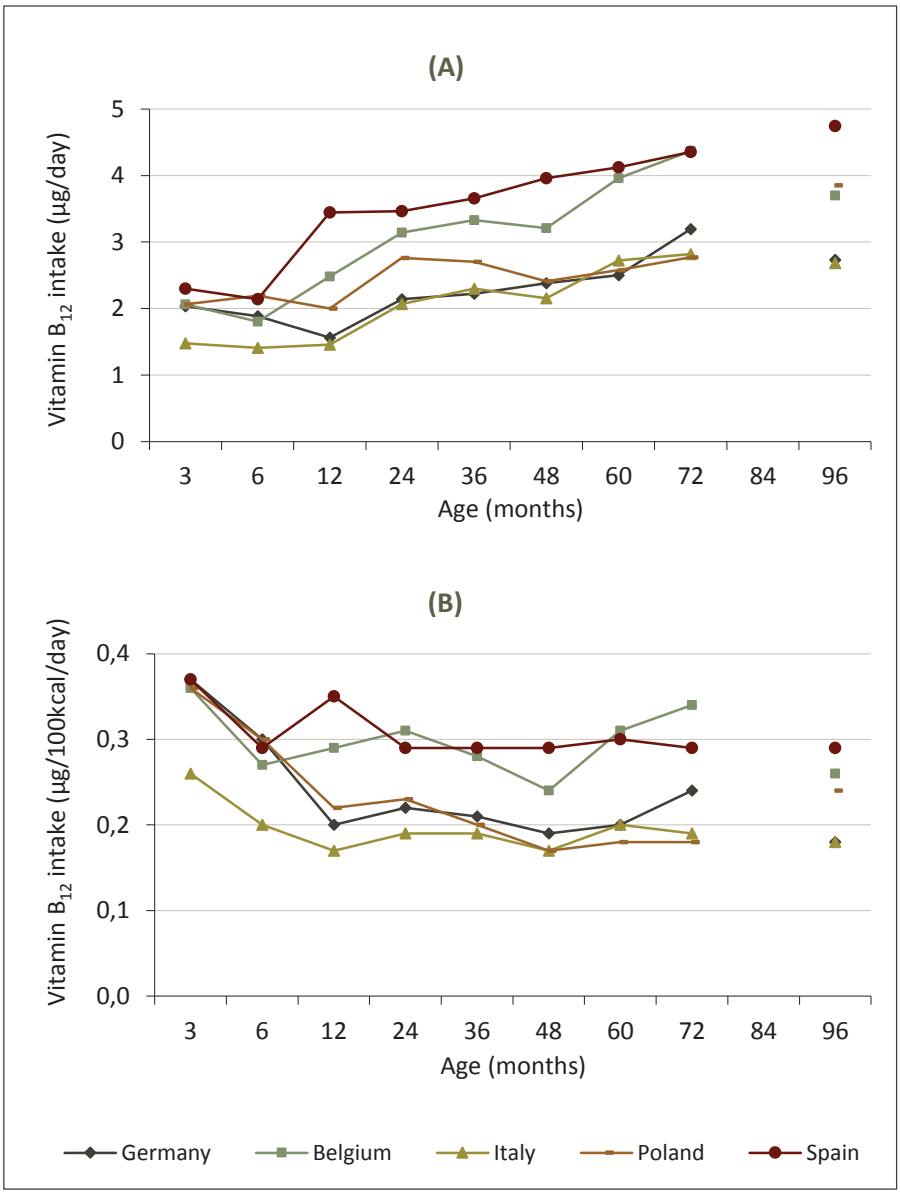


Figure A: Daily vitamin B_{12} intake by country (µg/day). Figure B: Daily vitamin B_{12} intake by country, adjusted by energy (µg/100Kcal/day).

Figure 18. Daily vitamin B_9 (folate) intake by country.

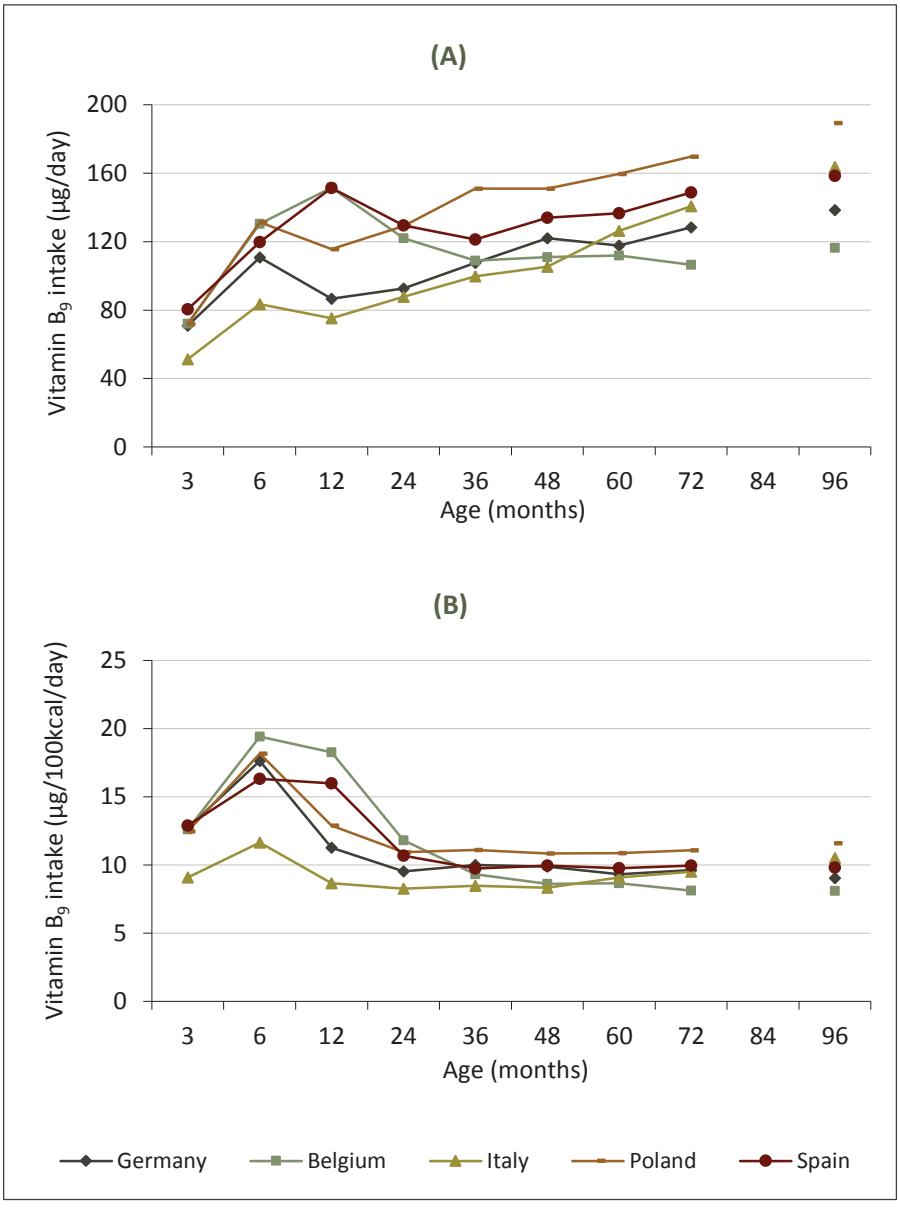


Figure A: Daily vitamin B_9 intake by country (µg/day). Figure B: Daily vitamin B_9 intake by country, adjusted by energy (µg/100Kcal/day).

Italians intake of vitamin B_9 was the lowest until 2 years ($p<0.001$), and lower than Polish and Spanish to 4 years ($p<0.001$). Spanish children were those with the highest intake at 3 ($p<0.05$) and 12 months ($p<0.001$ vs. Germany, Italy and Poland), but from 24 months onwards Polish reached the highest consumption of folate ($p<0.001$ at 36, 48 and 60 months, and $p<0.05$ at 72 and 96 months). Belgians started with a high consumption and finished with the lower. Trends did not change too much after adjusting by energy intake (Figure 18).

Polish and Belgians intake of vitamin A was the highest, but showed a strange behaviour of consumption. Polish consumed the highest amount of vitamin A during the first year ($p<0.001$ vs. all at 6, $p<0.05$ vs. Germany, Italy and Spain at 12 months), and Belgians did from the 4th year ($p<0.001$ vs. all at 60th and 72nd months, and $p<0.05$ at 96th month). In between, vitamin A intake was similar in both countries. Spanish, Italian and German children followed a similar line of consumption. Profile did not changed after adjusting by energy intake (Figure 19).

Looking at vitamin D intakes, we observed that all countries had similar behaviour, considering longitudinal graphs shapes. All children had a higher consumption of vitamin D during the first year of life, decreasing until 24th month of life, when it became more or less stable until the age of 8. There were relevant significant country differences between Spain and Italy ($p<0.001$ until 36th month, $p<0.05$ later on). And the same happened after adjusting by energy intake ($p<0.001$ between Spain and Italy) (Figure 20).

Figure 19. Daily vitamin A intake by country.

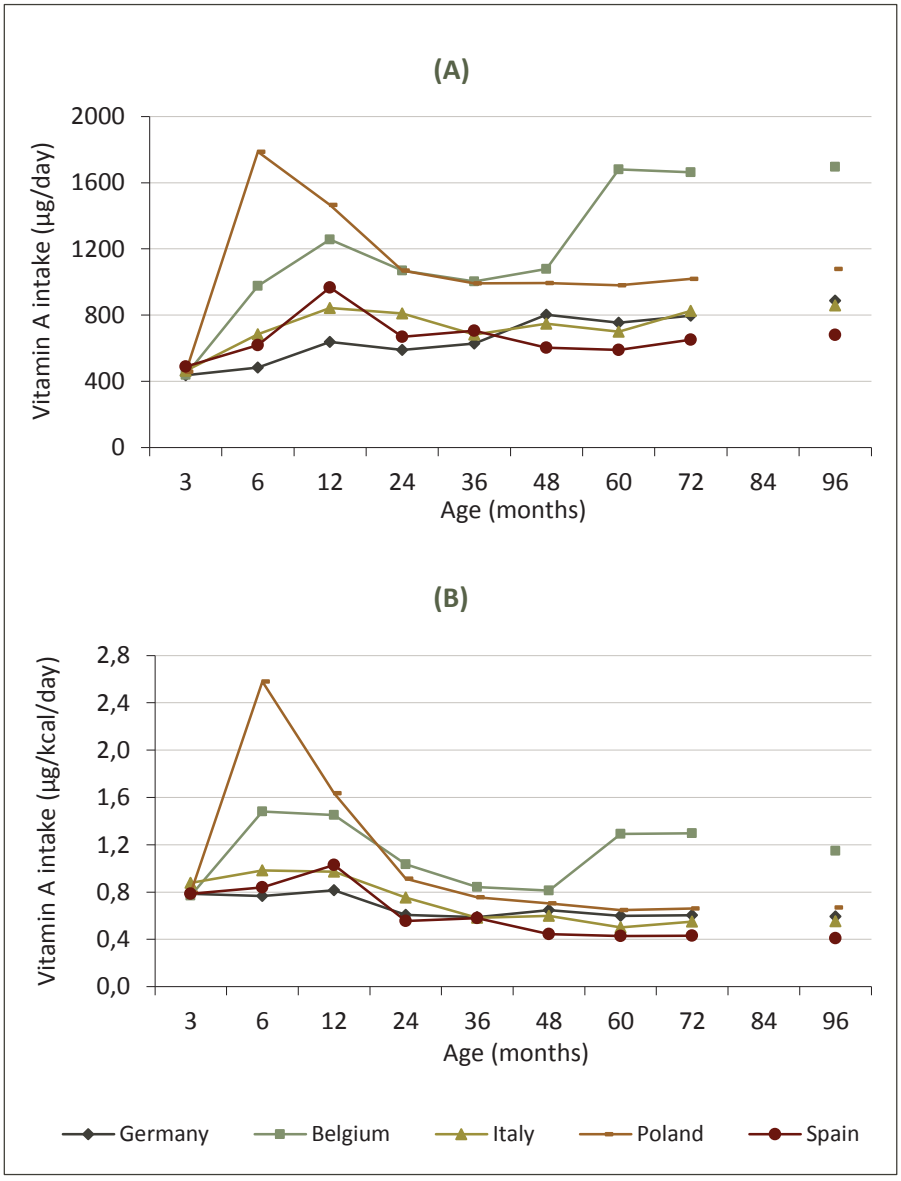


Figure A: Daily vitamin A intake by country (µg/day). Figure B: Daily vitamin A intake by country, adjusted by energy (µg/Kcal/day).

Figure 20. Daily vitamin D intake by country.

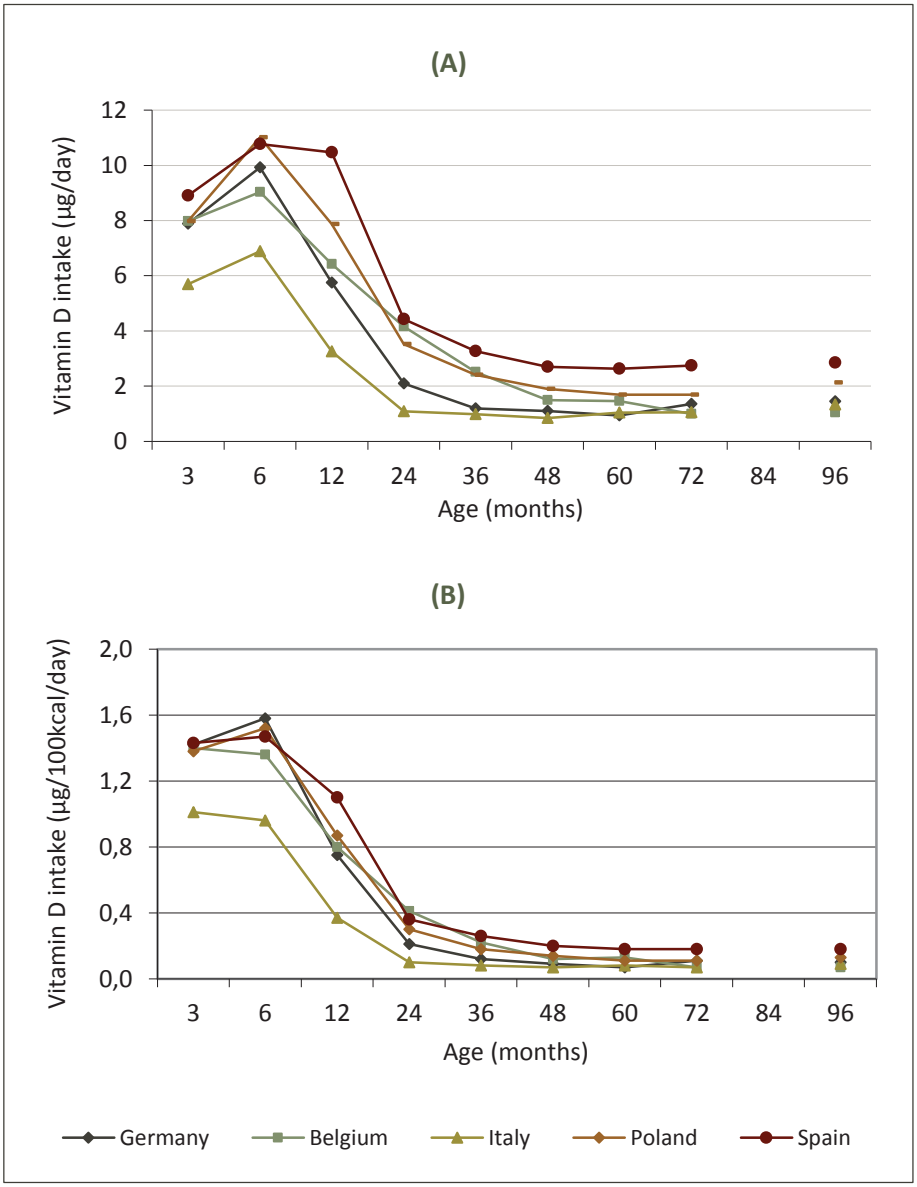


Figure A: Daily vitamin D intake by country (µg/day). Figure B: Daily vitamin D intake by country, adjusted by energy (µg/100Kcal/day).

4.2.4. Assessment of micronutrients intake adequacy to the recommendations

- Assessment of micronutrients intake adequacy at individual level

Coefficient of variation of intake was calculated for each micronutrient. According to it, quantitative assessment of adequacy at individual level was performed for: calcium, iron, zinc (except at 12 months), vitamin B_{12} (except at 1, 3 and 6 years) and folate comparing to FAO/WHO/UNU EAR. And for phosphorus, magnesium and iodine (except at 2 and at 4 years) comparing to FNB-IOM EAR.

Results are shown in Tables 20 to 29 one table for each micronutrient.

- Assessment of micronutrients intake adequacy at group level

Adequacy of the intakes to the requirements was assessed at group level by the EAR cut-point method. Results are shown in Tables 20 to 29, together with the individual assessment.

Table 20. Calcium intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 3 months (n=904)				
627.5 ±169.1	300	>95% 75-95% 50-75% 25-50% <25%	791 (87.5) 34 (3.8) 6 (0.6) 28 (3.1) 45 (5)	831 (91.9)
at 6 months (n=839)				
770.8 ±244.6	300	>95% 75-95% 50-75% 25-50% <25%	708 (84.4) 78 (9.3) 21 (2.5) 25 (3.0) 7 (0.8)	807 (96.2)
at 12 months (n=822)				
713.3 ±258.7	440	>95% 75-95% 50-75% 25-50% <25%	409 (49.8) 231 (28.1) 100 (12.1) 50 (6.1) 32 (3.9)	740 (90.0)
at 24 months (n=745)				
698.9 ±280.8	440	>95% 75-95% 50-75% 25-50% <25%	326 (43.8) 189 (25.3) 115 (15.4) 67 (9.0) 48 (6.5)	630 (84.6)
at 36 months (n=527)				
662.1 ±255.1	440	>95% 75-95% 50-75% 25-50% <25%	207 (39.3) 127 (24.1) 93 (17.6) 59 (11.2) 41 (7.8)	427 (81.0)

(Continued on next page)

*(**Table 20.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 48 months (n=503)				
656.4 ±253.9	440	>95%	197 (39.2)	398 (79.1)
		75-95%	125 (24.9)	
		50-75%	76 (15.1)	
		25-50%	59 (11.7)	
		<25%	46 (9.1)	
at 60 months (n=445)				
655.9 ±257.3	440	>95%	171 (38.4)	360 (80.9)
		75-95%	97 (21.8)	
		50-75%	92 (20.7)	
		25-50%	49 (11.0)	
		<25%	36 (8.1)	
at 72 months (n=468)				
647.7 ±251.5	440	>95%	175 (37.4)	375 (80.1)
		75-95%	104 (22.2)	
		50-75%	96 (20.5)	
		25-50%	62 (13.2)	
		<25%	31 (6.7)	
at 96 months (n=396)				
683.9 ±251.7	440	>95%	173 (43.7)	331 (83.6)
		75-95%	94 (23.7)	
		50-75%	64 (16.2)	
		25-50%	44 (11.1)	
		<25%	21 (5.3)	

EAR: Estimated Average Requirement from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31].

Table 21. Phosphorus intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis		
		At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 12 months (n=822)				
656.0 ±216.5	380	>95% 75-95% 50-75% 25-50% <25%	488 (59.4) 183 (22.2) 84 (10.2) 50 (6.1) 17 (2.1)	755 (91.8)
at 24 months (n=745)				
771.8 ±246.1	380	>95% 75-95% 50-75% 25-50% <25%	524 (70.3) 150 (20.2) 47 (6.3) 17 (2.3) 7 (0.9)	721 (96.8)
at 36 months (n=527)				
791.1 ±226.3	380	>95% 75-95% 50-75% 25-50% <25%	409 (77.6) 83 (15.8) 27 (5.1) 7 (1.3) 1 (0.2)	519 (98.5)
at 48 months (n=503)				
823.81 ±232.0	405	>95% 75-95% 50-75% 25-50% <25%	375 (74.6) 101 (20.0) 20 (4.0) 4 (0.8) 3 (0.6)	496 (98.6)
at 60 months (n=445)				
838.6 ±229.1	405	>95% 75-95% 50-75% 25-50% <25%	348 (78.2) 75 (16.9) 19 (4.2) 3 (0.7) -	442 (99.3)

(Continued on next page)

*(**Table 21.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 72 months (n=468)				
859.6 ±210.8	405	>95%	414 (88.5)	466 (99.6)
		75-95%	43 (9.2)	
		50-75%	9 (1.9)	
		25-50%	2 (0.4)	
		<25%	-	
at 96 months (n=396)				
934.6 ±236.0	405	>95%	364 (91.9)	395 (99.7)
		75-95%	24 (6.1)	
		50-75%	7 (1.7)	
		25-50%	-	
		<25%	1 (0.3)	

EAR: Estimated Average Requirement from the American Institute of Medicine (FNB-IOM)[34]. There were not EAR available for phosphorus for ages below 12 months from FNB-IOM. There were not EAR available for phosphorus from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation.

Table 22. Iron intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 6 months (n=839)				
9.61 ±3.66	7.2	>95%	264 (31.5)	662 (78.9)
		75-95%	250 (29.8)	
		50-75%	148 (17.6)	
		25-50%	104 (12.4)	
		<25%	73 (8.7)	
at 12 months (n=822)				
8.50 ±4.78	4.6	>95%	292 (35.5)	668 (81.3)
		75-95%	243 (29.6)	
		50-75%	133 (16.2)	
		25-50%	115 (14.0)	
		<25%	39 (4.7)	
at 24 months (n=745)				
6.61 ±3.89	4.6	>95%	164 (22.0)	507 (68.1)
		75-95%	146 (19.6)	
		50-75%	197 (26.5)	
		25-50%	193 (25.9)	
		<25%	45 (6.0)	
at 36 months (n=527)				
6.51 ±2.76	4.6	>95%	160 (30.4)	391 (74.2)
		75-95%	120 (22.7)	
		50-75%	111 (21.1)	
		25-50%	100 (19.0)	
		<25%	36 (6.8)	
at 48 months (n=503)				
6.87 ±2.83	5.0	>95%	125 (24.9)	377 (75.0)
		75-95%	129 (25.6)	
		50-75%	123 (24.5)	
		25-50%	98 (19.4)	
		<25%	28 (5.6)	

(Continued on next page)

*(**Table 22.** Continued)*

Intake	EAR	Adequacy analysis		
		At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 60 months (n=445)				
7.22 ±2.69	5.0	>95%	148 (33.3)	360 (80.9)
		75-95%	131 (29.4)	
		50-75%	84 (18.9)	
		25-50%	60 (13.5)	
		<25%	22 (4.9)	
at 72 months (n=468)				
7.67 ±2.81	5.0	>95%	184 (39.3)	408 (87.2)
		75-95%	137 (29.3)	
		50-75%	87 (18.6)	
		25-50%	54 (11.5)	
		<25%	6 (1.3)	
at 96 months (n=396)				
9.01 ±3.10	7.1[a]-12[b]	>95%	62 (15.7)	169 (42.7)
		75-95%	60 (15.1)	
		50-75%	47 (11.9)	
		25-50%	45 (11.3)	
		<25%	182 (46)	

EAR: Estimated Average Requirement from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation(FAO/WHO/UNU)[31]. There were not EAR available for iron for ages below 6 months, neither from FAO/WHO/UNU nor from the American Institute of Medicine. . [a]: EAR for male. [b]: EAR for female.

Table 23. Zinc intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 3 months (n=899)				
3.85 ±1.16	0.64[a]-0.57[b]	>95%	117 (13.0)	610 (67.5)
		75-95%	276 (30.7)	
		50-75%	217 (24.1)	
		25-50%	146 (16.2)	
		<25%	143 (16.0)	
at 6 months (n=829)				
5.21 ±6.21	0.25	>95%	582 (70.2)	789 (95.2)
		75-95%	181 (21.8)	
		50-75%	26 (3.2)	
		25-50%	30 (3.6)	
		<25%	10 (1.2)	
at 12 months (n=812)				
8.63 ±22.58	0.28	>95%	*	727 (89.5)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 24 months (n=733)				
7.18 ±12.83	0.28	>95%	193 (26.3)	645 (88.0)
		75-95%	241 (32.9)	
		50-75%	211 (28.8)	
		25-50%	82 (11.2)	
		<25%	6 (0.8)	
at 36 months (n=512)				
6.13 ±5.87	0.28	>95%	138 (27.0)	420 (82.0)
		75-95%	164 (32.0)	
		50-75%	118 (23.0)	
		25-50%	72 (14.1)	
		<25%	20 (3.9)	

(Continued on next page)

*(**Table 23.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 48 months (n=491)				
6.28 ±2.59	0.23	>95% 75-95% 50-75% 25-50% <25%	175 (35.6) 181 (36.9) 90 (18.3) 39 (8.0) 6 (1.2)	446 (90.8)
at 60 months (n=439)				
6.51 ±2.33	0.23	>95% 75-95% 50-75% 25-50% <25%	152 (34.6) 143 (32.6) 83 (18.9) 42 (9.6) 19 (4.3)	378 (86.1)
at 72 months (n=462)				
6.84 ±2.47	0.23	>95% 75-95% 50-75% 25-50% <25%	128 (27.7) 151(32.7) 101 (21.9) 54 (11.7) 28 (6.0)	380 (82.3)
at 96 months (n=394)				
7.62 ±3.30	0.18	>95% 75-95% 50-75% 25-50% <25%	149 (37.8) 116 (29.5) 77 (19.5) 33 (8.4) 19 (4.8)	342 (86.8)

EAR: Estimated Average Requirement from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31]. . [a]: EAR for male. [b]: EAR for female. *Adequacy of zinc at 12 months could not be assessed at individual level because CV of intake was >70.

Table 24. Magnesium intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 12 months (n=822)				
107.2 ±45.7	65	>95% 75-95% 50-75% 25-50% <25%	340 (41.4) 213 (25.9) 139 (16.9) 92 (11.2) 38 (4.6)	692 (84.2)
at 24 months (n=745)				
140.3 ±46.6	65	>95% 75-95% 50-75% 25-50% <25%	526 (70.6) 156 (20.9) 43 (5.8) 18 (2.4) 2 (0.3)	725 (97.3)
at 36 months (n=527)				
151.3 ±40.4	65	>95% 75-95% 50-75% 25-50% <25%	457 (86.7) 55 (10.4) 13 (2.5) 1 (0.2) 1 (0.2)	525 (99.6)
at 48 months (n=503)				
164.2 ±45.0	110	>95% 75-95% 50-75% 25-50% <25%	261 (51.9) 131 (26.0) 68 (13.5) 33 (6.6) 10 (2.0)	460 (91.5)
at 60 months (n=445)				
175.4 ±48.6	110	>95% 75-95% 50-75% 25-50% <25%	261 (58.7) 109 (24.5) 48 (10.8) 21 (4.7) 6 (1.3)	418 (93.9)

(Continued on next page)

*(**Table 24.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 72 months (n=468)				
183.5 ±47.8	110	>95%	302 (64.5)	454 (97.0)
		75-95%	112 (24.0)	
		50-75%	40 (8.5)	
		25-50%	11 (2.4)	
		<25%	3 (0.6)	
at 96 months (n=396)				
203.3 ±49.9	110	>95%	311 (78.5)	392 (99.0)
		75-95%	68 (17.2)	
		50-75%	13 (3.3)	
		25-50%	2 (0.5)	
		<25%	2 (0.5)	

EAR: Estimated Average Requirements from Food and Nutrition Board of the American Institute of Medicine(FNB-IOM)[78–81]. There were not EAR available for magnesium for ages below 12 month from FNB-IOM. There were not EAR available for magnesium from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation.

Table 25. Iodine intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 12 months (n=822)				
62.0 ±30.7	65	>95%	57 (6.9)	351 (42.7)
		75-95%	104 (12.7)	
		50-75%	190 (23.1)	
		25-50%	183 (22.3)	
		<25%	288 (35.0)	
at 24 months (n=745)				
58.2 ±48.4	65	>95%	*	238 (31.9)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 36 months (n=527)				
58.8 ±75.0	65	>95%	39 (7.4)	144 (27.3)
		75-95%	44 (8.3)	
		50-75%	61 (11.6)	
		25-50%	135 (25.6)	
		<25%	248 (47.1)	
at 48 months (n=503)				
58.0 ±43.2	65	>95%	*	144 (28.6)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 60 months (n=445)				
60.6 ±39.0	65	>95%	31 (7.0)	136 (30.6)
		75-95%	44 (9.9)	
		50-75%	61 (13.7)	
		25-50%	128 (28.8)	
		<25%	181 (40.6)	

(Continued on next page)

*(**Table 25.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 72 months (n=468)				
62.1 ±44.2	65	>95%	30 (6.4)	140 (29.9)
		75-95%	33 (7.1)	
		50-75%	77 (16.5)	
		25-50%	171 (36.5)	
		<25%	157 (33.5)	
at 96 months (n=396)				
63.4 ±42.3	65	>95%	34 (8.6)	111 (28.0)
		75-95%	27 (6.8)	
		50-75%	50 (12.6)	
		25-50%	148 (37.4)	
		<25%	137 (34.6)	

EAR: Estimated Average Requirement from the American Institute of Medicine (FNB-IOM)[171]. There were not EAR available for iodine for ages below 12 months from FNB-IOM. There were not EAR available for iodine from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation. *Adequacy of iodine at 24 and at 48 months could not be assessed at individual level because CV of intake was >70.

Table 26. Vitamin B_{12} intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 3 months (n=904)				
1.94 ±0.70	0.3	>95%	824 (91.2)	828 (91.6)
		75-95%	1 (0.1)	
		50-75%	3 (0.3)	
		25-50%	19 (2.1)	
		<25%	57 (6.3)	
at 6 months (n=839)				
1.85 ±0.82	0.3	>95%	733 (87.4)	796 (94.9)
		75-95%	50 (6.0)	
		50-75%	13 (1.5)	
		25-50%	43 (5.1)	
		<25%	-	
at 12 months (n=822)				
2.17 ±3.59	0.7	>95%	*	742 (90.3)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 24 months (n=745)				
2.67 ±1.89	0.7	>95%	326 (43.8)	712 (95.6)
		75-95%	264 (35.4)	
		50-75%	122 (16.4)	
		25-50%	33 (4.4)	
		<25%	-	
at 36 months (n=527)				
2.80 ±2.01	0.7	>95%	*	508 (96.4)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	

(Continued on next page)

*(**Table 26.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 48 months (n=503)				
2.85 ±1.84	1	>95%	236 (46.9)	465 (92.4)
		75-95%	153 (30.4)	
		50-75%	76 (15.1)	
		25-50%	37 (7.4)	
		<25%	1 (0.2)	
at 60 months (n=445)				
3.18 ±1.71	1	>95%	240 (53.9)	436 (98.0)
		75-95%	143 (32.2)	
		50-75%	53 (11.9)	
		25-50%	8 (1.8)	
		<25%	1 (0.2)	
at 72 months (n=468)				
3.42 ±2.94	1	>95%	*	458 (97.9)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 96 months (n=396)				
3.80 ±2.39	1.5	>95%	150 (37.9)	372 (93.9)
		75-95%	142 (35.8)	
		50-75%	80 (20.2)	
		25-50%	24 (6.1)	
		<25%	-	

EAR: Estimated Average Requirement from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31].
*Adequacy of vitamin B_{12} at 12, 36 and at 72 months could not be assessed at individual level because CV of intake was >70.

Table 27. Vitamin B_9 (folate) intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 3 months (n=904)				
67.8 ±24.3	65	>95%	86 (9.5)	635 (70.2)
		75-95%	267 (29.5)	
		50-75%	282 (31.2)	
		25-50%	144 (16.0)	
		<25%	125 (13.8)	
at 6 months (n=839)				
110.8 ±46.6	65	>95%	416 (49.6)	738 (88.0)
		75-95%	224 (26.7)	
		50-75%	98 (11.7)	
		25-50%	41 (4.8)	
		<25%	60 (7.2)	
at 12 months (n=822)				
111.4 ±54.0	120	>95%	74 (9.0)	329 (40.0)
		75-95%	124 (15.1)	
		50-75%	131 (15.9)	
		25-50%	138 (16.8)	
		<25%	355 (43.2)	
at 24 months (n=745)				
110.0 ±48.1	120	>95%	71 (9.5)	248 (33.3)
		75-95%	74 (10.0)	
		50-75%	103 (13.8)	
		25-50%	154 (20.7)	
		<25%	343 (46.0)	
at 36 months (n=527)				
115.6 ±43.1	120	>95%	60 (11.4)	210 (39.8)
		75-95%	61 (11.6)	
		50-75%	89 (16.8)	
		25-50%	111 (21.1)	
		<25%	206 (39.1)	

(Continued on next page)

*(**Table 27.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 48 months (n=503)				
123.5 ±45.8	160	>95%	18 (3.6)	88 (17.5)
		75-95%	33 (6.5)	
		50-75%	37 (7.4)	
		25-50%	71 (14.1)	
		<25%	344 (68.4)	
at 60 months (n=445)				
132.1 ±46.9	160	>95%	19 (4.3)	113 (25.4)
		75-95%	43 (9.6)	
		50-75%	51 (11.5)	
		25-50%	68 (15.3)	
		<25%	264 (59.3)	
at 72 months (n=468)				
144.0 ±51.3	160	>95%	41 (8.8)	163 (34.8)
		75-95%	56 (11.9)	
		50-75%	66 (14.1)	
		25-50%	72 (15.4)	
		<25%	233 (49.8)	
at 96 months (n=396)				
159.7 ±56.3	250	>95%	5 (1.3)	23 (5.8)
		75-95%	6 (1.5)	
		50-75%	12 (3.0)	
		25-50%	26 (6.6)	
		<25%	347 (87.6)	

EAR: Estimated Average Requirement from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31].

Table 28. Vitamin A intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis		
		At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 3 months (n=904)				
463.3 ±87.4	180	>95%	*	903 (99.8)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 6 months (n=839)				
860.0 ±827.0	180	>95%	*	838 (99.9)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 12 months (n=822)				
998.4 ±1314.3	200	>95%	*	811 (98.7)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 24 months (n=745)				
817.3 ±1281.0	200	>95%	*	723 (97.0)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 36 months (n=527)				
765.0 ±1239.2	200	>95%	*	514 (97.5)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	

(Continued on next page)

*(**Table 28.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 48 months (n=503)				
787.0 ±672.5	200	>95%	*	493 (98.0)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 60 months (n=445)				
821.0 ±882.3	200	>95%	*	428 (96.2)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 72 months (n=468)				
897.6 ±954.7	200	>95%	*	452 (96.6)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 96 months (n=396)				
905.3 ±933.2	250	>95%	*	379 (95.7)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	

EAR: Estimated Average Requirement from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31].
* Adequacy could not be assessed at individual level because vitamin A CV of intake was >70.

Table 29. Vitamin D intake adequacy to the recommendations.

Intake	EAR	Adequacy analysis		
		At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 12 months (n=822)				
6.60 ±5.07	10	>95%	*	136 (16.5)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 24 months (n=745)				
2.85 ±3.47	10	>95%	*	25 (3.4)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 36 months (n=527)				
1.97 ±2.00	10	>95%	*	3 (0.6)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 48 months (n=503)				
1.64 ±1.63	10	>95%	*	3 (0.6)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 60 months (n=445)				
1.62 ±1.70	10	>95%	*	2 (0.4)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	

(Continued on next page)

*(**Table 29.** Continued)*

Intake	EAR	Adequacy analysis: At individual level		At group level
(mg/day) mean ±SD	(mg/day)	Probability of adequate intake	N (%)	Prevalence of adequate intake N (%)
at 72 months (n=468)				
1.67 ±1.56	10	>95%	*	2 (0.4)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	
at 96 months (n=396)				
2.07 ±1.83	10	>95%	*	3 (0.8)
		75-95%	*	
		50-75%	*	
		25-50%	*	
		<25%	*	

EAR: Estimated Average Requirement from the American Institute of Medicine (FNB-IOM)[34,172]. There were not EAR available for vitamin D for ages below 12 months from FNB-IOM. There were not EAR available for vitamin D from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation * Adequacy could not be assessed at individual level because vitamin D CV of intake was >70.

4.2.5. Assessment of micronutrients intake adequacy to the recommendations by country

Figures 21 to 28 show country prevalence of the probability of adequate intake categories for each micronutrient assessed at individual level, thus available for calcium, iron, zinc (except at 12 months), vitamin B_{12} (except at 1, 3 and 6 years) and folate according to FAO/WHO/UNU EAR and for phosphorus, magnesium and iodine (except at 2 and 4 years) according to FNB-IOM EAR.

Annex VII Provides complete information on probability of adequacy for each micronutrient at each timepoint and for each country. Country differences are also provided.

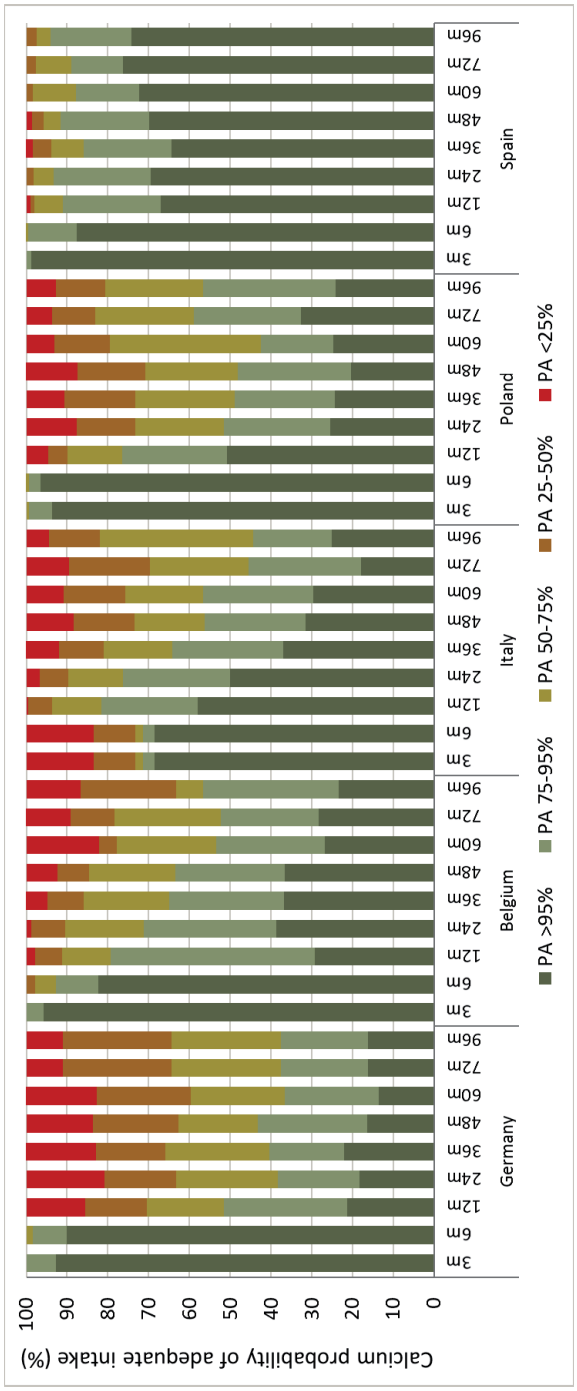


Figure 21. Calcium probability of adequate intake by country.

Adequacy assessed at individual level using EAR from Food and Agriculture Organization of the United Nations/World Health Organization/ United Nations University joint consultation[31].

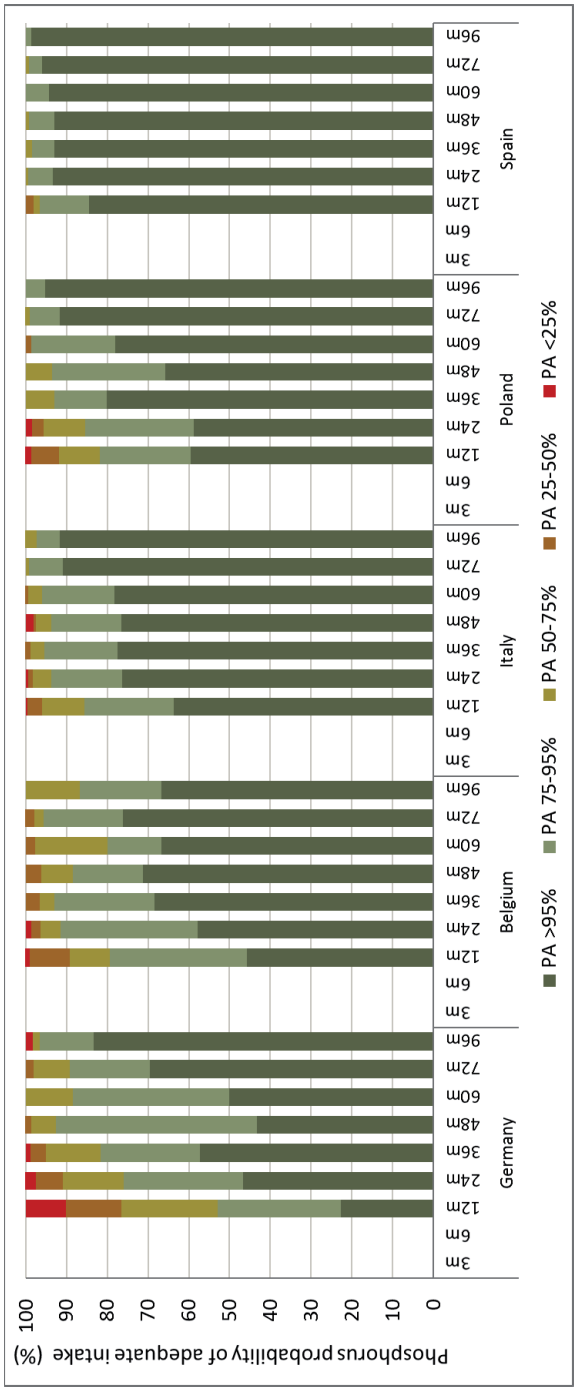


Figure 22. Phosphorus probability of adequate intake by country.

Adequacy assessed at individual level using EAR from the American Institute of Medicine[34]. There were not EAR available for ages below 12 months.

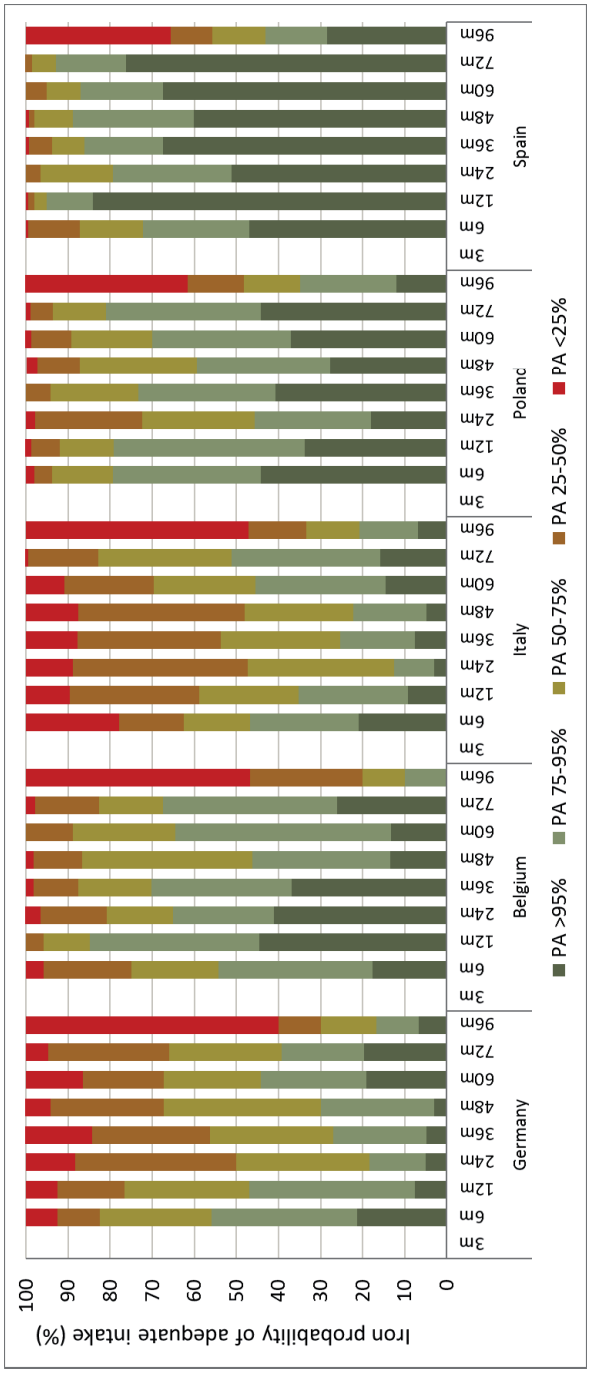


Figure 23. Iron probability of adequate intake by country.

Adequacy assessed at individual level using Estimated Average Requirements from Food and Agriculture Organization of the United Nations/World Health Organization[31]. There were not EAR available for ages below 6 months.

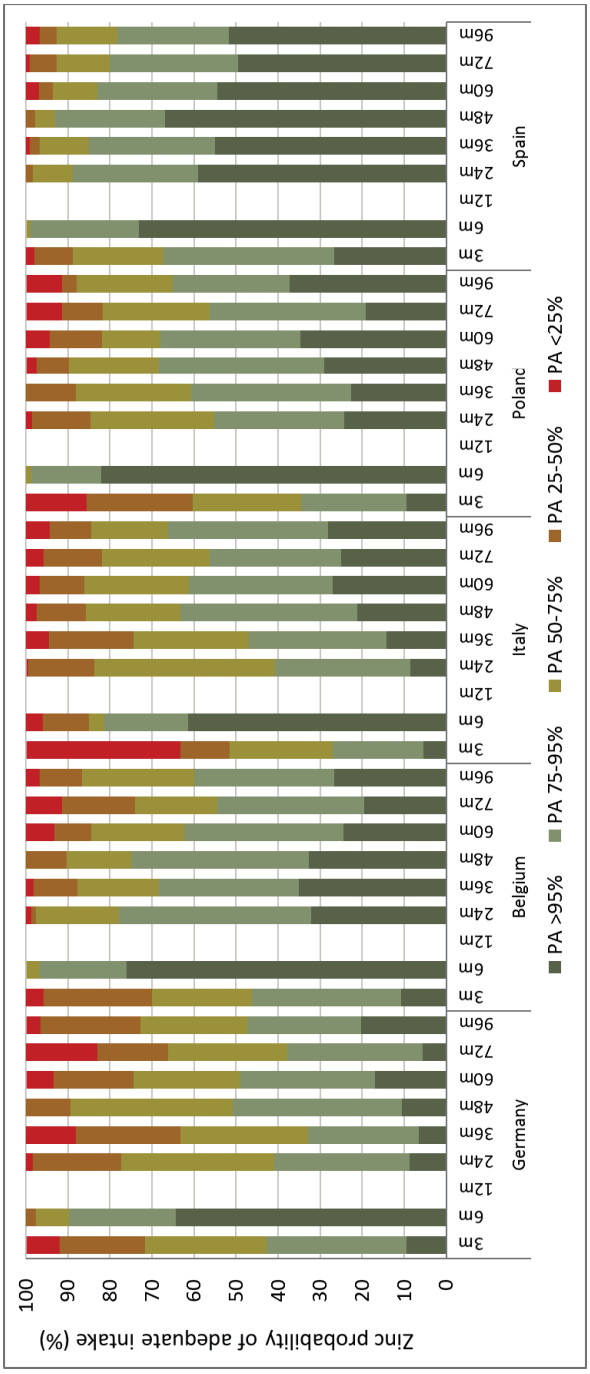


Figure 24. Zinc probability of adequate intake by country.

Adequacy assessed at individual level using Estimated Average Requirements from Food and Agriculture Organization of the United Nations/World Health Organization[31]. Assessment of probability of adequate intake could not be calculated at 12 months because zinc intake CV was >70.

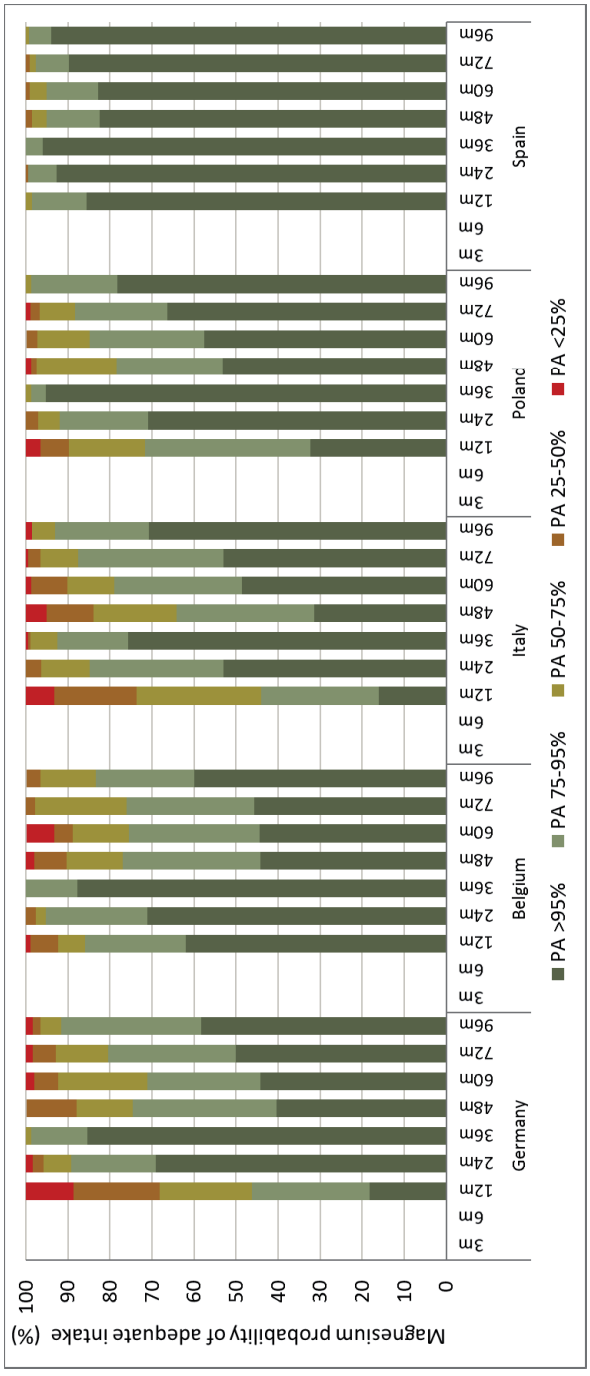


Figure 25. Magnesium probability of adequate intake by country.

Adequacy assessed at individual level using EAR from the American Institute of Medicine[34]. There were not EAR available for ages below 12 months

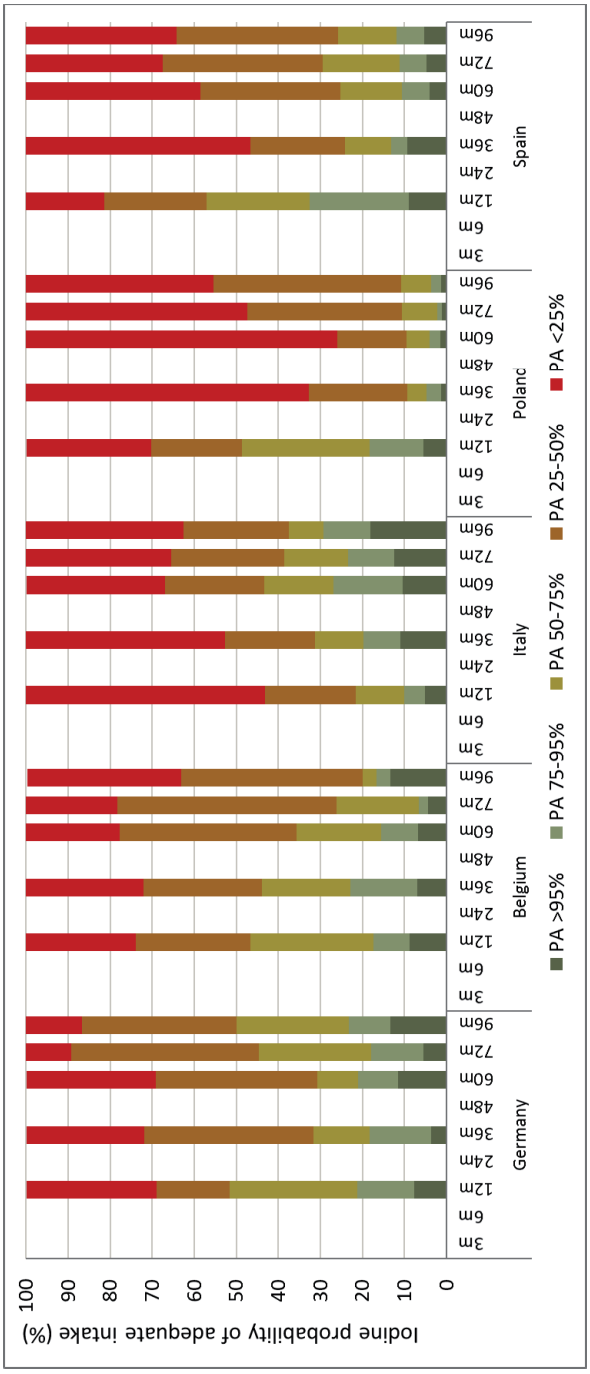
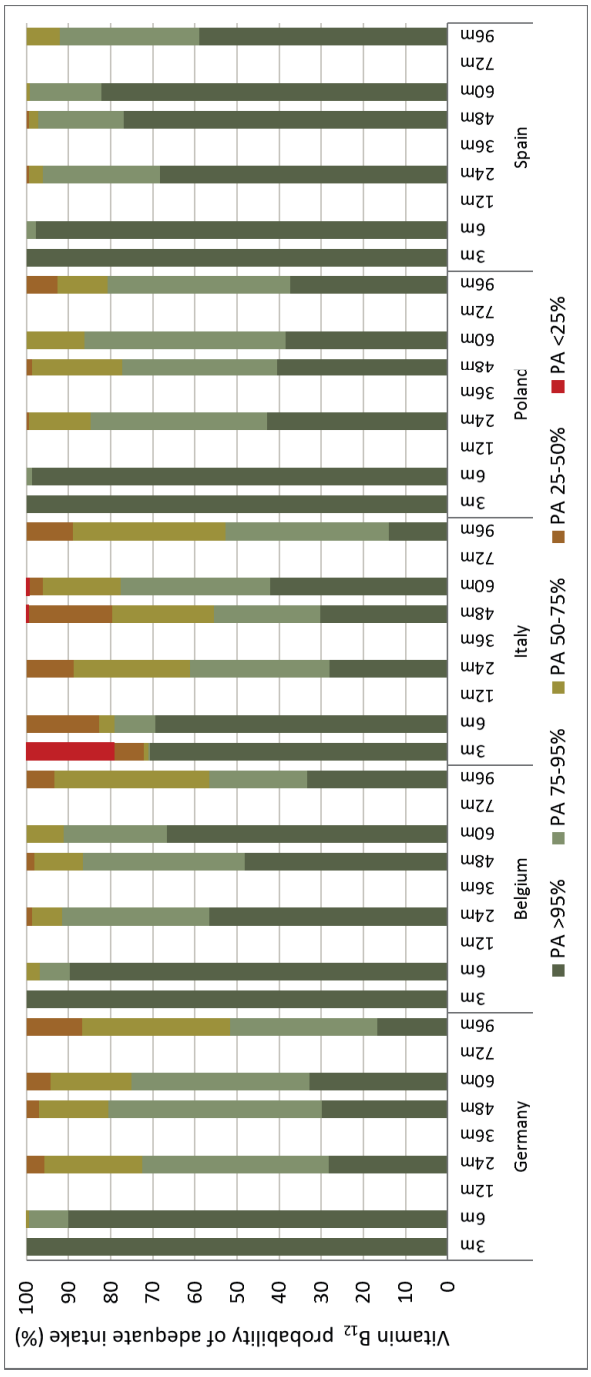


Figure 26. Iodine probability of adequate intake by country.

Adequacy assessed at individual level using EAR from the American Institute of Medicine[174]. There were not EAR available for ages below 12 months. Assessment of probability of adequate intake could not be calculated at 24 and 48 months because iodine CV of intake was <70.

Figure 27. Vitamin B_{12} (cobalamin) probability of adequate intake by country.



Adequacy assessed at individual level using Estimated Average Requirements from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31]. Assessment of probability of adequate intake could not be calculated at 24 and 48 months because vitamin B_{12} CV of intake was <70.

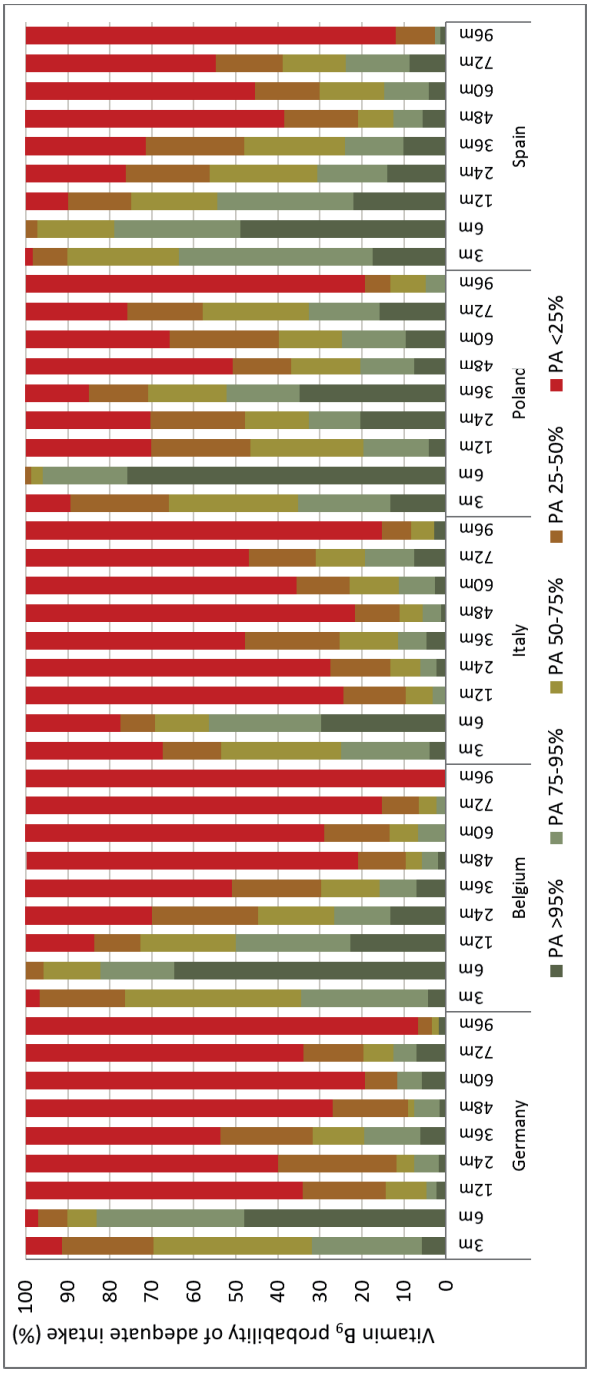


Figure 28. Vitamin B_9 (folate) probability of adequate intake by country.

Adequacy assessed at individual level using Estimated Average Requirements from Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University joint consultation[31]

4.3. CALCIUM INTAKE ASSOCIATION WITH BONE MINERAL DENSITY

4.3.1. Description of dual-energy x-ray absorptiometry results

Results of the bone mineral assessment performed in the Spanish subsample of the EU CHOP project at 7 years of age are shown in Table 30. Prevalence of children with osteopenia and low bone mineral density for age was assessed and it is shown in Table 31.

Table 30. Dual-energy X-ray Absorptiometry results.

	N	Bone mineral content (g) (mean ±SD)	Bone mineral density (g/cm^2) (mean ±SD)	Bone mineral density z-score (mean ±SD)
Lumbar Spine at L1-L4	179	18.5 ±3.0	0.70 ±0.07	-0.004 ±0.999
Whole body	179	867.4 ±125.1	0.84 ±0.04	-0.003 ±0.999

Bone mineral density z-scores were calculated internally.

Table 31. Prevalence of osteopenia and low mineral density for age.

	N	Normal bone mineralization N (%)	Osteopenia N (%)	Low mineral density for age N (%)
Lumbar Spine at L1-L4	179	152 (84.9)	23 (12.8)	4 (2.2)
Whole body	179	152 (84.9)	23 (12.8)	4 (2.2)

Normal bone mineralization: Bone mineral density z-scores >-1. Osteopenia: Bone mineral density z-scores between -1 and -2. Low mineral density for age: Bone mineral density z-scores under -2.

4.3.2. Calcium intake and bone mineral density

Associations between lumbar spine (LS) and whole body (WB) bone mineral density (BMD) and calcium intake during the previous years before DXA examination were explored.

Correlation analyses between calcium daily mean intake and bone mineral density, found significant relationships of calcium intake at 6 years of age (72 months) with lumbar spine bone mineral density and with lumbar spine bone mineral density z-score ($R=0.205$, $p=0.030$ and $R=0.203$, $p=0.031$; respectively).

Linear regression analyses were performed to assess the effect of calcium intake on lumbar spine bone mineral density z-score (LS-BMD z-score) (Table 32).

The first model showed a significant direct effect of calcium intake at 6 years of age ($p=0.031$) on the LS_BMD z-score, explaining up to 3.3% of its variability. A second model adjusting by body mass index z-score at 7 years of age ($p<0.001$), explained up to 19.4% of lumbar bone mass density z-score variability. Although BMI was the variable with the highest effect on BMD, calcium maintained its significant effect. An increase of 100 mg of calcium in the diet modified BMD z-score in 0.1 units.

Other models adjusting the effects of calcium intake at 6 years on LS-BMD z-score, by physical activity, protein, phosphorus and vitamin D intake did not improved the predictive models.

Table 32. Calcium intake effect on lumbar bone mineral density z-score.

	B	Confidence interval 95% (min, max)	p-value	R^2
Models with effect on lumbar bone mineral density z-score				
Ca intake at 6 years (mg/day)	0.001	(0.000, 0.002)	0.031	3.3
Ca intake at 6 years (mg/day)	0.001	(0.000, 0.002)	0.011	19.4
BMI z-score at 7 years	0.343	(0.202, 0.484)	<0.001	

Ca: Calcium. BMI: Body Mass Index. Each lineal regression model separated by a line. β is the effect of calcium intake on lumbar spine bone mineral density z-score, either unadjusted and adjusted by BMI model.

Neither regression models assessing the association of calcium intake at earlier ages (5 or at 4 years of age) showed any effect on LS_BMD, nor the models assessing the association of calcium intake at 6, 5 or at 4 years showed any effect on whole body mineral density z-score.

4.3.3. Calcium intake adequacy to the recommendations and bone mineral density

Associations between lumbar spine (LS) and whole body (WB) bone mineral density (BMD) and calcium adequacy to the recommendations (calculated at individual level) during the previous years before DXA examination were explored.

Correlation analysis between calcium probability of adequacy at 4, 5 or 6 years of age and BMD z-scores, did not show any significant relationship, neither at lumbar spine not at whole body level.

We grouped the children in two groups according to their calcium intake adequacy to the recommendations. In one group children with "high probability of adequate intake" (PAI>95%) and in the other, all those with PAI<95%. We also created two longitudinal variables grouping together the children with PAI>95% during two consecutive years (at 5 and at 6 years) and during three consecutive years (at 4, 5 and at 6 years). Table 33 shows the number of children in each group at each age.

Table 33. Prevalence of children with high probabilities of calcium adequate intake by age.

	N	Calcium PAI>95% N, (%)	Calcium PAI<95% N, (%)
4 years	123	86 (69.9)	37 (30.1)
5 years	110	82 (74.5)	28 (25.5)
6 years	112	89 (79.5)	23 (20.5)
5 & 6 years	94	63 (67.0)	31 (33.0)
4 & 5 & 6 years	78	42 (53.8)	36 (46.2)

Calcium PAI>95% = High probability of calcium adequate intake (Probability of adequate intake of calcium higher than 95%).

We looked for differences in bone mineral density z-scores between groups of children according to probability of adequate intake (PAI>95% and PAI<95%). At 4, at 5 and at 6 years of age, the means of LS and WB bone mineral density trend to be higher in the "high probability of adequate intake" group versus the other group, although the differences were not significant (Figure 29).

When, we analysed whether children with high probabilities of calcium adequate intake during longer periods (at 6 and at 5, or at 6, 5 and 4 years), would have higher BMD than those who only had high probability of adequate intake at one of those timepoints or at none of those timepoints, differences became significant. Children with high probabilities of calcium adequate intake at 6 and 5 years showed significantly higher LS and WB BMD z-scores compared to those that only had high probability of adequate intake at one of those ages or at any of those ages. The same happened when comparing children who reached high probabilities of calcium adequacy at 6, 5 and at 4 years of age to those who did not. Figure 29(A) summarizes differences in bone mineral density z-score according to high probability of calcium adequate intake at lumbar spine level; and Figure 29(B) at whole body level.

Figure 29. Bone mineral density z-score according to probability of adequate intake group.

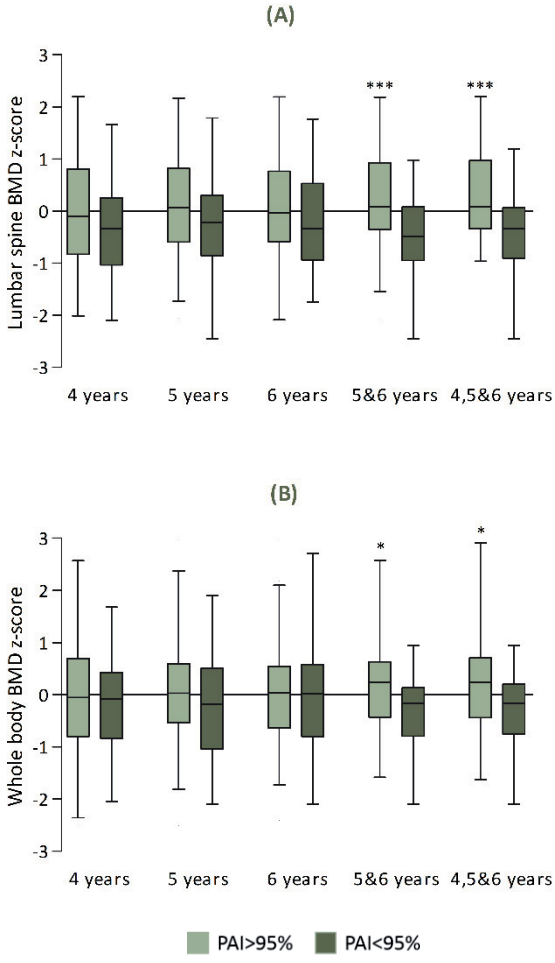


Figure A: Lumbar spine BMD z-score differences according to probability of adequate intake (PAI) group. Figure B: Whole body BMD z-score differences according to PAI group. BMD: Bone mineral density. PAI>95%= Probability of adequate intake of calcium higher than 95%. PAI<95% = Probability of adequate intake of calcium lower than 95%. *: p<0.05 and ***: p<0.001 vs. children with PAI<95%.

Differences in the factors that could influence BMD, such as protein, vitamin D or phosphorus intake, physical activity and BMI where analysed between both groups of children. At 4 and at 5 years, children with high probability of calcium adequate intake had higher intakes of protein, phosphorus and vitamin D than those with PAI<95%. At 6 years only significantly higher intakes of protein and phosphorus were found in the PAI>95% group vs. the PAI<95% group. Children with continued adequate intake probabilities over 95% at 5 and at 6 years of age also showed higher intakes of protein, phosphorus and vitamin D in comparison with those that only reached PAI>95% at 6 or at 5 years or at none. And the same happened with children with PAI>95% during three consecutive years. No differences in BMI, fat mass index and lean mass index or physical activity were found at any age.

Linear regression analyses were performed to determine the effect of calcium high probability of adequate intake on bone mineral density z-score at both, lumbar spine and whole body levels.

Having calcium high probability of adequate intake during two or during three consecutive years explains up to 26.3 and 24.9% of lumbar spine BMD variation, respectively ($p<0.0001$), when adjusting by BMI and by dietary factors (protein, phosphorus and vitamin D intake). Whole body bone mineral density variation was explained up to a 17.4 and 20.0% ($p=0.001$) when calcium high probability of adequate intake was reached during two or three consecutive years, respectively.

Table 34 details the models performed assessing the effects of calcium high prevalence of adequate intakes on lumbar spine bone mineral density, and Table 35 those assessing the effects on whole body bone mineral density.

Table 34. High probability of calcium adequate intake effect on lumbar bone mineral density z-score.

	β	Confidence interval 95% (min, max)	p-value	R^2	Model p-value
Models with effect on lumbar spine bone mineral density z-score at 7 years					
Calcium PAI>95% at 6 years [1]	0.348	(-0.116, 0.813)	0.220	1.1	ns
Calcium PAI>95% at 6 years [2]	0.407	(-0.018, 0.833)	0.061	17.2	<0.001
Calcium PAI>95% at 6 years [3]	0.313	(-0.123, 0.749)	0.158	18.6	<0.001
Calcium PAI>95% at 5 & 6 years [1]	0.711	(0.297, 1.125)	0.001	11.3	0.001
Calcium PAI>95% at 5 & 6 years [2]	0.715	(0.331, 1.098)	<0.001	23.9	<0.001
Calcium PAI>95% at 5 & 6 years [3]	0.669	(0.202, 1.137)	0.006	26.3	<0.001
Calcium PAI>95% at 4, 5 & 6 years [1]	0.760	(0.340, 1.180)	0.001	14.5	0.001
Calcium PAI>95% at 4, 5 & 6 years [2]	0.744	(0.342, 1.147)	<0.001	21.6	<0.001
Calcium PAI>95% at 4, 5 & 6 years [3]	0.773	(0.282, 1.264)	0.002	24.9	<0.001

Calcium PAI>95% = High probability of calcium adequate intake. [1]: simple model. [2]: adjusted by BMI. [3]: adjusted by BMI and dietary factors. β is the effect of having high probability on adequate intake on lumbar spine bone mineral density z-score, either unadjusted ([1]) and adjusted models ([2] and [3]).

Table 35. High probability of calcium adequate intake effect on whole body mineral density z-score.

	β	Confidence interval 95% (min, max)	p-value	R^2	Model p-value
Models with effect on whole body mineral density z-score at 7 years					
Calcium PAI>95% at 5 & 6 years [1]	0.484	(0.077, 0.891)	0.020	5.2	0.020
Calcium PAI>95% at 5 & 6 years [2]	0.490	(0.100, 0.880)	0.014	13.1	0.001
Calcium PAI>95% at 5 & 6 years [3]	0.559	(0.089, 1.028)	0.020	17.4	0.001
Calcium PAI>95% at 4, 5 & 6 years [1]	0.489	(0.082, 0.897)	0.019	6.3	0.019
Calcium PAI>95% at 4, 5 & 6 years [2]	0.480	(0.079, 0.880)	0.020	9.8	0.011
Calcium PAI>95% at 4, 5 & 6 years [3]	0.668	(0.197, 1.140)	0.006	20.0	0.001

Calcium PAI>95% = High probability of calcium adequate intake. [1]: simple model. [2]: adjusted by BMI. [3]: adjusted by BMI and dietary factors. β is the effect of having high probability on adequate intake on whole body bone mineral density z-score, either unadjusted ([1]) and adjusted models ([2] and [3]).

Later, we looked if prevalence of osteopenia and low bone mineralization for age were different according to probability of adequate intake group (PAI>95% or PAI>95%). There proportion of children with osteopenia and low mineralization for age were lower in the group of children with calcium PAI>95% than in those with calcium PAI<95% at 4, at 5, at 6 years, and during two or three consecutive years, at 5 and 6 and at 4, 5 and 6 years. The differences between groups were only statistically significant at lumbar spine level when PAI was consecutively >95% during 2 and 3 years. Figure 30(A) summarizes differences in osteopenia prevalence according to high probability of calcium adequate intake at lumbar spine level; and Figure 30(B) at whole body level.

Logistic regressions analyses were performed to determine the effect of calcium high probability of adequate intake on the risk of having osteopenia (and/or low bone mineralization for age) at both, lumbar spine and whole body levels.

Children having a calcium PAI>95% during two consecutive years (5 & 6 years) had 6.8 fold reduced risk of osteopenia at lumbar level. Models adjusted by BMI and dietary factors explained up to 48.6% of the osteopenia variation; in this model, children with calcium PAI>95% at 5 and 6 years had a 13.8 fold reduced risk. Having a calcium PAI>95% during two consecutive years (5 & 6 years) only had effects on the risk of osteopenia at whole body level when the models were adjusted by BMI and dietary factors, the decrease of risk was 12.3 fold.

Table 36 details the models performed assessing the effects of calcium high prevalence of adequate intakes on the risk of having osteopenia at lumbar and at whole body level.

Figure 30. Osteopenia prevalence according to probability of adequate intake group.

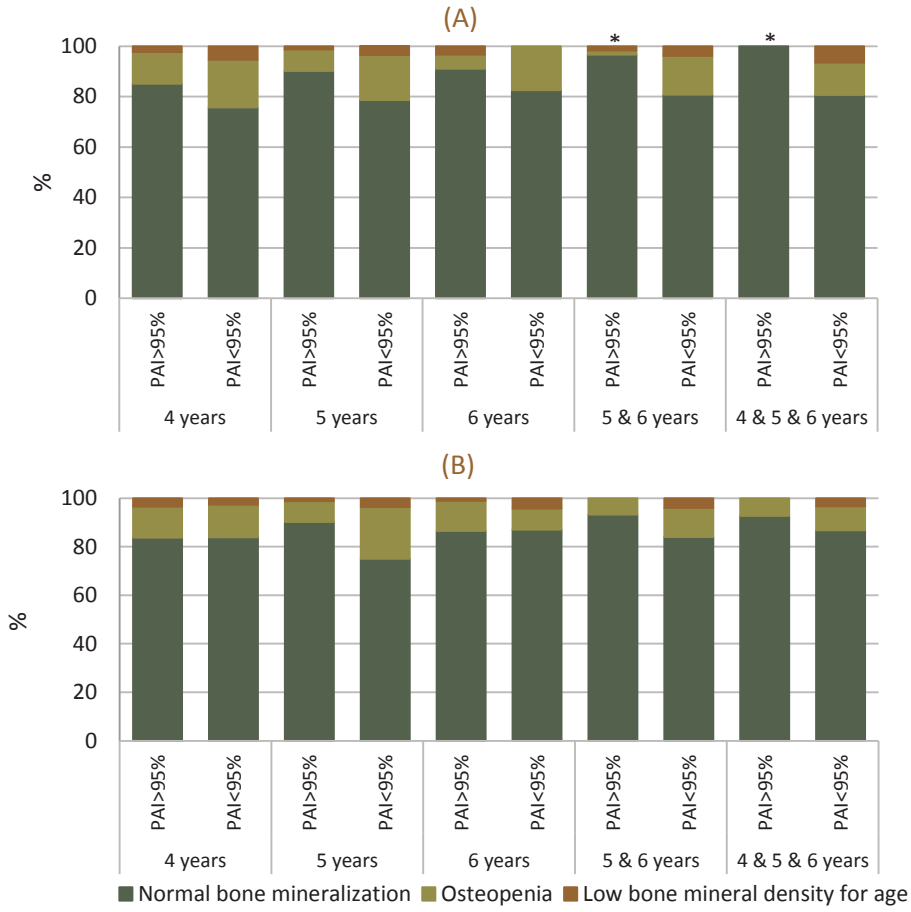


Figure A: Prevalence of lumbar spine normal mineralization, osteopenia and low mineralization for age according to probability of adequate intake group. Figure B: Prevalence of whole body normal mineralization, osteopenia and low mineralization for age according to probability of adequate intake group. PAI>95: Probability of adequate intake of calcium higher than 95%. PAI<95% = Probability of adequate intake of calcium lower than 95%. *: p<0.05 vs. children with PAI<95%.

Table 36. High probability of calcium adequate intake effect on the risk of having osteopenia.

	β	Odds ratio	Confidence interval 95% (min, max)	p-value	R^2 Nagelkerke	Model p-value
Models with effect on lumbar spine osteopenia at 7 years						
Calcium PAI>95% at 5 & 6 years [1]	1.915	6.786	(1.222, 37.685)	0.029	14.3	0.020
Calcium PAI>95% at 5 & 6 years [2]	2.351	10.497	(1.500, 73.476)	0.018	35.9	0.001
Calcium PAI>95% at 5 & 6 years [3]	2.628	13.844	(1.083, 176.979)	0.043	48.6	0.001
Models with effect on whole body osteopenia at 7 years						
Calcium PAI>95% at 5 & 6 years [1]	0.963	2.619	(0.600, 11.440)	0.201	4.0	ns
Calcium PAI>95% at 5 & 6 years [2]	1.069	2.91	(0.629, 13.475)	0.172	14.5	ns
Calcium PAI>95% at 5 & 6 years [3]	2.506	12.262	(1.007, 149.276)	0.049	29.8	0.028

Calcium PAI>95% = High probability of calcium adequate intake. [1]: simple model. [2]: adjusted by BMI. [3]: adjusted by BMI and dietary factors. β is the effect of having high probability of adequate intake on lumbar spine and whole body osteopenia, either unadjusted ([1]) and adjusted models ([2] and [3]). Odds ratio is the reduction of risk of having osteopenia reached by having a calcium PAI>95%.

Discussion

5. DISCUSSION

To our knowledge, the present study is the first describing micronutrients intake and analysing the probability of adequate intake of micronutrients, using dietary intake data of healthy children from five different European countries (Belgium, Germany, Italy, Poland and Spain), collected simultaneously and longitudinally from birth to 8 years of age, and utilising the same standardised methodology in all study centres.

Vitamins A, D, B_{12} and folate, and sodium, potassium, calcium, phosphorus, iron, zinc, magnesium and iodine intake information was obtained through 3-day food records. The same food composition tables (with some adaptation explained elsewhere in the present dissertation) were utilised by all participating centres and nutritional data was analysed together to describe the micronutrients intake and to determine the probability of adequate intake in comparison to the Dietary Reference Intakes.

This effort provides a European overview of childhood micronutrients intake and adequacy to the recommendations, obtained and assessed by unified and standardized methodology; hence, it provides a valuable resource of data for further evaluations.

5.1. METHODOLOGY USED

Epidemiological studies often obtain data from different sources and comparing those data becomes so challenging. Dietary data are usually collected with different dietary assessment methods in the different participating locations. Food frequency questionnaires (FFQ), 24h recalls, diet history and estimated food records (diaries) are the main used methods to assess dietary intake[275]. Anyway, even when the same method is used, it could be conducted so differently. For instance, in the case of 24h recall, it can be completed on a single day with little detail or instead on several days and completed with multiple passes interview to obtain as much information as possible. For FFQ, the number of items would be the determining factor[276].

Despite all methods have their weaknesses and strengths, food records have been traditionally considered as the reference method and utilised as reference for validating other dietary assessment methods. A review performed by Ortiz-Andrellucchi et al. in 2009[277] stated that validation studies of micronutrients intake measured with biomarkers gave better correlations when compared to intakes assessed with food records than to those assessed with FFQ. Other authors have also demonstrated that 3-day food records are superior to FFQ as a tool to validate nutrient intake[278]. Therefore, assessing dietary intake with food records would be a good election. In addition, food records are a prospective dietary assessment method, consequently, information collection does not rely on participants' short term or long term memory as it does in the case of 24h recall and FFQ or Diet history.

In contrast, assessing dietary intake with food records could modify spontaneous diet habit and drive to underrating and/or underreporting. Nevertheless, the European Food Safety Authority (EFSA) considers that exclusion of under- or over- reporters from the datasets introduces unknown bias; therefore recommends to report the proportion of underreporters but to preserve them in the sample for analysis. Currently most studies use the Goldberg cut-off method, explained elsewhere[263]. Mertz et al.[279] performed a study in adults evaluating the relation between energy intake calculated from diet records and real intake determined to maintain body weight, and found that an 81% of the individuals reported less energy intake than that determined to maintain weight, what was translated in a difference of 18% of the energy reported versus the real energy intake. In a review of 2009, Poslusna et al.[280] reported a percentage of underreporters from 11.9 to 44% in studies using estimated food records, and from 14.3 to 38.5% in studies with weighed food records. In that case, underreporting was translated into an energy intake underestimation of 12.2 and 18%, respectively. Our results concerning energy intake underestimation around 13% are consistent with those others consulted. Information on underreporting energy intake is quite limited; few studies aim validating reported intake of micronutrients. Poslusna et al. concluded that micronutrient misreporting goes together with misreporting of energy. Therefore, we could conclude that micronutrient underreporting in our study was around 13%, as well.

In addition, it is known that assessing dietary intake with food records is high burden and very time consuming for both the subject and the investigator, then it is necessary to optimize the number of days registered.

The number of days of dietary record needed to accurately assess nutrient intake in infants and children varies according to intra and interindividual variation of intake. In a study performed by Lanigan et al. in 2004[281] they stated that from 6 months to 2 years of age, period in which infants' diets are quite uniform by nature, the number of days needed to ensure r≥0.9 in the assessment of the intake of several micronutrients was 2. Days needed to assess energy and macronutrients were a little bit higher. And, as variation increases with age, necessary days do too, and so more days are needed in children over 2.

For the present study, we collected 3 consecutive days of food records, including one weekend and two week days. All the participating centres across Europe used the same diary format, adapted at each age (Annex I) and used the same tools to help participants to complete the records. A scale to weigh foods, a validated food picture atlas to estimate serving sizes (self-developed by the EU CHOP nutritionists) and detailed instructions to correctly complete the required information were provided to participants[265].

Season, day of the week or being a working or a holiday period are also important factors influencing collection of dietary intake data. Herein, data from 3, 6 and 12 months, and data collected once a year from 2 to 8 years is shown.

Individual intake of micronutrients could be influenced by season, but at group level we assume that this variation source was neutralised because our subjects were assessed through all the seasons. Furthermore, currently, modern food distribution and importations guarantees access to almost all food groups in countries with short growing seasons and during the whole year.

Food composition databases utilised to convert food intake into nutrient intake are also a source of variation in epidemiologic studies that get data from, for example, national surveys. Differences in dietary intake in different studies can be due to the use of different food composition data bases. In the EU CHOP we developed an own software (NutrCalc®) to convert food items into nutrients. NutrCalc software initially contained the German BLS II.3 food composition table[256] with around 10000 food items, and subsequently, study nutritionists added the nutritional composition of more than 2200 local food items, according to information from the manufacturers, local nutrition composition tables[257–262] or ingredient details avaliable in the product label. Fortified foods and products were also included in the food composition database in NutrCalc software, but we could not analyse the intake of micronutrients from foods and from fortified foods separately.

All the procedures, including the calculation of grams of food from the food records, the choice of an item from the NutrCalc, as well as the introduction of new food items into the NutrCalc original food composition database, or the review and depuration of final databases, were accurately specified by comprehensive Standard Operating Procedure manuals developed before, during and after the study period.

For this dissertation we did not consider the consumption of supplements nor medications. Anyway, there is some controversy in the importance of assessing mineral and vitamin supplements. As described recently by Mensink et al.[187] for some micronutrients, supplements consumption increased its intake markedly. However, in most cases, supplements made little difference to the proportion of individuals reaching or not dietary recommendations. Furthermore, it has been highlighted that subjects who consume supplements are normally those who are concerned about healthy lifestyle and consequently are not at risk of any deficient intake[282]. In a study performed by Murphy et al. in 2007[283], individuals who were usual consumers of supplements only showed an increase of 2% in the probability of adequate intake of micronutrients from food compared to those who were not supplement consumers. Micronutrients intake adequacy of supplement consumers considering food intake and supplement consumption together, was only an 8% higher than in those not supplemented. So, supplements consumption consideration may be important for assessing micronutrients intake, but not that much when assessing micronutrient adequacy to recommendations, and nor assessing the risk of micronutrient deficiencies.

5.2. MICRONUTRIENTS INTAKE

In this work we provide a description of European children detailed intake data for 12 micronutrients (sodium, potassium, calcium, phosphorus, iron, zinc, magnesium, iodine, vitamin B12, folate, vitamin A and vitamin D) at 3, 6, 12, 24, 36, 48, 60, 72 and 96 months of age. Furthermore, we add annex information of the intakes adjusted by energy, and separated by gender and by country.

To our knowledge, there are up to date 3 publications reporting grouped intake data from European children[187,199,200]. From those, two publications, the one from Elmadfa in 2004[199] and the metaanalysis by Mensink et al. in 2013[187], provide information of our interest for many micronutrients (Na, K, Ca, Fe, Mg, I, folate and vitamin D; and K, Ca, Fe, Zn, Mg, I, vitamin B_{12}, folate, vitamin A and vitamin D, respectively). Elmadfa study was published within the European Nutrition and Health Report of the European Academy of Nutritional Science, and it grouped together the results of several national nutrition surveys in Austria, Belgium, Denmark, Finland, Germany, Greece, Hungry; Italy, Norway, Spain and United Kingdom. Results were shown from children aged 1 to 14 years (all together). Mensink et al. metaanalysis compiled dietary survey data from Belgium, Denmark, France, Germany, The Nederlands, Poland, Spain and the United Kingdom. This metaanalysis was performed from 1 year-olds to elderly; and childhood data of our interest was provided separated by age ranges from 1 to 3 and from 4 to 10 years of age. The third publication, from Kaganov et al. in 2015[200] was a review that only included the results of Elmadfa and Mensink publications mentioned above, and only provided values of our interest for

vitamin D and folate, and only on 4 to 9 years-old children. Therefore, the 2013 metaanalysis was the most adequate to compare the results obtained in our study on micronutrients intake from 12 months onwards.

A not-published draft review from Nissenshon et al., available as part of a Report in the EURRECA project website[284], summarized micronutrient intake data during the first year of life from 6 studies in healthy full term children[285–290]. From those, the studies reporting data from the Avon Longitudinal Study of Parents and Children (ALSPAC study) at 4 and 8 months of age, published by Noble et al. in 2001 and 2006, were those with information on more nutrients, including: K, Ca, Fe, Zn, I, vitamin B_{12}, folate, vitamin A and vitamin D among others. The other four studies mentioned, all provided information on calcium and some of them on iron, zinc, vitamin C or vitamin D.

When we compared micronutrients intake in the EU CHOP at of 3 and 6 months of life to those measured in the ALSPAC study at 4 and at 8 months, we found almost exact intakes for iron, zinc and vitamin B_{12} (8.03 vs. 8.03 mg/day, 4.18 vs. 4.18 mg/day and 1.89 vs. 1.78 µg/day for iron, zinc and vitamin B_{12}, in EU CHOP vs. ALSPAC, respectively). We also found very similar intakes of potassium (1003.0 vs. 946.5 mg/day) and iodine (60.9 vs. 66.8 mg/day). The EU CHOP children showed slightly higher intake of calcium (699.2 vs. 593.5 mg/day) and vitamin D (8.43 vs. 6.80 µg/day), and in contrast, lower vitamin A (661.5 vs. 908.0 µg/day). The strong similarity found between micronutrients intake in the EU CHOP and the ASLPAC children supports the consistency of our results.

From 12 months of life onwards, we compared the intakes of micronutrients of the EU CHOP children to those reported in the supplemental tables of the metaanalysis by Mensink et al.[187]. In general, vitamin B_{12} and folate intakes were almost equal in both studies, what makes our results coherent with those published before. Zinc intake was equal in both datasets from 1 to 3 years of age, then it was slightly lower in EU CHOP sample (average exactly 5.9 mg/day in both studies at 1-3 years, and 6.8 vs. 7.6 mg/day, from 4 years in EU CHOP vs. Mensink's, respectively). Concerning calcium, our results were very similar to those reviewed for the first 3 years, later, from 4 to 8 years, our results were a little bit lower than those reported by Mensink et al. (691.4 vs. 744 mg/day at 1-3 years, and 660.9 vs. 841.2 mg/day from 4 years onwards). The other way round, magnesium intake of EU CHOP children was quite lower during the first period and then became almost equal in both samples (132.9 vs. 173.5 mg/day at 1-3 years, and 181.6 vs. 229.4 mg/day from 4 years onwards). For vitamin D, its intake was very much higher in EU CHOP children during the first 3 years of life and later became almost equal to the European mean showed in the metaanalysis (3.81 vs. 1.8 µg/day from 1-3 years, and 1.75 vs. 2.1 µg/day). Vitamin A was consumed in higher amounts by EU CHOP participants during all the period (860.2 vs. 661.0 µg/day, and 852.7 vs. 752.7 µg/day, during the first and the second age ranges, respectively), and the contrary happened with iodine (59.6 vs. 108.5 µg/day from 1-3 years, and 61.0 vs. 118.5 µg/day from 4 years onwards), in comparison with the data provided in the review.

Sodium and phosphorus were not analysed by the comprehensive review performed by Mensink et al., neither were they analysed by Noble et al. in the ALSPAC study during the first year of life. Anyway, about sodium, we must clarify that we did not register table salt consumption what did probably provide very low intakes of salt compared to the real intakes, especially from 24 months of age onwards when cultural habit of not adding salt to children meals may disappear.
Despite for vitamin A and vitamin D, and iodine from 12 months onwards, our results were very consistent in comparison to those published before.

Concerning country differences, it is relevant that public health initiatives in Europe to address common nutritional deficiencies vary by country. Differences in fortification policies are likely to explain some portion of the disparity in the intakes of some nutrients among children living in Europe[200]. For example, iodine fortification of salt is mandatory in some countries such as Denmark but voluntary in others, such as Finland or Italy. Fortification of milk with vitamin D is required or strongly encouraged in most countries but fortification of milk products or margarine with vitamin A or E is less common[200]. Consequently, in our study country differences were statistically significant for all the 12 micronutrients analysed in our study and at all timepoints. Anyway, those differences were sometimes only between one country versus all the others, sometimes three countries versus the other two, and so on. In general, for most of the nutrients, Spanish and Belgian children were those with higher intakes of micronutrients, followed by Polish and Germans, and Italians were those with lower intakes; with some exceptions, of course.

We also looked for gender differences and, after adjusting by energy intake, found that there were not micronutrient intake gender differences beyond 3 years of age. We only found some punctual differences for sodium intake at 12 and at 24 months, potassium at 12 months, zinc, folate and vitamin D at 24 months and for iodine and vitamin B_{12} at 36 months of age. In all the cases, girls were those with statistically significant higher intakes in comparison with boys. As these differences between genders were found after adjusting micronutrient intakes by energy, we could speculate that the differences in micronutrient intakes between genders at early ages, are due to the quality of diet not to the quantity.

5.3. MICRONUTRIENTS INTAKE ADEQUACY TO THE RECOMMENDATIONS

Even in regions as Europe, where nutritional deficiencies are expected to be uncommon due to the availability and average consume of fruits, fresh vegetables and proteins; dietary choices made by the individuals may lead to nutritional deficiencies, including nutritional deficiencies concurrent with excess body weight.

Many studies deal with the assessment of micronutrient intake adequacy for individual countries[291,292], or for vulnerable population either for a disease, pregnancy[293], for socio-economic position or for geographical challenging environments[112,181,294], and many others focus only on specific disease investigation where diet may have a role[16,75]. Furthermore, the comparison of dietary intake adequacy across countries is complicated by the different methods used to analyse dietary intake[277], the variety of food composition tables to calculate nutritional composition of diet, the multiplicity of dietary recommendations and the diversity of methods to assess nutrients intake adequacy available[179,180,184]. Anyway, studies comparing information from different European countries on micronutrients adequacy to the recommendations have been published.

In 2011, Roman-Viñas et al.[184] published a review assessing the prevalence of nutrient intake inadequacy in European adults. Vitamins C, D, B_{12} and folic acid, and calcium, iron, zinc, selenium, copper and iodine were considered and the Nordic and FNB-IOM DRI were utilised for the adequacy assessment. That review concluded that vitamin C, vitamin D, folate, calcium, selenium and iodine had high prevalence if inadequate intakes in

Europe, up to 20% of individuals under the EAR; what means the same that an 80% of individuals had adequate intakes (being over the EAR). That review was only focused in adults, but some studies have been focused on children.

In 2013, Mensink et al.[187] presented a metaanalysis comparing dietary survey data from 8 countries in Europe (Belgium, Denmark, France, Germany, The Nederlands, Poland, Spain and the United Kingdom) considering calcium, copper, iodine, iron, magnesium, potassium, selenium, zinc and vitamins A, B_1, B_2, B_6, folate, B_{12}, C, D and E. This review presented data from 1 year of age to elderly, separated by age ranges as follows: 1-3 years, 4-10 years, 11-17 years, 18-60 years and above 60 years. United Kingdom DRI were used when available, and Nordic DRI when needed. That review, concluded, in general, that for the studied minerals, the risk of low intakes (inadequacy prevalence >20%) was likely to appear more often in specific groups, as for instance: high prevalence of inadequate intake of calcium in Polish aged 4 to 10 years, of iodine in Polish and Germans aged 1- 10 years, of iron in Belgians and Polish aged 1 to 10 years. And that with the exception of vitamin D, which showed prevalence of inadequate intake near to the 100%, the current intakes of vitamin from foods lead to low risk of low intakes in all age and sex groups.

Another study in paediatric population was performed before by Elmadfa and published in 2004 within the European Nutrition and Health Report of the European Academy of Nutritional Sciences[197]. It grouped together the results of several national nutrition surveys and concluded that vitamin D and folate were low in children from 1 to 14 years of age in all countries

assessed (Austria, Belgium, Denmark, Finland, Germany, Greece, Hungary, Italy, Norway, Spain and United Kingdom). Insufficient potassium intakes were found in Austrian, German, Hungarian and Italian children. In all countries, calcium intake of younger children was on average higher and more sufficient than that of older children, being insufficient in Austrian, German, Hungarian, Italian and Norwegian children. Iron was low in girls at fertile age.

Focusing in our study, in one hand, giving that it was performed in five European countries, the international dietary reference intakes developed by the joint expert consultation of Food and Drug Administration, World Health Organization and United Nations University (FAO/WHO/UNU) were chosen as the reference. When FAO/WHO/UNU DRI were not available for a determined micronutrient (phosphorus, magnesium, iodine and vitamin D), those from FNB-IOM were utilised instead. We did not use the dietary recommendations from the European Food Safety Authority (EFSA), because their guidelines were limited to infants and children of 36 months of age or younger[295] and furthermore they were under construction and review during the development of our analysis.

In the other hand, as explained before in this dissertation, the adequacy of micronutrient intakes to the recommendations was assessed according to the American Institute of Medicine Methodology (IOM)[173], once at group level following the “EAR cut-point method” and once at individual level using the “quantitative EAR method for individual assessment of adequacy”.

The IOM "quantitative EAR method for individual assessment of adequacy" is only applicable to assess the adequacy of micronutrients which intake distribution is normal and its coefficient of variation is under 70. We assessed the distribution of our data with Q-Q graphs and saw that data adjusted well to the normal distribution for most of the nutrients at most timepoints. In addition, we calculated the coefficient of variation of micronutrients intake and determined that the individual method could be applied for: calcium, phosphorus, iron, zinc (except at 12 months), magnesium and iodine (except at 2 and at 4 years), and for vitamin B_{12} (except at 1, 3 and 6 years) and folate.

Therefore, we obtained 2 sets of results about adequacy of intake: one at group level and one at individual level.

5.3.1. Prevalence of adequate intakes at group level

All the review studies mentioned above deal with the assessment of adequacy at group level using the EAR cut-point method, they measured the % of individuals with micronutrient intake over (prevalence of adequacy) or under (prevalence of inadequacy) the EAR. Those nutrients with prevalence of inadequate intake over 20% (20% of individuals with intakes under the EAR) were considered to be those worrying. Consequently, those nutrients with prevalence of adequate intake over 80% were considered adequate.

In our study, results of intake adequacy at group level were obtained for calcium, phosphorus, iron, zinc, magnesium, iodine, vitamin B12, folate, vitamin A and vitamin D. Our European children had, in general, adequate intakes for all micronutrients with the exception of iron, iodine, folate and vitamin D, which showed a prevalence of adequate intake under the 80% of children at almost all ages, so were deficient. Calcium prevalence of adequate intake was high during the first year of life, later it was around the 80% during the rest of the study period, at the limit.

Iron prevalence of adequate intake was high during the first year of life, under 80% from 2 to 4 years, and around 40% at 8 years. In the case of iodine and folate, deficiency was more marked with prevalence of adequate intake between 27 and 43% for iodine during all the study, and between 17 and 40% for folate. Nevertheless, folate prevalence of adequate intake was fluctuating from around 80% during the first 6 months, through 17-40% in most of the ages in between and to 5.8% at 8 years.
Vitamin D was the most worrying micronutrient, with very low prevalence of adequate intakes. More than 99% of children had inadequate intakes from 3 to 8 years of age; more than 96% at 2 years, and 83.5% at 12 months.

Therefore, in our population, the most concerning nutrients were calcium, iron, iodine, folate and vitamin D, coinciding with Mensink et al.[187] who also found low vitamin D at all ages, and calcium, iron and iodine low adequacies although only in some countries (including Poland, Germany and Belgium). Our results were also consistent with Eldmadfa[199], who also found low vitamin D and folate, low iron and curiously, higher calcium adequacy at younger ages in comparison to older children, being inappropriate at some

ages. Although Roman-Viñas results were from adult population, they also reported high prevalence of inadequacy for calcium, iodine, folate and vitamin D[184]. Hence, our prevalence of adequacy assessment results at group level were coherent with the other adequacy assessments performed in children throughout Europe.

5.3.2. Probability of adequate intake at individual level

Probabilities of adequate intake at individual level according to the IOM "quantitative EAR method for individual assessment of adequacy", could be calculated for calcium, phosphorus, iron, zinc (except at 12 months), magnesium, iodine (except at 2 and 4 years), vitamin B12 (except at 1, 3 and 5 years) and folate. Vitamin A and D could not be assessed at individual level because coefficient of variation of the intakes were over 70, thus the method was not applicable.

From the individual assessment we obtained probability of adequate intake, and categorised the results in five probability groups: >95% of adequate intake, 75-95%, 50-75%, 25-50% and <25%. For comparison with the results at group level, we considered that the better approximation would be to consider a nutrient as consumed adequately when the number of individuals with probability of adequate intake >95% + number of individuals with probability of adequate intake between 75 and 95% was over the 80%.

Individual assessment found prevalence of adequate intake under the 80% for all the nutrients, except for phosphorus. The best adequacy results were for phosphorus, magnesium and vitamin B_{12}. Phosphorus had high proportion of children with adequate intakes at all ages. Magnesium intakes were adequate at almost all timepoints, prevalence was inadequate only at 12 months (70%). And, vitamin B_{12} intakes were adequate at almost all timepoints, and when they were not adequate, they had borderline prevalence of adequate intake, over 73%.

As happened with the assessment at group level, calcium prevalence of adequate intake was higher during the first year of life. Intake was adequate from 3 to 12 months, later, the proportion of children with high probability of adequate intake decreased to be between 60 and 70% during the rest of the study period.

Iron intake adequacy was low during all the study period, being between 30 and 65%. The lowest prevalence of iron adequate intake was at 8 years, when it decreased to a 30.8%. Zinc intake only reached acceptable adequacy of intake at 6 months of life, later on prevalence of adequate intake were around 60%, so intake was inadequate. At 3 months was the age when zinc intake was more inadequate, with a prevalence of adequate intakes of 43.4%.

Finally, as happened with the assessment of adequacy at group level, iodine and folate were two nutrients with very low proportion of high probabilities of adequate intake. Iodine intakes were frequently inadequate during all the study, with proportions of adequacy under 20% at all ages. Vitamin B_9

(folate) intakes were very inadequate during all the study period, proportion of children with high prevalence of adequate intake were under 20% at almost all timepoints.

Therefore, when assessing micronutrient intake adequacy in our population at individual level, concerns arose for calcium, iron, zinc, iodine and folate.

In general the assessment at individual level detected higher proportions of inadequate intakes than the assessment at group level; prevalence of inadequate intake was on average a 20% higher than those calculated at group level. The differences between both methods, made that for micronutrients for which intakes were borderline inadequate or only happening at some timepoints when assessed at group level, became fully inadequate with the methodology at individual level; as happened for instance for calcium, iron or zinc.

Therefore, the individual assessment of adequacy is stricter than the EAR cut-point method in detecting proportion of individuals at risk of deficiency. Consequently, when the objective is to study the effect of dietary intake adequacy of a determined nutrient on a specific health outcome, it becomes necessary to have information at individual level and being able to classify individuals into those meeting the recommendations and those not meeting the recommendations; or, what is the best approximation, classifying individuals into those with a high probability of adequate intakes and those with high probabilities of inadequate intakes.

For this thesis dissertation individual assessment was needed, hence the EAR quantitative assessment method at individual level described by the IOM[173] was chosen because it is the most accurate methodology to assess micronutrient intake adequacy, minimizing the effects of inter and intra individual variation as well as taking into account the EAR variation, so, theoretically its results are more consistent than those obtained at group level.

If we take into account that our study results obtained at individual level showed higher prevalence of inadequate intakes, we can speculate that all the studies that give adequacy data assessed at group level are underestimating the real prevalence of inadequate intake.

With both methods, the nutrients for which low prevalence of adequate intake were found were calcium, iron, zinc, iodine and folate. Vitamin A and vitamin D were not assessed for adequacy at individual level because the coefficient of variation of their intake was over 70, thus the individual IOM method was not applicable. But vitamin D was the micronutrient with lower prevalence of adequacy when assessed at group level, under 1%; so although it could not be assessed at individual level, the adequacy results at individual level can be supposed to be even worse.

- Calcium

Prevalence of calcium inadequate intakes have been described to be around 30% in children aged 4-10 years[187]. In our sample, when calcium adequacy was assessed at group level, prevalence of adequate intake was over 90% during the first year and over 80% during the rest of the study, so completely adequate.

Focusing in the most accurate methodology results, those obtained at individual level; we studied calcium adequacy of intake longitudinally from 3 months to 8 years, and found that a high proportion of the children showed high probabilities of adequate intake during the first year of life (91.3, 93.7 and 77.9% at 3, 6 and 12 months, respectively), later the proportion of children with good probability of adequacy decreased under 80% for the rest of study period. In the same direction, Elmadfa et al.[199] reported that calcium adequacy was better in younger children.

In a report of the IOM, a high percentage of the population had inadequate intakes of calcium[34], even in countries where diary consumption was encouraged[37,296,297]. This is coherent with our adequacy results calculated with the individual methodology. At individual level calcium probabilities of adequate intake were low after the first year of life, only between 60 and 70% of children had adequate intakes of calcium during the remaining study period. Germany and Poland were the countries with lower proportion of adequate intakes, being around 60%, while it was over 70% in Belgium and Italy, and over 90% in Spain. Around a 30-40% of EU CHOP study participants had inadequate intakes of calcium from 24 months of life onwards.

In fact, calcium intake of the EU CHOP children started to decrease from 6 months of life, coinciding with the introduction of complementary feeding[298]. The decrease in calcium intake was parallel in all participating countries, but calcium intake by calorie of Germans and Polish children was the lowest. Hence, we could speculate that in Germany and Poland there was a greater substitution of milk and dairy products by other foods during the complementary feeding introduction than in the other countries, what might be the cause of lowest calcium intake registered. Since milk and dairy products are rich in protein, consequently, German and Polish children diet might be lower in protein intake as well in comparison with the other countries; and in fact, according to EU CHOP study results, protein intake of Germans was the lowest (data not published, shown in ANNEX V). As published by Koletzko et al. in 2009[254], a higher consume of protein was related to higher BMI at two years of life, so, German children may have a lower BMI in comparison with the other countries. Then, if we take into account adequacy results at group level (those utilised for most publications), although calcium intake decreased from 6 months, proportion of children with calcium intakes adequate to the recommendations remained acceptable (over 80%). So, consuming lower amount of milk and dairy products from the introduction of complementary feeding, entails lower consume of protein and consequently could protect against later obesity without compromising compliance with calcium dietary recommendations. To reinforce this hypothesis, it is important to mention that Spanish participants of EU CHOP study were those with highest calcium intake and with the highest proportion of children with high probabilities of calcium adequate intake and with highest BMI as well.

In the comparison of our results with those published before, mean intakes of calcium reported by Mensink et al[187] at European level were sufficient and the proportions of intakes below the EAR were low in almost all countries except for Poland. And, if we look at our results by country we can see that Poland prevalence of high probability of adequate intake are very similar to those in Italy and Belgium, but significantly lower than those in Spain.

As it is widely known, calcium is responsible of bone structure and teeth, and it is also essential to vascular contraction and vasodilatation, muscular contraction (skeletal, smooth and heart muscle), neuronal transmission, neurotransmitters formation and glandular secretion (hormone and enzyme)[13,35–37]. It is necessary that calcium intake is well equilibrated during all growth process, especially along the first 2 years of life, the puberty as well as the adolescence too[37]. Given our results of calcium adequacy at individual level obtained from 24 months onwards, a proportion of EU CHOP children should be considered at risk. Later, in this dissertation, we will discuss the results we obtained when we studied the effects of calcium intake adequacy on bone mineral density.

- Iron

When iron adequacy was evaluated at group level, prevalence of adequacy was around 70-80% during most of the timepoints, with the exception of 8 years when it was considerably low 42.7%, being more than the half of the children showing low prevalence of adequate intake of iron. Our results at group level were more or less consistent to those presented for iron in the

metaanalysis of Mensink et. al[187] which reported between 45 to 77% of children with intakes over the EAR. With the individual assessment our results were magnified; the proportion of children with high probabilities of adequate intake ranged from 50 to 70% from 6 months to 6 years, and decreased to 30% at 8 years. This decrease of the proportion of children with adequate intakes of iron at 8 years is due to the increased iron recommendations for this pre-pubertal age. Although iron requirements increase at pre-pubertal ages and consequently dietary recommendations and intake have to be increased as well, children eating habits do not change from one day to the other. A transition period may be needed to allow children adapting their dietary intake, which was enough to cover EAR stablished for 4 to 6 years, but not enough to cover the suddenly increased EAR for 7 to 9 years-old children. Another hypothesis could be that at these ages, children main sources of protein from animal products might be milk and dairy products, which provide them high protein amounts but low iron. This can be corroborated by the adequate consume in vitamin B_{12} and vitamin A, which are found mainly in animal products such as milk and dairy a part from meat.

Infants, children, adolescents and women in childbearing age, especially pregnant women, are the population groups at highest risk of iron deficiency due to the physiologic increase of requirements. Iron deficiency anaemia is the most common nutritional deficiency in the world[13], also in children, particularly at pre-school ages. Our results on iron intake should be associated to blood analysis to determine if anaemia is present in our sample.

- Zinc

In relation to zinc, we obtained high prevalence of adequacy when it was assessed at group level, what was consistent with the results of the European metaanalysis by Mensink et al.[187] which showed very low percentages of individuals with zinc intakes under the EAR. However, our assessment at individual level, gave results around the 40% of prevalence of inadequate intakes for most timepoints, and even higher at 3 months when it was the 56.6%. There are many publications affirming that nearly half of the world's population is at risk to inadequate zinc intake[72,299,300].

Although severe zinc deficiency is uncommon in Europe, marginal insufficiency is likely to be much more prevalent[301], with associations to immune system dysfunction and restricted physical development[68]. Zinc deficiency during periods of high skeletal mineral accumulation as is pre-puberty years has also been associated with slower skeletal growth, maturation, and reduced bone mineralisation. This assumption are supported by few reports on zinc deficiency or supplementation in monkeys and humans[70,71].

We should go over our participants' medical history to check up on if children with lower probability of adequate intake have more periods of illness or infections prevalence.

- Iodine

A third part of the world population, approximately 2 billion people, is estimated to have a low iodine intake based on urinary iodine concentrations, 31.5% of school-aged children have insufficient iodine intakes.[302,303]. To reduce the risk of iodine deficiency and health related consequences, a worldwide strategy has been the intentional supply through salt iodisation, but iodine deficiency is still a problem in developing and developed countries. Iodine fortification of all food-grade salt is mandated in 120 countries, but the degree of implementation of these efforts in individual countries are unknown[84]. About 50% European population remains mildly iodine deficient, and iodine intake in other developed countries (USA, UK or Australia) has fallen in recent decades[85].

In our sample, we counted an 80 to 85% prevalence of inadequate intake of iodine when adequacy was assessed at individual level and a 60-70% at group level, thus very high proportions of inadequate intakes. This inadequacy results were much higher than those published before and mentioned above. It must be considered that our methodology had an important handicap concerning iodine adequacy assessment due that table salt addition nor the use of iodized salt were registered.

A joint FAO/WHO report detailed iodine intakes ranging from 2-3mg/day in Japan (due to seaweed and reef fish consumption) to 20-80 µg/day in some areas of Africa, Asia, Latin America and parts of Europe. Intake in Canada and the USA and some other parts of Europe, was described to be around 500 µg/day[31]. EU CHOP children intakes were estimated to be between 60 and 70 µg/day, consistent with those intakes detailed by FAO/WHO report

for some regions or European regions (20-80 µg/day), nevertheless, lower than those reported for other parts of Europe (500 µg/day). However, our results may underestimate real iodine intake, due to our methodologic limitation in assessing iodine intake. It is known that the majority of daily salt intake (75-80%) comes from processed foods, while only 12% comes from natural sources, and approximately 10% comes from salt added during food preparation or consumption[304]. Therefore, considering this 10% of salt that may come from food preparation and table salt addition, we speculate that EU CHOP children iodine consumption could be between 66 and 77 µg/day, so over the recommended 65µg/day.

Mild to moderate zinc deficiency occasions goitre and can affect cognitive development in infants and children[84,91,92] and severe iodine deficiency causes hypothyroidism, with mental and growth retardation, and can result in children born with cretinism related to maternal iodine deficiency[89]. Although in our study probabilities of inadequate intakes ranged from 60 to 85% there were not, up to date, reported cases of goitre within our sample nor of mental growth retardation. This reinforces the hypothesis that our zinc adequacy results may be biased by a methodological limitation.

- Vitamin B_9 (folate)

Folate prevalence of adequacy of our study sample were very low either when assessed at group level (ranging between 20 and 40% at most timepoints, and being under 20% at 4 years; and under 6% at 8 years), or at individual level (with values between 10 and 40% at almost all timepoints, and of 2.8% at 8 years). With both methods, the proportion of children with

high probabilities of adequate intake was less worrying at 6 months, being 88 and 76.3%, respectively). Mensink et al.[187] reported that folate inadequate intake was greater with increasing age; they gave no details about the concrete values but specified that inadequate intake prevalence ranged from 1% at 2-10 years to 63% at 11-17 years. Our inadequate intake prevalence were around 60-80% most of the time, and higher than 90% at 8 years. Thus, if it is true that folate inadequacy increases with the age, and considering that our children are quite below the ages 11 to 17, we could extrapolate that folate adequacy was worst in our sample in comparison with the metaanalysis results.

However, our results on folate intake were almost equal than those published by Mensink et al. in their metaanalysis[187] what somehow validates the correction of our folate intake assessment. The differences in adequacy between both studies may lay then on the different DRI utilised for comparison. Hence, once cancelled the possibility of a measurement error, it may be that the quality of diet of EU CHOP study participants may be the responsible of the low prevalence of adequacy intake of folate.

Folate deficiency hinders cellular synthesis and division, affecting spinal cord, a cell rapid turnover organ. Due RNA and protein synthesis are not completely impaired, big or amorphous blood cells appear (megaloblasts), resulting in megaloblastic anaemia. So, folate is needed by adults and children to produce normal blood cells and prevent from anaemia[13]. In relation to our sample, future plans may include the exploration of associations between folate adequacy of intake and blood analysis results on anaemia parameters collected at 5 and 8 years of age.

- Vitamin D

In our study we assessed vitamin D intake, thus we were able to determine the adequacy of vitamin D intake to the recommendations. However, as bioactive vitamin D highly depends on the UV exposure, we are not able to extrapolate if whether there was a risk of vitamin D deficiency or not. To determine if an individual is at risk of vitamin D deficiency it would be needed to analyse blood levels of calcidiol (25(OH)vitD), which are used as biomarker of vitamin D status, as it reflects both vitamin D dietary intake and UV exposure-derived production.

We determined that our study population had very low prevalence of vitamin D adequate intake. Recommendations were available only from the FNB-IOM and assessment could only be performed at group level because distribution of intake was skewed. Looking at group level adequacy results, vitamin D prevalence of adequate intake was very low, 16.5% at 12 months, 3.4% at 2 years, and lower than 1% at later ages. Our results were consistent with those reported for European population in the review published by Mensink et al.[187], with the proportions of population with vitamin intakes below the EAR and LRNI over the 90%. After the first year, when weaning process is supposed to be completely finished and complementary feeding completely introduced, supplemented infant milks and products may be replaced by familiar foods. Adequacy intake rates of vitamin D in our study sample were ridiculous at these age, almost all children had inadequate intakes of this vitamin, from 24 months onwards.

Unfortunately, recommendations for the first year of life (from birth to 12 months) are not available for vitamin D and we could not confirm this

hypothesis of being the replacement of supplemented infant milks the responsible of these low adequacy. Anyway, the mean vitamin D intake of the children in our study was of almost 7 µg/day the first year of life (quite more near to the 10µg/day recommended that the intake at later ages), and between 1.60 and 2.90 µg/day at later timepoints, consistent with the intake of the Australians reported by Nowson et al. in 2002[305], being of 2-3 µg/day of vitamin D from dietary sources.

In fact, it is known that skin exposure to the UVB radiation is the main source of vitamin D for human[146,147], the "sunshine vitamin"[143]. We have different sun exposures in the five different European countries taking part in EU CHOP study, so for sure, big differences could be detected assessing vitamin D blood levels. So may the vitamin D dietary recommendations be adapted to sun exposure times? However, depending on sun exposure to reach vitamin D requirements is a big deal since sun protection factors are mandatory to protect against cancerous skin damage, and so, widely used.

The consistency of our vitamin D intake and inadequacy results in comparison with those published before could drive us to speculate that vitamin D dietary recommendations may be too high considering the importance of skin synthesis of vitamin D induced by sun exposure, but we cannot confirm this because we do not have vitamin D serum determinations of calcidiol (25(OH)vitD). In fact, the current interest in vitamin D has produced an increase in vitamin D serum determinations, showing a huge prevalence of biochemical vitamin D deficiency[147]. Reports from across the world indicate that hypovitaminosis D is widespread and is re-emerging as a major health problem globally[160], particularly in

developing countries, at high latitudes and in countries where skin exposure to sunlight is discouraged[147,153]. Hence, from here we can speculate that vitamin D recommendations are adequate and it is vitamin D intake what should be improved urgently.

- Phosphorus

In our environment phosphorus does not represent a problem because it is widespread in the diet and deficiencies are very rare. Phosphorus is present in most foods as a natural component and as an additive in processed foods[46]. In fact, the consumption of phosphorus normally exceeds the nutrient requirements of most men and women[50]. Our results were consistent, being that phosphorus was the unique micronutrient within all those analysed for this thesis dissertation that had very high prevalence of adequate intake at all timepoints and with both assessment methodologies, at group and at individual level.

- Magnesium

Our results on magnesium adequacy were very good, with high proportion of individuals showing good probabilities of magnesium adequate intake either when assessed at group (84-99% of adequate intakes) or at individual level (around 80% of adequate intakes during all the study, except 67.3% at 12 months). Our results are quite consistent with Mensink et al.[187] that reported inadequacy prevalences from 2.5 to 5% at group level.

Anyway, nutritional monitoring programs have shown an inadequate dietary Mg intake in Europe and North America which leads to subclinical Mg deficiency[78]. Mg supplementation is important in the preadolescent group, given the suboptimal dietary Mg intake documented in food surveys in western countries[78]. Diseases as consequence of Mg deficiency have not been noticed to increase, maybe because they may be related to Mg insufficiency and not to a deficiency. However, low Mg status has been associated with chronic inflammatory stress conditions[306]. This inflammatory response could play a role in obesity in humans because obesity has been characterized as having a chronic low-grade inflammation component and an increased incidence of a low magnesium status[306]. We did not explore magnesium intake data relationships with anthropometric nor with inflammation results of our study, but could be interesting to do in a near future.

- Vitamin B_{12} (cobalamin)

Vitamin B_{12} intake in Europe, Canada and USA is higher than the dietary recommendations for adults, children and infants[115], only vegetarians might be at risk due that vitamin B_{12} is naturally present only in foods of animal origin[13,31,97,98]. It has been widely described that strict vegetarian individuals who consume diets completely free of animal-source foods are at high risk of vitamin B_{12} deficiency[98,100–105]; while lacto-ovo-vegetarians, who consume the vitamin in eggs, milk and other dairy products, are at lower risk of deficiency than vegans but still at greater risk of cobalamin depletion than omnivores[98,105–109].

In EU CHOP study, vitamin B_{12} intake was adequate to the recommendations, either when assessed at group or at individual level. Prevalence of adequate intake were very high at group level (over 90%), and they were borderline but still adequate (around 80%) when assessed at individual level. It is clear that animal products consumption in our environment is guaranteed. Our results at group level were consistent with those published by Mensink et al[187] in their metaanalysis, that were between 2 and 7% of intakes below EAR.

- Vitamin A

Vitamin A is a public health problem in developing countries but it is adequate in our environment[135,137,138]. According to the World health Organization (WHO), vitamin A deficiency is the first cause of childhood blindness, and is associated with reduced immune function and increased mortality risk from common childhood infections and measles in developing countries[131,136,138].

In our study, vitamin A intake was higher in comparison with other published results[187]. When we assessed vitamin A intake adequacy to the recommendations (only at group level because its distribution of intake was skewed) prevalence of adequate intake was over 95% at all ages. Our subjects prevalence of inadequate intake was then around 5%, lower than those reported by Mensink et al.[187], which ranged between 12 and 22% at 1-3 years, and between 1 and 30% at 4-10 years, being somehow higher in Belgium in comparison with other European countries.

Vitamin A as preformed retinoid is found in animal products such as human milk, whole milk, dairy products, liver and fish oils or egg yolk; and as carotenoids in green leafy vegetables, yellow vegetables and non-citrus fruits[129,130]. As mentioned above for vitamin B12, we could conclude that animal products consumption in our environment is guaranteed.

5.3.3. Country differences in adequacy

When we analysed if there were country differences in the probability of adequate intake assessed at individual level, we found that there were significant differences between countries for all micronutrients and at all timepoints; but as happened with country differences in the intake of micronutrients, those differences were sometimes only between one country versus all the others, sometimes three countries versus the other two, and so on.

In general, we found interesting to highlight some particularities, such as that Spanish children were those with highest probabilities of adequate intake for all micronutrients, what may be due to the higher energy intake of Spanish in comparison with the other countries. Following the same theory, the lower intake of energy of Italians in comparison with the other countries, may be the responsible of the specially lower probabilities of adequate intakes found in Italy for calcium, iron, zinc, vitamin B_{12} and folate at very early ages (3months) in comparison with the other countries; what must be also due the lower intake of energy of Italians given that at 3 months infants diet is very homogeneous (mostly formula fed). This differences in adequacy between countries due to energy intake differences

could be diluted if the DRI were stablished not only according to chronological age but also by kg of body weight or by energy intake.

Concerning phosphorus, it was noteworthy that although prevalence of high probability of adequate intake was high during all the study period, when data was analysed by country we detected a worst adequacy of phosphorus intake of German children at 12 months. Even though phosphorus is widespread through many food groups, it is known that in adults a 20-30% of phosphorus intake comes from milk and dairy products and another 20-30% from meat, poultry, grain products and pulses (protein rich foods)[47]; thus this could indicate that German children took smaller amounts of these foods at 12 months in comparison with the children from the other countries. Furthermore, calcium probabilities of adequate intake of Germans were also a little bit lower than in the other countries, what could support this speculation about lower consume of milk and dairy products.

5.3.4. Strengths and limitations

To our knowledge, the EU CHOP is the first study in collecting longitudinal dietary intake data (from birth to 8 years) of a big number of healthy children (from 904 at 3 months to 396 at 8 years) from five different European countries (Belgium, Germany, Italy, Poland and Spain) using an accurate and standardised common methodology in all the study centres. Furthermore, parallel examinations on growth, health and development

were performed. The EU CHOP study database brings an invaluable opportunity to explore diet relation with many health outcomes.

For this thesis work, assessment of dietary intake adequacy to the recommendations was evaluated using twice, using quite different methodologies. Once at individual level with the "quantitative EAR method for individual assessment of adequacy"; and once at group level through the "EAR cut-point method", which is the most utilised in most publications and was thus very useful for comparison of our results. The assessment at individual level gives a more realistic approximation to the probability of adequate intake of children, taking into account intra and interindividual variations of dietary intake and also standard deviation of EAR; so it would be more indicated to relate dietary intake adequacy to other health outcomes from the EU CHOP study.

Unfortunately, we did not measure calcidiol (25(OH)vitD) levels in blood, which is the best biomarker of vitamin D status, as it reflects vitamin D dietary intake and UV exposure-derived production. Although our results showed prevalences of inadequate intake of vitamin D over the 99% in most of the timepoints, we cannot conclude that there was a vitamin D deficiency through our population of study, because UVB radiation is the main source of vitamin D for human[146,147].

Concerning sodium and potassium, their adequacy of intake could not be assessed as there were not EAR available neither from FAO/WHO/UNO or FNB-IOM. However, sodium is not a nutrient of concern for deficiency risk, due that a typical modern diet provides a 10-fold excess of salt compared

with traditional diets during human evolution[85]. Furthermore, sodium intakes provided by EU CHOP study should be not considered as a reference given that neither table salt addition nor the use of iodized salt were registered.

Linked to this same methodologic limitation, iodine intakes and adequacy results of this thesis work may be biased and should be viewed with caution as iodine intake through iodine-enriched table salt addition was not registered.

We observed curious increases of inadequate intake prevalence at 8 years in several nutrients, such as iron, vitamin B_{12} and folate. In all cases this may be explained by the fact that requirements at pre-pubertal ages increase considerably and so they do the DRI established for 8 year old children. This drives us to think that may the recommendations be too high for the age due that pubertal changes have not yet started, or that maybe children eating habits do not adapt as fast as expected to this sudden increase of recommendations. A transition period may be needed to allow children to adapt their dietary intake, which had been until then enough to cover EAR stablished for 4 to 6 years to the suddenly increased EAR for 7 to 9 years and maybe require specific supplementation for those micronutrients in this age ranges.

About underreporting, in our sample there was a 13% of energy intake underreporting that could be translated into a 13% of micronutrients intake underreporting, thus into an overestimation of inadequacy rates. However, a publication of Tylavsky et al.[307] assessing micronutrient adequacy of intake, compared the results analysing complete data and excluding

underreporters and concluded than even when energy is affected by underreporting, the results on micronutrient adequacy are mantained[307]. Lower reported energy intake may or may not translate into a lower quality of diet, depending on selection of food, pertinent checking should be done in each particular scenario. Furthermore, in general, our results were consistent to those published before[187,199,200].

Reached this point, we could conclude that easy access to food does not ensure the achievement of an adequate nutrition. The coexistence of nutritional deficiencies with an obesity epidemic in childhood may have the main cause on the “good”-response to the marketing of prepared products rich in fat and with poor nutritional value.

5.4. EFFECTS OF CALCIUM INTAKE ON BONE MINERAL DENSITY

It has been widely described that calcium intake and/or calcium status is one of the factors influencing bone mineral content, together with BMI, protein, vitamin D, phosphorus and physical activity[110,147,206,212–217,220–223,230]. For this thesis dissertation the relation of calcium intake and calcium intake adequacy at different ages in childhood with bone mineral density at 7 years of age was analysed.

5.4.1. Calcium intake and bone mineral density

Many association studies between bone mineral density and calcium intakes have been performed in children. According to a review of Rizzoli et al. in 2010[230], most clinical studies suggest a positive association calcium intake - bone mass. However, after comparing 13 cross-sectional studies, Lanou et al.[228] concluded that 9 of them did not find a relationship between total dietary calcium intake and BMD or BMC in adolescent girls and women. The other 4 studies reported positive associations between dietary calcium intake and BMD in children aged 6 years or more. Lanou et al.[228] also compared 9 prospective studies addressing the influence of total calcium intake on bone mineralization in children or young adults. In this occasion, 8 studies concluded that calcium was not a predictor of BMD, no significant correlation or associations between calcium intake and BMD were found. Only one, from Lee at al. in 1993, positively associated longitudinal calcium intakes with later BMD[308].

Even considering the contrast of the previously published results, in our study we found positive calcium intake - bone mass associations. We found a significant relationship of calcium intake at 6 years with lumbar spine bone mineral density and with bone mineral density z-scores. Furthermore, it was determined that calcium intake at 6 years had an effect on lumbar spine BMD at 7 years. When the model was adjusted by BMI, a 19.4% of BMD variation was explained. An increase of 100 mg of calcium in the diet modified BMD z-score in 0.1 units. Contrarily, calcium intake was not associated nor had any effects on whole body bone mineral density.

5.4.2. Calcium intake adequacy to the recommendations and bone mineral density

However, as explained before in this dissertation, the same nutrient intake amount shall be adequate for an individual and inadequate for another, given that a nutrient requirement is different for each individual. Furthermore, to determine an exact value for nutritional requirements is impossible even for an individual, because metabolism and activity conditions change every day. So, it had sense to look for associations between calcium intake adequacy calculated at individual level and its corresponding BMD.

The study of bone mineralization was performed only in the Spanish subsample for which calcium mean probability of adequate intake was over 90% during all the study period. As proportion of children with low probability of adequate intakes was very low (5-20%) children was

categorised into two groups, in one group those with a probability of adequate intake over 95% (PAI>95%), and in the other those with PAI<95%. Although we did not find statistically significant differences in BMD between the groups, LS and WB BMD values were coherently higher in PAI>95% group vs. the others; at 4, 5 and at 6 years of age.

Children with consecutive calcium PAI>95% at 5 and at 6 years of age had significantly higher lumbar and whole body BMD ($p<0.001$ and $p=0.020$, respectively) in comparison with the other group. Considering longer periods, having consecutive calcium PAI>95% at 4, 5 and at 6 years, produced statistically significant differences in BMD at both, lumbar and whole body levels ($p<0.001$ and $p=0.019$, respectively). What lead us to conclude that not only calcium intake or calcium adequate intake are associated with BMD; having high probabilities of adequate intake during longer periods may improve BMD at lumbar and at whole body level.

Having a calcium high probability of adequate intake the year before the DXA examination had an effect on lumbar spine BMD, positively modulated by BMI and by protein intake, and explained up to 18.6% of lumbar spine BMD variation.

If we considered calcium high probability of adequate intake during two consecutive years prior to the DXA examination, the effects of calcium adequacy on BMD were even stronger, since calcium PAI>95% at 5 and at 6 years explained a 26.3% of LS BMD variation when adjusted by BMI and protein, phosphorus and vitamin D intake. Being that, the difference between having PAI>95% during 2 years or not produced a change in almost 0.7 units of lumbar spine BMD z-score. If further, calcium high probability

of adequate intake was maintained the three consecutive previous years to the DXA examination, the effects on lumbar BMD variation were of 24.9%.

Although the linear regression models assessing the effects of the longest period of consecutive PAI>95% explained less lumbar BMD variability, calcium maintained PAI>95% at 4, 5 and 6 years produced an increase of 0.8 units of BMD z-score, even better than with calcium maintained PAI>95%at 5 and 6 years. Thus, the model including longer periods of calcium adequate intake became slightly worst because the influence of the other dietary factors by which the models were adjusted had less effect on BMD, whereas calcium effect was consistently stronger.

However, having a calcium high probability of adequate intake at 6 years (the year previous to DXA examination) did not have any effect on whole body BMD. But, when we analysed calcium high probability of adequate intake during two consecutive years before the DXA examination, the effects of calcium on whole body BMD were positive, explaining a 17.4% of its variation when adjusted by BMI and protein, phosphorus and vitamin D intake. Being that, the difference between having PAI>95% during 5 and 6 years or not, produced a change in almost 0.6 units of whole body BMD z-score. If further, calcium high probability of adequate intake was maintained during the three consecutive previous years to the DXA examination, the effects on BMD variation were of 20.0%, producing an increase of almost 0.7 units of whole body bone mineral density z-score.

Thus, calcium high probability of adequate intake effects on lumbar spine and whole body BMD were better when PAI>95% was maintained during

long periods of time. From here we could conclude that calcium absorption into the bone is a continuous and dynamic process and it is necessary to have a longitudinal view of calcium intake over time. To detect changes in bone mineral density calcium intake needs to be adequate during long periods of time, not only at punctual timepoint. Furthermore, we found a slight different effect of having calcium PAI>95% on lumbar spine and whole body mineral density; while having calcium PAI>95% only at 6 years had a significant effect on the lumbar spine, it was necessary having such calcium PAI>95% during at least two years to affect whole body BMD. This is consistent with the physiological bone mineralization of the different body areas, since it is well known that lumbar spine is the area of the body with the fastest bone turnover, where recent changes in bone mineralization can be observed earlier[309].

These results were consistent with a review published by Abrams in 2010, in which it is explained how DXA evaluation was used to assess changes in total body calcium. Changes in calcium absorption occurred slowly and needed to be measured over separated months[310]. In addition, in a longitudinal study from Lee at al. in 1993, performed in Chinese children from birth to five years, cumulative calcium intake from birth to 5 years was positively associated with BMD measured at 5 years; however, current calcium intake at 5 years was not associated with BMD[308].

5.4.3. Early calcium intake influence on bone mineral density

There are two critical periods in the bone mass peak obtainment, during the three first years of life and in puberty. If bone mass accumulation is deficient during the first period of life, BMD defects may be balanced later on[33]. Nevertheless, if BMD defects occur during puberty, they may produce an osteoporosis risk, difficult to compensate[33].

We looked for associations between calcium intake during early life and BMD at 7 years of age and did not find any relationship (data not shown). Anyway, we did not expect to find any relationship at early ages due that proportion of children with calcium high probabilities of adequate intake was very high, almost a 90% of children had probability of adequate intake over 95%.

To follow-on our sample evolution it would be needed to perform another DXA evaluation during puberty. At the time of this writing DXA measurement had been repeated in our study sample at 11 years of age, but data was still not available for analysis.

In addition, it is not clear that the inherent bioavailability of calcium in currently used infant formulas is substantially different than from human milk. Because the intake of calcium is much higher from all infant formulas marketed in the United States, the net calcium absorption and retention is higher in formula-fed infants[310] (an average retention of 100mg daily calcium accretion during exclusive breast-feeding and 140 mg daily on mixed feedings can be estimated). Thus children formula-fed children might

have better BMD. Furthermore, in 2011, Jones[311] mentioned that breastfed children may have lower bone mass than bottle-fed children at early ages, but longer-term studies suggest that they have higher bone mass (size adjusted) by age 8 years, especially in children born at term.

As our study design included two groups of formula-fed children and a group of breastfed children, we compared BMD at 7 years within those groups but we did not find any difference, neither between formula-fed and breastfed infants nor between infants fed higher protein and those fed lower protein formulas. So being breastfed did not program to have higher BMD later in life (we did not assess BMD at early ages), neither did the intake of higher amounts of protein (data not shown).

5.4.4. Analysis of osteopenia and low bone mineral density for age

Osteoporosis is an important health problem throughout the world. About 33% of women and 20% of men from the age of 50 onwards undergo some kind of fracture[251]. Given that the BMD along life depends upon the peak bone mass reached by the end of the second decade of life, osteoporosis is not only an adulthood and elderly concern; it might be prevented from childhood, guaranteeing the adequate conditions to ensure the development of the best bone mass possible[252].

During childhood osteopenia tends to be asymptomatic, thus it is difficult to identify the affected patients. The primary sign of osteopenia is the occurrence of fractures after light traumas or during daily activity[245]. In the

Spanish subsample of EU CHOP study, we performed DXA evaluation to 179 7 years old healthy children, and we detected a 12.8% of children with osteopenia and a 2.2% of children with low bone mineral density for age at both, lumbar and whole body levels. This data has to be considered in the context of internal BMD z-scores.

Then we looked if prevalence of osteopenia was different according to calcium intake adequacy, and found that prevalence of osteopenia at lumbar spine and whole body trend to be lower among children with calcium PAI>95% at 4, at 5 and at 6 years, and among those with maintained calcium PAI>95% during the period from 5 to 6 and from 4 to 6 years, than among those with calcium PAI<95% at the same ages. Differences were only statistically significant at lumbar level between children with and without calcium PAI>95% during 5 and at 6 years.

Binary logistic regression analyses adjusted by BMI and dietary factors did not show any effect on the risk of having osteopenia when calcium high adequacy of intake was only maintained during one timepoint. However, children with calcium PAI>95% maintained during the period from 5 to 6 years showed more than 13 fold reduced risk of having osteopenia at lumbar level and a reduction around 12 fold in whole body. So, having longer periods of high probability of calcium adequate intake may reduce the risk of osteopenia.

Conclusions

6. CONCLUSIONS

On dietary intake adequacy to the recommendations

- The consumption of phosphorus, magnesium, vitamin B_{12} and vitamin A among European children was adequate to dietary reference intakes for their age.

- Micronutrients with high prevalence of inadequate intake among European children and on which we should concern are calcium, iron, zinc, folate and vitamin D. Being folate and vitamin D those with worst adequacy levels.

- Dietary intake of iodine and iodine intake adequacy to dietary recommendations were very low, without considering iodised salt addition.

- Spanish children are those with highest micronutrient intakes and thus highest prevalence of adequate intakes.

On calcium effects on bone health

- Calcium intake during childhood affects lumbar and whole body bone mineral density at 7 years.
- Having probability of adequate calcium intakes above 95% maintained during 2 and 3 years (at 5 and 6, and at 4, 5 and 6 years) is associated to better lumbar spine and whole body BMD at 7 years.
- Calcium supply need to be measured during long periods previously to the DXA evaluation to detect visible changes in bone mineralization.
- Associations of calcium high prevalence of intake are visible earlier on lumbar bone than on whole body bone.
- Early feeding in infancy, neither breast feeding, protein intake nor calcium intake, did not program to have higher BMD in later childhood.
- Having probability of adequate calcium intakes above 95% maintained during 2 years (at 5 and 6years) may reduce in 13 fold the risk of osteopenia at lumbar level and in 12 fold the risk of osteopenia at whole body level.

Future Plans

7. FUTURE PLANS

This thesis dissertation was the first exploration of micronutrient intake data collected within the EU CHOP study from birth to 8 years of age.

The first step was to describe micronutrient intakes, the second to analyse if micronutrient intakes of EU CHOP children were adequate to Dietary Reference Intakes, and from here onwards, the possibility of further analysis was huge. We started studying the relationships of calcium intake on bone mineral density, but considering all data available, future analysis plans may be uncountable.

EU CHOP study has followed-on this cohort of European children during 11 years, focusing in metabolic programming, as well as in obesity risk factors and mental development. Abundant data is still waiting to be explored; and looking for relations of our longitudinal dietary intake data with all the other health outcomes assessed could take a great part of my professional future.

Our immediate future plans are:

- To determine food sources of micronutrients in our study sample and to study the different food patterns in relation to other health outcomes assessed.
- To explore iron, iodine, zinc and folate intakes in relation to children mental performance.
- To explore iron, folate and vitamin B12 adequacy in relation to blood analysis results on anaemia parameters.

References

8. REFERENCES

1. Beauman C, Cannon G, Elmadfa I, Glasauer P, Hoffmann I, Keller M, et al. The principles, definition and dimensions of the new nutrition science. Public Health Nutr. Cambridge Journals Online; 2005;8(6a):695–8.

2. Herman D, Taylor Baer M, Adams E, Cunningham-Sabo L, Duran N, Johnson D, et al. Life Course Perspective: Evidence for the Role of Nutrition. Matern Child Heal J. Springer US; 2013;1–12.

3. Abete I, Cuervo M, Alves M, Martinez JA. Fundamentos de Nutricion. In: Federacion Española de Sociedades de Nutricion Alimentacion y Dietetica (FESNAD), editor. Ingestas Dieteticas de Referencia (IDR) para la poblacion española. Pamplona: Ediciones Universidad de Navarra S.A; 2010. p. 15–74.

4. World Health Organization. Basic definitions on nutrition [Internet]. [cited 2012 Nov 5]. Available from: http://www.who.int/topics/nutrition/en/

5. World Health Organization. WHO Nutrition. Micronutrients. [Internet]. 2015 [cited 2015 May 19]. Available from: http://www.who.int/nutrition/topics/micronutrients/en/

6. Gil Hernández A, Sánchez de Medina Contreras F. Funciones y metabolismo de los nutrientes. In: Gil Hernández A, editor. Tratado de Nutrición Tomo 1: Bases Fisiológicas y Bioquimicas de la Nutrición. Madrid: Acción Médica; 2005. p. 19–52.

7. Golden MHN, Golden BE. Trace elements. Potential importance in human nutrition with particular reference to zinc and vanadium. Br Med Bull. 1981 Jan 1;37(1):31–6.

8. López Novoa JM. Metabolismo hidromineral: agua y electrólitos. In: Gil Hernández Á, editor. Tratado de Nutrición Tomo 1: Bases Fisiológicas y Bioquimicas de la Nutrición. Madrid: Acción Médica; 2005. p. 825–64.

9. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ. 2013 Jan;346:f1326.

10. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. Int J Epidemiol. 2009 Jun;38(3):791–813.

11. Anderson CAM, Appel LJ, Okuda N, Brown IJ, Chan Q, Zhao L, et al. Dietary sources of sodium in China, Japan, the United Kingdom, and the United States, women and men aged 40 to 59 years: the INTERMAP study. J Am Diet Assoc. 2010 May;110(5):736–45.

12. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr. 1984 Oct;40(4):786–93.

13. Food and Nutrition Board. Institute of Medicine., Otten JJ, Pitzi Hellwig J, Meyers LD. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Otten JJ, Pitzi Hellwig J, Meyers LD, editors. Washington, D.C.: National Academies Press; 2006. 560 p.

14. Caregaro L, Di Pascoli L, Favaro A, Nardi M, Santonastaso P. Sodium depletion and hemoconcentration: overlooked complications in patients with anorexia nervosa? Nutrition. 2005 Apr;21(4):438–45.

15. Elliott P, Stamler J, Nichols R, Dyer AR, Stamler R, Kesteloot H, et al. Intersalt revisited: further analyses of 24 hour sodium excretion and blood pressure within and across populations. Intersalt Cooperative Research Group. BMJ. 1996 May 18;312(7041):1249–53.

16. Geleijnse JM, Grobbee DE, Hofman A. Sodium and potassium intake and blood pressure change in childhood. BMJ. 1990 Apr 7;300(6729):899–902.

17. Cutler JA, Follmann D, Allender PS. Randomized trials of sodium reduction: an overview. Am J Clin Nutr. 1997 Feb;65(2 Suppl):643S – 651S.

18. He FJ, MacGregor GA. How far should salt intake be reduced? Hypertension. 2003 Dec;42(6):1093–9.

19. He FJ, Li J, Macgregor GA. Effect of longer-term modest salt reduction on blood pressure. Cochrane database Syst Rev. 2013 Jan;4:CD004937.

20. Khan E, Spiers C, Khan M. The heart and potassium: a banana republic. Acute Card Care. 2013 Mar;15(1):17–24.

21. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ. 2013 Jan;346:f1378.

22. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, et al. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr. 2005 Feb;81(2):341–54.

23. American Medical Association. Research letters. Suboptimal potassium intake and potential impact on population blood pressure. Arch Intern Med. 2010 Sep 13;170(16):1501–10.

24. Energy and Nutrient Intake in the European Union. Ann Nutr Metab. 2004;48(suppl 2(Suppl. 2):8–10.

25. D'Elia L, Barba G, Cappuccio FP, Strazzullo P. Potassium intake, stroke, and cardiovascular disease a meta-analysis of prospective studies. J Am Coll Cardiol. 2011 Mar 8;57(10):1210–9.

26. FAO/WHO. Diet, Nutrition and the Prevention of chronic Disease. Report of the joint WHO/FAO expert consultation. In: World Health Organization, editor. WHO Technical Report Series Num 916. TRS916 ed. Geneva; 2003.

27. Houston MC. The importance of potassium in managing hypertension. Curr Hypertens Rep. 2011 Aug;13(4):309–17.

28. Cappuccio FP, MacGregor GA. Does potassium supplementation lower blood pressure? A meta-analysis of published trials. J Hypertens. 1991 May;9(5):465–73.

29. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens. 2003 Jul;17(7):471–80.

30. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, et al. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. JAMA. 1997 May 28;277(20):1624–32.

31. FAO/WHO. Vitamin and mineral requirements in human nutrition. 2nd edition. Bangkok, Thailand; 2004.

32. Pérez Llamas F, Garaulet Aza M, Gil Hernández A, S. ZN. Calcio, fósforo, magnesio y flúor. Metabolismo óseo y su regulación. In: A. Gil Hernández, editor. Tratado de Nutrición Tomo 1: Bases Fisiológicas y Bioquimicas de la Nutrición. Madrid: Acción Médica; 2010.

33. Alonso Franch M, Redondo Del Río MP, Suárez Cortina L. [Nutrition and bone health in children]. An Pediatr (Barc). 2010 Jan;72(1):80.e1–11.

34. Food and Nutrition Board. Institute of Medicine. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 1997. 448 p.

35. Krey JF, Dolmetsch RE. Molecular mechanisms of autism: a possible role for Ca2+ signaling. Curr Opin Neurobiol. 2007 Feb;17(1):112–9.

36. Lohmann C. Calcium signaling and the development of specific neuronal connections. Prog Brain Res. 2009 Jan;175:443–52.

37. Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, et al. Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr. 2010 Apr;140(4):817–22.

38. Wardlaw G.M., Hampl J.S., Disilvestro R.A. Perspectivas en nutrición. 6th ed. México: Mc Graw-Hill; 2004.

39. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. FASEB J. 2000 Jun;14(9):1132–8.

40. Zemel MB, Richards J, Milstead A, Campbell P. Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res. 2005 Jul;13(7):1218–25.

41. Zemel MB. Proposed role of calcium and dairy food components in weight management and metabolic health. Phys Sportsmed. 2009 Jun;37(2):29–39.

42. Hanks LJ, Casazza K, Willig AL, Cardel MI, Beasley TM, Fernandez JR. Associations among calcium intake, resting energy expenditure, and body fat in a multiethnic sample of children. J Pediatr. 2010 Sep;157(3):473–8.

43. Whiting SJ, Wood RJ. Adverse effects of high-calcium diets in humans. Nutr Rev. 1997 Jan;55(1 Pt 1):1–9.

44. Daly RM, Ebeling PR. Is excess calcium harmful to health? Nutrients. 2010 May;2(5):505–22.

45. Bergman C, Gray-Scott D, Chen J-J, Meacham S. What is next for the Dietary Reference Intakes for bone metabolism related nutrients beyond calcium: phosphorus, magnesium, vitamin D, and fluoride? Crit Rev Food Sci Nutr. 2009 Feb;49(2):136–44.

46. Uribarri J, Calvo MS. Dietary phosphorus intake and health1-3. Am J Clin Nutr. 2014 Feb;99(2):247–8.

47. Calvo MS, Park YK. Changing phosphorus content of the U.S. diet: potential for adverse effects on bone. J Nutr. 1996 Apr;126(4 Suppl):1168S – 80S.

48. Karp H, Ekholm P, Kemi V, Itkonen S, Hirvonen T, Närkki S, et al. Differences among total and in vitro digestible phosphorus content of plant foods and beverages. J Ren Nutr. 2012 Jul;22(4):416–22.

49. Moe SM, Zidehsarai MP, Chambers MA, Jackman LA, Radcliffe JS, Trevino LL, et al. Vegetarian compared with meat dietary protein source and phosphorus homeostasis in chronic kidney disease. Clin J Am Soc Nephrol. 2011 Mar;6(2):257–64.

50. Calvo MS, Uribarri J. Public health impact of dietary phosphorus excess on bone and cardiovascular health in the general population. Am J Clin Nutr. 2013 Jul;98(1):6–15.

51. Uribarri J. Dietary phosphorus and kidney disease. Ann N Y Acad Sci. 2013 Oct;1301:11–9.

52. Yamamoto KT, Robinson-Cohen C, de Oliveira MC, Kostina A, Nettleton JA, Ix JH, et al. Dietary phosphorus is associated with greater left ventricular mass. Kidney Int. 2013 Apr;83(4):707–14.

53. Chang AR, Lazo M, Appel LJ, Gutiérrez OM, Grams ME. High dietary phosphorus intake is associated with all-cause mortality: results from NHANES III. Am J Clin Nutr. 2014 Feb;99(2):320–7.

54. Ruan L, Chen W, Srinivasan SR, Xu J, Toprak A, Berenson GS. Relation of serum phosphorus levels to carotid intima-media thickness in asymptomatic young adults (from the Bogalusa Heart Study). Am J Cardiol. 2010 Sep 15;106(6):793–7.

55. Kendrick J, Ix JH, Targher G, Smits G, Chonchol M. Relation of serum phosphorus levels to ankle brachial pressure index (from the Third National

Health and Nutrition Examination Survey). Am J Cardiol. 2010 Aug 15;106(4):564–8.

56. Tuttle KR, Short RA. Longitudinal relationships among coronary artery calcification, serum phosphorus, and kidney function. Clin J Am Soc Nephrol. 2009 Dec;4(12):1968–73.

57. Block GA, Ix JH, Ketteler M, Martin KJ, Thadhani RI, Tonelli M, et al. Phosphate homeostasis in CKD: report of a scientific symposium sponsored by the National Kidney Foundation. Am J Kidney Dis. 2013 Sep;62(3):457–73.

58. Calvo MS, Kumar R, Heath H. Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. J Clin Endocrinol Metab. 1990 May;70(5):1334–40.

59. Georgieff MK. The role of iron in neurodevelopment: fetal iron deficiency and the developing hippocampus. Biochem Soc Trans. 2008 Dec;36(Pt 6):1267–71.

60. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007 Apr;85(4):931–45.

61. Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E, et al. Iron deficiency anemia and iron therapy effects on infant developmental test performance. Pediatrics. 1987 Jun;79(6):981–95.

62. Burden MJ, Westerlund AJ, Armony-Sivan R, Nelson CA, Jacobson SW, Lozoff B, et al. An event-related potential study of attention and recognition memory in infants with iron-deficiency anemia. Pediatrics. 2007 Aug;120(2):e336–45.

63. Gibson RS. Zinc: the missing link in combating micronutrient malnutrition in developing countries. Proc Nutr Soc. 2006 Feb;65(1):51–60.

64. Kumar P, Lal NR, Mondal AK, Mondal A, Gharami RC, Maiti A. Zinc and skin: a brief summary. Dermatol Online J. 2012 Mar;18(3):1.

65. Kawade R. Zinc status and its association with the health of adolescents: a review of studies in India. Glob Health Action. 2012 Jan;5:7353.

66. Levenson CW, Morris D. Zinc and neurogenesis: making new neurons from development to adulthood. Adv Nutr. 2011 Mar;2(2):96–100.

67. Bhatnagar S, Taneja S. Zinc and cognitive development. Br J Nutr. 2001 May;85 Suppl 2:S139–45.

68. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr. 1998 Aug;68(2 Suppl):447S – 463S.

69. Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, et al. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 2004 Mar;25(1 Suppl 2):S99–203.

70. Bouglé DL, Sabatier J-P, Guaydier-Souquières G, Guillon-Metz F, Laroche D, Jauzac P, et al. Zinc status and bone mineralisation in adolescent girls. J Trace Elem Med Biol. 2004 Jan;18(1):17–21.

71. King J. Does poor zinc nutriture retard skeletal growth and mineralization in adolescents? Am J Clin Nutr. 1996 Sep 1;64(3):375–6.

72. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2002 Jun;75(6):1062–71.

73. Bhutta ZA, Black RE, Brown KH, Gardner JM, Gore S, Hidayat A, et al. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. J Pediatr. 1999 Dec;135(6):689–97.

74. Fischer Walker C, Black RE. Zinc and the risk for infectious disease. Annu Rev Nutr. 2004 Jan;24:255–75.

75. Black MM. The evidence linking zinc deficiency with children's cognitive and motor functioning. J Nutr. 2003 May;133(5 Suppl 1):1473S – 6S.

76. Volpe SL. Magnesium in disease prevention and overall health. Adv Nutr. 2013 May;4(3):378S – 83S.

77. Witkowski M, Hubert J, Mazur A. Methods of assessment of magnesium status in humans: a systematic review. Magnes Res. 2011 Dec;24(4):163–80.

78. Castiglioni S, Cazzaniga A, Albisetti W, Maier JAM. Magnesium and osteoporosis: current state of knowledge and future research directions. Nutrients. 2013 Aug;5(8):3022–33.

79. Kass L, Weekes J, Carpenter L. Effect of magnesium supplementation on blood pressure: a meta-analysis. Eur J Clin Nutr. 2012 Apr;66(4):411–8.

80. Houston M. The role of magnesium in hypertension and cardiovascular disease. J Clin Hypertens (Greenwich). 2011 Nov;13(11):843–7.

81. Dibaba DT, Xun P, He K. Dietary magnesium intake is inversely associated with serum C-reactive protein levels: meta-analysis and systematic review. Eur J Clin Nutr. 2014 Apr;68(4):510–6.

82. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel DP. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr. 1999 Apr;69(4):727–36.

83. Wang MC, Moore EC, Crawford PB, Hudes M, Sabry ZI, Marcus R, et al. Influence of pre-adolescent diet on quantitative ultrasound measurements of the calcaneus in young adult women. Osteoporos Int. 1999 Jan;9(6):532–5.

84. Leung AM, Braverman LE, Pearce EN. History of U.S. iodine fortification and supplementation. Nutrients. 2012 Nov;4(11):1740–6.

85. Campbell NRC, Dary O, Cappuccio FP, Neufeld LM, Harding KB, Zimmermann MB. Need for coordinated programs to improve global health by optimizing salt and iodine intake. Rev Panam Salud Publica. 2012 Oct;32(4):281–6.

86. Aburto NJ, Abudou M, Candeias V, Wu T. Effect of salt iodization to prevent iodine deficiency disorders: a systematic review with meta-analyses. World health Organization, editor. Geneva, Switzerland: World Health Organization; 2014. 142 p.

87. Leung AM, Braverman LE. Consequences of excess iodine. Nat Rev Endocrinol. 2014 Mar;10(3):136–42.

88. World Health Organization, Unicef. Iodine deficiency in Europe; a continuing public health problem. Andersson M, de Benoist B, Darnton-Hill I, Delange F, editors. Geneva, Switzerland: World Health Organization - Unicef; 2007. 30 p.

89. Zimmermann MB. Iodine deficiency. Endocr Rev. 2009 Jun;30(4):376–408.

90. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. Lancet. 2008 Oct 4;372(9645):1251–62.

91. Melse-Boonstra A, Jaiswal N. Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development. Best Pract Res Clin Endocrinol Metab. 2010 Feb;24(1):29–38.

92. Bougma K, Aboud FE, Harding KB, Marquis GS. Iodine and mental development of children 5 years old and under: a systematic review and meta-analysis. Nutrients. 2013 Apr;5(4):1384–416.

93. Ghirri P, Lunardi S, Boldrini A. Iodine supplementation in the newborn. Nutrients. 2014 Jan;6(1):382–90.

94. Wolff J, Chaikoff IL. Plasma inorganic iodide as a homeostatic regulator of thyroid function. J Biol Chem. 1948 Jun;174(2):555–64.

95. Galofré JC, Fernández-Calvet L, Ríos M, García-Mayor R V. Increased incidence of thyrotoxicosis after iodine supplementation in an iodine sufficient area. J Endocrinol Invest. 1994 Jan;17(1):23–7.

96. Todd CH, Allain T, Gomo ZA, Hasler JA, Ndiweni M, Oken E. Increase in thyrotoxicosis associated with iodine supplements in Zimbabwe. Lancet. 1995 Dec 9;346(8989):1563–4.

97. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr. 2004 Jan;24:299–326.

98. Allen LH. Causes of vitamin B12 and folate deficiency. Food Nutr Bull. 2008 Jun;29(2 Suppl):S20–34; discussion S35–7.

99. Morris MC. Nutritional determinants of cognitive aging and dementia. Proc Nutr Soc. Cambridge Journals Online; 2012 Feb;71(01):1–13.

100. Miller DR, Specker BL, Ho ML, Norman EJ. Vitamin B-12 status in a macrobiotic community. Am J Clin Nutr. 1991 Feb;53(2):524–9.

101. Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR. Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. Am J Clin Nutr. 1999 Sep;70(3 Suppl):586S – 593S.

102. Waldmann A, Koschizke JW, Leitzmann C, Hahn A. Homocysteine and cobalamin status in German vegans. Public Health Nutr. 2004 May;7(3):467–72.

103. Dagnelie PC, van Staveren WA. Macrobiotic nutrition and child health: results of a population-based, mixed-longitudinal cohort study in The Netherlands. Am J Clin Nutr. 1994 May;59(5 Suppl):1187S – 1196S.

104. Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneede J, Ueland PM, et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr. 2000 Sep;72(3):762–9.

105. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. Am J Clin Nutr. 2003 Jul;78(1):3–6.

106. Koebnick C, Hoffmann I, Dagnelie PC, Heins UA, Wickramasinghe SN, Ratnayaka ID, et al. Long-term ovo-lacto vegetarian diet impairs vitamin B-12 status in pregnant women. J Nutr. 2004 Dec;134(12):3319–26.

107. Löwik MR, Schrijver J, Odink J, van den Berg H, Wedel M. Long-term effects of a vegetarian diet on the nutritional status of elderly people (Dutch Nutrition Surveillance System). J Am Coll Nutr. 1990 Dec;9(6):600–9.

108. Alexander D, Ball MJ, Mann J. Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. Eur J Clin Nutr. 1994 Aug;48(8):538–46.

109. Narayanan MN, Dawson DW, Lewis MJ. Dietary deficiency of vitamin B12 is associated with low serum cobalamin levels in non-vegetarians. Eur J Haematol. 1991 Aug;47(2):115–8.

110. Allen LH. Vitamin B12 metabolism and status during pregnancy, lactation and infancy. Adv Exp Med Biol. 1994 Jan;352:173–86.

111. Casterline JE, Allen LH, Ruel MT. Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. J Nutr. 1997 Oct;127(10):1966–72.

112. Allen LH, Rosado JL, Casterline JE, Martinez H, Lopez P, Muñoz E, et al. Vitamin B-12 deficiency and malabsorption are highly prevalent in rural Mexican communities. Am J Clin Nutr. 1995 Nov;62(5):1013–9.

113. Savage DG, Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects. Baillieres Clin Haematol. 1995 Sep;8(3):657–78.

114. Varela Moreiras G. Ácido fólico y vitamina B12. In: Gil Hernández Á, editor. Tratado de Nutrición Tomo 1: Bases Fisiológicas y Bioquimicas de la Nutrición. Madrid: Acción Médica; 2005. p. 731–54.

115. Food and Nutrition Board. Institute of Medicine. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate Other B Vitamins and Choline. Subcommittee on Upper Reference Levels of Nutrients. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 1998. 592 p.

116. Simpson JL, Bailey LB, Ietrzik K, Shane B, Holzgreve W, Simpson JL. Micronutrients and women of reproductive potential: required dietary intake and consequences of dietary deficiency or excess. Part I – Folate, Vitamin B12, Vitamin B6. J Matern Neonatal Med. Informa Clin Med; 2010 Apr 7;23(12):1323–43.

117. Pfeiffer CM, Rogers LM, Bailey LB, Gregory JF. Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. Am J Clin Nutr. 1997 Dec 1;66(6):1388–97.

118. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC. Folate requirement and metabolism in nonpregnant women. Am J Clin Nutr. 1987 Dec 1;46(6):1016–28.

119. Centers for Disease Control. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Recomm Rep. 1992 Sep 11;41(RR-14):1–7.

120. National Health and Medical Research Council. Nutrient Reference Values for Australia and New Zeland. Executive Summary. 2005.

121. Food Safety Authority of Ireland. Recommended Dietary Allowances for Ireland. Dublin; 1999.

122. Health Council of the Nederlands. Towards an optimal use of folic acid. The Hague; 2008.

123. Scientific Advisory Committee on Nutrition. Folate and disease prevention. Norwich; 2006.

124. Smithells RW, Sheppard S, Schorah CJ, Seller MJ, Nevin NC, Harris R, et al. Possible prevention of neural-tube defects by periconceptional vitamin supplementation. Lancet. 1980;1(8164):339–40.

125. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med. 1999 Nov 11;341(20):1485–90.

126. Botto LD, Mulinare J, Erickson JD. Do multivitamin or folic acid supplements reduce the risk for congenital heart defects? Evidence and gaps. Am J Med Genet. 2003 Aug 30;121A(2):95–101.

127. Giovannucci E. Epidemiologic Studies of Folate and Colorectal Neoplasia: a Review. J Nutr. 2002 Aug 1;132(8):2350S – 2355S.

128. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, et al. Improvement in Stroke Mortality in Canada and the United States, 1990 to 2002. Circulation. 2006 Mar 14;113(10):1335–43.

129. Dawson MI. The importance of vitamin A in nutrition. Curr Pharm Des. 2000 Feb;6(3):311–25.

130. U.S. Department of Health & Human Services. National Institutes of Health. Office of Dietary Supplements. [Internet]. [cited 2015 May 19]. Available from: http://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/#h3

131. Haskell MJ. The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion--evidence in humans. Am J Clin Nutr. 2012 Nov;96(5):1193S – 203S.

132. Sommer A, Vyas KS. A global clinical view on vitamin A and carotenoids. Am J Clin Nutr. 2012 Nov 1;96(5):1204S – 6S.

133. U.S. Department of Agriculture. Agricultural Research Service. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, by Gender and Age, What We Eat in America, NHANES 2009-2010. [Internet]. 2012 [cited 2015 May 20]. Available from: www.ars.usda.gov/ba/bhnrc/fsrg

134. Jynto. Jynto images [Internet]. [cited 2015 Aug 15]. Available from: https://commons.wikimedia.org/wiki/Category:Images_by_Jynto

135. Gutierrez-Mazariegos J, Theodosiou M, Campo-Paysaa F, Schubert M. Vitamin A: A multifunctional tool for development. Semin Cell Dev Biol. 2011 Aug;22(6):603–10.

136. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr. 1994 Feb 1;59(2):517S – 522S.

137. World Health Organization. WHO Nutrition. Micronutrient deficiencies. Vitamin A deficiency. [Internet]. Available from: http://www.who.int/nutrition/topics/vad/en/

138. Beaton G, Martorell R, L'Abbe K, Edmonston B, McCabe G, Ross A, et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. Geneva, Switzerland; 1993.

139. Teratology Society. Teratology Society position paper: recommendations for vitamin A use during pregnancy. Teratology. 1987 Apr;35(2):269–75.

140. Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. JAMA. 2002 Jan 2;287(1):47–54.

141. Novotny JA, Harrison DJ, Pawlosky R, Flanagan VP, Harrison EH, Kurilich AC. Beta-carotene conversion to vitamin A decreases as the dietary dose increases in humans. J Nutr. 2010 May 1;140(5):915–8.

142. Martínez-Agustin O, Puerta Fernández V, Suárez Ortega MD. Vitamina D. In: Gil Hernández A, editor. Tratado de Nutrición Tomo 1: Bases Fisiológicas y Bioquimicas de la Nutrición. Madrid: Acción Médica; 2005. p. 789–824.

143. Borradale D, Kimlin M. Vitamin D in health and disease: an insight into traditional functions and new roles for the "sunshine vitamin." Nutr Res Rev. Cambridge Journals Online; 2009;22(02):118–36.

144. Wolpowitz D, Gilchrest BA. The vitamin D questions: How much do you need and how should you get it? J Am Acad Dermatol. 2006 Feb;54(2):301–17.

145. Al Mheid I, Patel RS, Tangpricha V, Quyyumi AA. Vitamin D and cardiovascular disease: is the evidence solid? Eur Heart J. 2013 Dec;34(48):3691–8.

146. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem. Wiley Subscription Services, Inc., A Wiley Company; 2003 Feb 1;88(2):296–307.

147. Shaw NJ, Mughal MZ. Vitamin D and child health Part 1 (skeletal aspects). Arch Dis Child. 2013 May 1;98(5):363–7.

148. Armas LAG, Dowell S, Akhter M, Duthuluru S, Huerter C, Hollis BW, et al. Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: The effect of UVB dose and skin color. J Am Acad Dermatol. 2007 Oct;57(4):588–93.

149. Shrapnel W, Truswell S. Vitamin D deficiency in Australia and New Zealand: What are the dietary options? Nutr Diet. 2006 Dec 1;63(4):206–12.

150. Weisberg P, Scanlon KS, Li R, Cogswell ME. Nutritional rickets among children in the United States: review of cases reported between 1986 and 2003. Am J Clin Nutr. 2004 Dec;80(6 Suppl):1697S – 705S.

151. Shaw NJ, Mughal MZ. Vitamin D and child health: part 2 (extraskeletal and other aspects). Arch Dis Child. 2013 May 1;98(5):368–72.

152. McGrath JJ, Kimlin MG, Saha S, Eyles DW, Parisi A V. Vitamin D insufficiency in south-east Queensland. Med J Aust. 2001 Feb 5;174(3):150–1.

153. Mawer E, Davies M. Vitamin D deficiency, rickets and osteomalacia, a returing problem worldwide. In: Norman A, Bouillon R, Thomasset M, editors. Vitamin D chemistry, biology and clinical applications of the steroid hormone. Riverside, California: University of California; 1997. p. 889–906.

154. Specker BL, Tsang RC, Hollis BW. Effect of race and diet on human-milk vitamin D and 25-hydroxyvitamin D. Am J Dis Child. 1985 Nov;139(11):1134–7.

155. Dawodu A, Zalla L, Woo JG, Herbers PM, Davidson BS, Heubi JE, et al. Heightened attention to supplementation is needed to improve the vitamin

D status of breastfeeding mothers and infants when sunshine exposure is restricted. Matern Child Nutr. 2012 Jun 19;

156. Holick MF. The role of vitamin D for bone health and fracture prevention. Curr Osteoporos Rep. 2006 Sep;4(3):96–102.

157. Tanzer F, Sunel S. Calcium, magnesium and phosphorus concentrations in human milk and in sera of nursing mothers and their infants during 26 weeks of lactation. Indian Pediatr. 1991 Apr;28(4):391–400.

158. Aksnes L, Aarskog D. Plasma concentrations of vitamin D metabolites in puberty: effect of sexual maturation and implications for growth. J Clin Endocrinol Metab. 1982 Jul;55(1):94–101.

159. Landrier J-F, Marcotorchino J, Tourniaire F. Lipophilic micronutrients and adipose tissue biology. Nutrients. 2012 Nov;4(11):1622–49.

160. Mithal A, Wahl DA, Bonjour J-P, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009 Nov;20(11):1807–20.

161. Mataix J. Requerimientos nutricionales e ingestas recomendadas. In: Serra Majem L, Aranceta J, Mataix J, editors. Nutrición y salud publica Métodos, bases científicas y aplicaciones. Barcelona: Masson; 1995.

162. Gibney MJ, Serra-Majem L, Kearney JM, Becker W, Löwik MRH, Wiseman M, De Henauw S, Haraldsdóttir J, Flynn MAT, Graça P, Kafatos A, Koenig J, Leclercq C, Lambe J, Ribas L, Román B, Valsta JL VWS. Por una política de nutrición en Europa en el siglo XXI: desarrollo de unas guías alimentarias basadas en el consumo de alimentos. Nutr Clin. 2000;20(3):29–46.

163. Corbalan M, Cuervo M, Baladia E, Martinez JA. Ingestas Dieteticas de Referencia (IDR): Conceptos y Evolucion Historica. In: Federacion Espanola de Sociedades de Nutricion Alimentacion y Dietetica (FESNAD), editor. Ingestas Dieteticas de Referencia (IDR) para la poblacion española. 1st ed. Pamplona: Ediciones Universidad de Navarra S.A; 2010. p. 75–102.

164. Aggett PJ, Bresson J, Haschke F, Hernell O, Koletzko B, Lafeber HN, et al. Recommended Dietary Allowances (RDAs), Recommended Dietary Intakes (RDIs), Recommended Nutrient Intakes (RNIs), and Population Reference Intakes (PRIs) are not “recommended intakes.” JPediatrGastroenterolNutr. 1997 Aug;25(0277-2116 (Print)):236–41.

165. Pavlovic M, Prentice A, Thorsdottir I, Wolfram G, Branca F. Challenges in Harmonizing Energy and Nutrient Recommendations in Europe. Ann Nutr Metab. 2007;51(2):108–14.

166. FAO/WHO/UNU. Human Energy Requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. 1st ed. FAO Food and Nutrition Technical Report Series. Rome: FAO Food and Nutrition Tecnical Report Series; 2004.

167. FAO/WHO. Calorie requirements: Report of the Committee on calorie requirements. FAO Nutritional Studies num 5. Rome; 1950.

168. Cuervo M, Corbalan M, Baladia E, Cabrerizo L, Formiguera X, Iglesias C, et al. Comparison of dietary reference intakes (DRI) between different countries of the European Union, The United States and the World Health Organization. NutrHosp. 2009 Jul;24(0212-1611 (Print)):384–414.

169. FAO/WHO. Protein and amino acid requirements in human nutrition. Report of a joint FAO/WHO expert consultation. Technical Report Series num 935. Singapore; 2007.

170. Food and Nutrition Board. Institute of Medicine. Subcommittee on the Tenth Edition of the Recommended Dietary Allowances. Commission on Life Sciences. National Research Council. Recommended Dietary Allowances. 10th ed. Washington, D.C.: National Academies Press; 1989. 302 p.

171. Health and Welfare Canada. Nutrition recommendations: the report of the Scientific Review Committee. Otawa; 1990.

172. Food and Nutrition Board. Institute of Medicine., Catharine Ross A, Taylor CL, Yaktine AL, Del Valle HB, Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin D. Catharine Ross A, Taylor CL, Yaktine AL, Del Valle HB, editors. Washington, D.C.: National Academies Press; 2011. 1132 p.

173. Food and Nutrition Board. Institute of Medicine. Subcommittee on Interpretation and Uses of Dietary Reference Intakes and Upper Reference Levels of Nutrients. Standing Committee on the Scientific Evaluation of DRI. Dietary Reference Intakes: Applications in Dietary Assessment. National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 2000. 306 p.

174. Food and Nutrition Board. Institute of Medicine. Panel on Micronutrients. Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary reference Intakes. Standing Committee on the Scientific Evaluation of Dietary Reference. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 2001. 800 p.

175. Food and Nutrition Board. Institute of Medicine. Panel on Macronutrients. Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Use of Dietary Reference Intakes. Standing Committee on the Scientific Evaluation of Dietary Reference In. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 2002. 1357 p.

176. Food and Nutrition Board. Institute of Medicine. Panel on Dietary Reference Intakes for Electrolytes and water. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 2004. 640 p.

177. Food and Nutrition Board. Institute of Medicine. Panel on Dietary Antioxidants and Related Compounds. Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs. Standing Committee on the Scientific Evaluation of DRI. Dietary Reference Intakes for vitamin C, vitamin E, Selenium and Carotenoids. National Academy of Sciences, editor. Washington, D.C.: National Academies Press; 2000. 529 p.

178. Scientific Committee for foods of the European Community. Proposed nutrient and energy intakes for the European Communnity: A Report of the Scientific Committtee for foods of the European Community. Nutr Rev. 1993 Jul;51(7):209–12.

179. Roman-Vinas B, Serra-Majem L, Ribas-Barba L, Ngo J, Garcia-Alvarez A, Wijnhoven TM, et al. Overview of methods used to evaluate the adequacy of nutrient intakes for individuals and populations. BrJNutr. 2009 Jul 19;101 Suppl(1475-2662 (Electronic)):S6–11.

180. Tabacchi G, Wijnhoven TMA, Branca F, Román-Viñas B, Ribas-Barba L, Ngo J, et al. How is the adequacy of micronutrient intake assessed across

Europe? A systematic literature review. BrJNutr. 2009 Jul 19;101 Suppl(1475-2662 (Electronic)):S29–36.

181. Arsenault JE, Yakes EA, Islam MMM, Hossain MB, Ahmed T, Hotz C, et al. Very Low Adequacy of Micronutrient Intakes by Young Children and Women in Rural Bangladesh Is Primarily Explained by Low Food Intake and Limited Diversity. J Nutr. 2013 Feb 1;143(2):197–203.

182. Carriquiry AL. Assessing the prevalence of nutrient inadequacy. Public Heal Nutr. 1999 Mar 19;2(1368-9800 (Print)):23–33.

183. Murphy SP, Poos MI. Dietary Reference Intakes: summary of applications in dietary assessment. Public Heal Nutr. Cancer Research Center of Hawaii, University of Hawaii, Honolulu, USA; 2002 Dec 19;5(1368-9800 (Print)):843–9.

184. Roman Viñas B, Ribas Barba L, Ngo J, Gurinovic M, Novakovic R, Cavelaars A, et al. Projected prevalence of inadequate nutrient intakes in Europe. Ann Nutr Metab. 2011 Jan;59(2-4):84–95.

185. Serra-Majem L, Bes-Rastrollo M, Román-Viñas B, Pfrimer K, Sánchez-Villegas A, Martínez-González MA. Dietary patterns and nutritional adequacy in a Mediterranean country. Br J Nutr. 2009 Jul;101 Suppl:S21–8.

186. World Health Organization. The world health report. Reducing risks, promoting healthy life: overview. Geneva, Switzerland; 2002.

187. Mensink GBM, Fletcher R, Gurinovic M, Huybrechts I, Lafay L, Serra-Majem L, et al. Mapping low intake of micronutrients across Europe. Br J Nutr. 2013 Aug;110(4):755–73.

188. De Groot LC, Hautvast JG, van Staveren WA. Nutrition and health of elderly people in Europe: the EURONUT-SENECA Study. Nutr Rev. 1992 Jul;50(7):185–94.

189. Essama-Tjani JC, Guilland JC, Potier de Courcy G, Fuchs F, Richard D. Folate status worsens in recently institutionalized elderly people without evidence of functional deterioration. J Am Coll Nutr. 2000 Jun;19(3):392–404.

190. Hallberg L. Results of surveys to assess iron status in Europe. Nutr Rev. 1995 Nov;53(11):314–22.

191. Hannon EM, Kiely M, Harrington KE, Robson PJ, Strain JJ, Flynn A. The North/South Ireland Food Consumption Survey: mineral intakes in 18-64-year-old adults. Public Health Nutr. 2001 Oct;4(5A):1081–8.

192. O'Brien MM, Kiely M, Harrington KE, Robson PJ, Strain JJ, Flynn A. The North/South Ireland Food Consumption Survey: vitamin intakes in 18-64-year-old adults. Public Health Nutr. 2001 Oct;4(5A):1069–79.

193. Van der Wielen RP, Löwik MR, van den Berg H, de Groot LC, Haller J, Moreiras O, et al. Serum vitamin D concentrations among elderly people in Europe. Lancet. 1995 Jul 22;346(8969):207–10.

194. Serra-Majem L. Vitamin and mineral intakes in European children. Is food fortification needed? Public Heal Nutr. 2001 Feb;4(1368-9800 (Print)):101–7.

195. Samuelson G. Dietary habits and nutritional status in adolescents over Europe. An overview of current studies in the Nordic countries. Eur J Clin Nutr. 2000 Mar;54 Suppl 1:S21–8.

196. Cruz JA. Dietary habits and nutritional status in adolescents over Europe--Southern Europe. Eur J Clin Nutr. 2000 Mar;54 Suppl 1:S29–35.

197. Parízková J. Dietary habits and nutritional status in adolescents in Central and Eastern Europe. Eur J Clin Nutr. 2000 Mar;54 Suppl 1:S36–40.

198. Rolland-Cachera MF, Bellisle F, Deheeger M. Nutritional status and food intake in adolescents living in Western Europe. Eur J Clin Nutr. 2000 Mar;54 Suppl 1:S41–6.

199. Elmadfa I. Energy and Nutrient Intake in the European Union. Ann Nutr Metab. 2004;48(Suppl 2):1–16.

200. Kaganov B, Caroli M, Mazur A, Singhal A, Vania A. Suboptimal Micronutrient Intake among Children in Europe. Nutrients. 2015 Jan;7(5):3524–35.

201. Doets EL, de Wit LS, Dhonukshe-Rutten RA, Cavelaars AE, Raats MM, Timotijevic L, et al. Current micronutrient recommendations in Europe: towards understanding their differences and similarities. EurJNutr. 2008 Apr;47 Suppl 1(1436-6207 (Print)):17–40.

202. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, et al. Timing of peak bone mass in Caucasian females and its implication for the

prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest. 1994 Feb;93(2):799–808.

203. Teegarden D, Proulx WR, Martin BR, Zhao J, McCabe GP, Lyle RM, et al. Peak bone mass in young women. J Bone Miner Res. 1995 May;10(5):711–5.

204. Ott SM. Bone density in adolescents. N Engl J Med. 1991 Dec 5;325(23):1646–7.

205. Teegarden D, Lyle RM, McCabe GP, McCabe LD, Proulx WR, Michon K, et al. Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. Am J Clin Nutr. 1998 Sep;68(3):749–54.

206. Soyka LA, Fairfield WP, Klibanski A. Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. J Clin Endocrinol Metab. 2000 Nov;85(11):3951–63.

207. Manzoni P, Brambilla P, Pietrobelli A, Beccaria L, Bianchessi A, Mora S, et al. Influence of body composition on bone mineral content in children and adolescents. Am J Clin Nutr. 1996 Oct;64(4):603–7.

208. Correa Rodríguez M, Rueda Medina B, González Jiménez E, Navarro Pérez CF, Schmidt-RioValle J. The levels of bone mineralization are influenced by body composition in children and adolescents. Nutr Hosp. 2014 Jan;30(4):763–8.

209. Del Rio L, Carrascosa A, Pons F, Gusinyé M, Yeste D, Domenech FM. Bone mineral density of the lumbar spine in white Mediterranean Spanish children and adolescents: changes related to age, sex, and puberty. Pediatr Res. 1994 Mar;35(3):362–6.

210. Bachrach LK, Guido D, Katzman D, Litt IF, Marcus R. Decreased bone density in adolescent girls with anorexia nervosa. Pediatrics. 1990 Sep;86(3):440–7.

211. Clemmons DR, Seek MM, Underwood LE. Supplemental essential amino acids augment the somatomedin-C/insulin-like growth factor I response to refeeding after fasting. Metabolism. 1985 Apr;34(4):391–5.

212. Bonjour J-P. Dietary protein: an essential nutrient for bone health. J Am Coll Nutr. 2005 Dec;24(6 Suppl):526S – 36S.

213. Beasley JM, Ichikawa LE, Ange BA, Spangler L, LaCroix AZ, Ott SM, et al. Is protein intake associated with bone mineral density in young women? Am J Clin Nutr. 2010 May;91(5):1311–6.

214. Cooper C, Atkinson EJ, Hensrud DD, Wahner HW, O'Fallon WM, Riggs BL, et al. Dietary protein intake and bone mass in women. Calcif Tissue Int. 1996 May;58(5):320–5.

215. Lacey JM, Anderson JJ, Fujita T, Yoshimoto Y, Fukase M, Tsuchie S, et al. Correlates of cortical bone mass among premenopausal and postmenopausal Japanese women. J Bone Miner Res. 1991 Jul;6(7):651–9.

216. Vatanparast H, Bailey DA, Baxter-Jones ADG, Whiting SJ. The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. J Nutr. 2007 Dec;137(12):2674–9.

217. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000 Dec;15(12):2504–12.

218. Jesudason D, Clifton P. The interaction between dietary protein and bone health. J Bone Miner Metab. 2011 Jan;29(1):1–14.

219. Sebastian A. Dietary protein content and the diet's net acid load: opposing effects on bone health. Am J Clin Nutr. 2005 Nov;82(5):921–2.

220. Huncharek M, Muscat J, Kupelnick B. Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. Bone. 2008 Aug;43(2):312–21.

221. Winzenberg T, Shaw K, Fryer J, Jones G. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. BMJ. 2006 Oct 14;333(7572):775.

222. Bonjour JP, Carrie AL, Ferrari S, Clavien H, Slosman D, Theintz G, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest. 1997 Mar 15;99(6):1287–94.

223. Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, et al. Calcium supplementation and increases in bone mineral density in children. N Engl J Med. 1992 Jul 9;327(2):82–7.

224. More J. Children's bone health and meeting calcium needs. J Fam Health Care. 2008 Jan;18(1):22–4.

225. Khadilkar A, Kadam N, Chiplonkar S, Fischer PR, Khadilkar V. School-based calcium-vitamin D with micronutrient supplementation enhances bone mass in underprivileged Indian premenarchal girls. Bone. 2012 Jul;51(1):1–7.

226. Ondrak KS, Morgan DW. Physical activity, calcium intake and bone health in children and adolescents. Sports Med. 2007 Jan;37(7):587–600.

227. Vatanparast H, Whiting SJ. Calcium supplementation trials and bone mass development in children, adolescents, and young adults. Nutr Rev. 2006 Apr;64(4):204–9.

228. Lanou AJ, Berkow SE, Barnard ND. Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. Pediatrics. 2005 Mar;115(3):736–43.

229. Bonjour JP, Chevalley T, Ammann P, Slosman D, Rizzoli R. Gain in bone mineral mass in prepubertal girls 3.5 years after discontinuation of calcium supplementation: a follow-up study. Lancet. 2001 Oct 13;358(9289):1208–12.

230. Rizzoli R, Bianchi ML, Garabédian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone. 2010 Feb;46(2):294–305.

231. Calvo MS, Heath H. Acute effects of oral phosphate-salt ingestion on serum phosphorus, serum ionized calcium, and parathyroid hormone in young adults. Am J Clin Nutr. 1988 Jun;47(6):1025–9.

232. Portale AA, Halloran BP, Morris RC. Physiologic regulation of the serum concentration of 1,25-dihydroxyvitamin D by phosphorus in normal men. J Clin Invest. 1989 May;83(5):1494–9.

233. Wood RJ, Sitrin MD, Rosenberg IH. Effect of phosphorus on endogenous calcium losses during total parenteral nutrition. Am J Clin Nutr. 1988 Sep;48(3):632–6.

234. Daly RM, Stenevi-Lundgren S, Linden C, Karlsson MK. Muscle determinants of bone mass, geometry and strength in prepubertal girls. Med Sci Sports Exerc. 2008 Jun;40(6):1135–41.

235. Zhang P, Hamamura K, Yokota H. A brief review of bone adaptation to unloading. Genomics Proteomics Bioinformatics. 2008 Mar;6(1):4–7.

236. MacKelvie KJ, McKay HA, Petit MA, Moran O, Khan KM. Bone mineral response to a 7-month randomized controlled, school-based jumping intervention in 121 prepubertal boys: associations with ethnicity and body mass index. J Bone Miner Res. 2002 May;17(5):834–44.

237. Fuchs RK, Snow CM. Gains in hip bone mass from high-impact training are maintained: a randomized controlled trial in children. J Pediatr. 2002 Sep;141(3):357–62.

238. MacKelvie KJ, Khan KM, Petit MA, Janssen PA, McKay HA. A school-based exercise intervention elicits substantial bone health benefits: a 2-year randomized controlled trial in girls. Pediatrics. 2003 Dec;112(6 Pt 1):e447.

239. McKay HA, Petit MA, Schutz RW, Prior JC, Barr SI, Khan KM. Augmented trochanteric bone mineral density after modified physical education classes: a randomized school-based exercise intervention study in prepubescent and early pubescent children. J Pediatr. 2000 Feb;136(2):156–62.

240. Weeks BK, Young CM, Beck BR. Eight months of regular in-school jumping improves indices of bone strength in adolescent boys and Girls: the POWER PE study. J Bone Miner Res. 2008 Jul;23(7):1002–11.

241. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. Bone. 2007 Jan;40(1):14–27.

242. Gunter K, Baxter-Jones ADG, Mirwald RL, Almstedt H, Fuller A, Durski S, et al. Jump starting skeletal health: a 4-year longitudinal study assessing the effects of jumping on skeletal development in pre and circum pubertal children. Bone. 2008 Apr;42(4):710–8.

243. Gunter K, Baxter-Jones AD, Mirwald RL, Almstedt H, Fuchs RK, Durski S, et al. Impact exercise increases BMC during growth: an 8-year longitudinal study. J Bone Miner Res. 2008 Jul;23(7):986–93.

244. World Health Organization. Global recommendations on physical activity for health. Geneva, Switzerland; 2010.

245. Campos LMA, Liphaus BL, Silva CAA, Pereira RMR. [Osteoporosis in childhood and adolescence]. J Pediatr (Rio J). Jan;79(6):481–8.

246. Frisch RE, Gotz-Welbergen A V, McArthur JW, Albright T, Witschi J, Bullen B, et al. Delayed menarche and amenorrhea of college athletes in relation to age of onset of training. JAMA. 1981 Oct 2;246(14):1559–63.

247. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med. 1993 Jun;94(6):646–50.

248. World Health Organization. Prevention and management of osteoporosis. Geneva, Switzerland; 2003.

249. World Health Organization. Guidelines for preclinical evaluation and clinical trials in osteoporosis. Geneva, Switzerland; 1998.

250. Gordon CM, Bachrach LK, Carpenter TO, Crabtree N, El-Hajj Fuleihan G, Kutilek S, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2007 ISCD Pediatric Official Positions. J Clin Densitom. 2008 Jan 1;11(1):43–58.

251. Svedbom A, Hernlund E, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: a compendium of country-specific reports. Arch Osteoporos. 2013 Jan;8(1-2):137.

252. Van der Sluis IM, de Muinck Keizer-Schrama SM. Osteoporosis in childhood: bone density of children in health and disease. J Pediatr Endocrinol Metab. Jan;14(7):817–32.

253. European Commission. Commission Directive 91/321/EEC of 14 May 1991 on infant and follow-on formulae. [Internet]. Official Journal of the European Commission. 1991 [cited 2015 May 6]. p. 0035–49. Available from: http://ec.europa.eu/food/fs/sc/scf/out09_en.html

254. Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, et al. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. Am J Clin Nutr. 2009 Jun;89(6):1836–45.

255. Lubchenco LO, Hansman C, Dressler M, Boyd E. Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation. Pediatrics. 1963 Nov 1;32(5):793–800.

256. Federal Institute for Health Protection of Consumers and Veterinary Medicine. The German Food Code and Nutrient Data Base: conception, structure and documentation of the data base blsdat. 3rd ed. Berlin, Germany.: BgVV Publications; 1999.

257. Insitut Paul Lambin. Composition des Aliments. 1 ed. Brussels: Institut Paul Lambin; 1998.

258. Van Havere R, Muls E, Seeuws C. Table belge de composition des aliments. 3 ed. Brussels: NUBEL v.z.w.; 1999.

259. Kunachowicz H, Nadolna I, Przybyla B, Iwanow K. Tabele wartosci odzywczej produktow spozywczych (Food composition tables). Instytut Zywnoasci i Zywienia (National Food and Nutrition Institute), editor. Warsaw: Instytut Zywnoasci i Zywienia (National Food and Nutrition Institute); 1998.

260. Mataix J, Mañas M, Llopis J, Martínez de Victoria E, Sánchez J, Borregon A. Tabla de Composicion de Alimentos Españoles. 4 ed. Editorial de la Universidad de Granada, editor. Granada: Editorial de la Universidad de Granada; 2003.

261. Gnagnarella P, Salvini S, Parpinel M. Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia [Internet]. Version 1. [cited 2015 Aug 11]. Available from: http://www.bda-ieo.it/

262. Istituto Nazionele di Ricerca per gli Alimenti e la Nutrizione. Tabelle di composizione degli alimenti [Internet]. 2000 [cited 2015 Aug 11]. Available from: ut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html

263. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr. 1991 Dec;45(12):569–81.

264. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr. 1985 Jan;39 Suppl 1:5–41.

265. Verwied-Jorky S, Schiess S, Luque V, Grote V, Scaglioni S, Vecchi F, et al. Methodology for longitudinal assessment of nutrient intake and dietary habits in early childhood in a transnational multicenter study. J Pediatr Gastroenterol Nutr. 2011 Jan;52(1):96–102.

266. Lohman TG, Roche AF MR. Anthropometric standardization reference manual. Human Kinetics Books. Champaign, IL; 1988.

267. National Center for Health Statistics. NCHS Growth Curves for Children. Birth-18 Years. United States. Series II, number 165. Hyattsville; 1977.

268. Wells JCK, Williams JE, Chomtho S, Darch T, Grijalva-Eternod C, Kennedy K, et al. Body-composition reference data for simple and reference techniques and a 4-component model: a new UK reference child. Am J Clin Nutr. 2012 Dec;96(6):1316–26.

269. Janz KF, Lutuchy EM, Wenthe P, Levy SM. Measuring activity in children and adolescents using self-report: PAQ-C and PAQ-A. Med Sci Sports Exerc. 2008 Apr;40(4):767–72.

270. Kowalski K, Crocker P, Donen R. The Physical Activity Questionnaire for Older Children (PAQ-C) and Adolescents (PAQ-A) Manual. College of Kinesiology. University of Saskatchewan.

271. World Health Organization. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ. 2001 Jan;79(4):373–4.

272. McIntosh N, Bates P, Brykczynska G, Dunstan G, Goldman A, Harvey D, et al. Guidelines for the ethical conduct of medical research involving children. Royal College of Paediatrics, Child Health: Ethics Advisory Committee. Arch Dis Child. 2000 Feb;82(2):177–82.

273. Begg C, Cho M, Eastwood S, Horton R, Moher D, Olkin I, et al. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. JAMA. 1996 Aug 28;276(8):637–9.

274. EURopean micronutrients RECcommendations Aligned. Eurreca Micronutrient database [Internet]. [cited 2015 May 14]. Available from: http://www.serbianfood.info/eurreca/

275. Thompson FE, Moler JE, Freedman LS, Clifford CK, Stables GJ, Willett WC. Register of dietary assessment calibration-validation studies: a status report. Am J Clin Nutr. 1997 Apr;65(4 Suppl):1142S – 1147S.

276. Koebnick C, Wagner K, Thielecke F, Dieter G, Höhne A, Franke A, et al. An easy-to-use semiquantitative food record validated for energy intake by

using doubly labelled water technique. Eur J Clin Nutr. 2005 Sep;59(9):989–95.

277. Ortiz-Andrellucchi A, Henriquez-Sanchez P, Sanchez-Villegas A, Pena-Quintana L, Mendez M, Serra-Majem L. Dietary assessment methods for micronutrient intake in infants, children and adolescents: a systematic review. BrJNutr. 2009 Dec;102 Suppl(1475-2662 (Electronic)):S87–117.

278. Roman-Vinas B, Ortiz-Andrellucchi A, Mendez M, Sanchez-Villegas A, Pena QL, Aznar LA, et al. Is the food frequency questionnaire suitable to assess micronutrient intake adequacy for infants, children and adolescents? Matern Nutr. 2010 Oct 19;6 Suppl 2(1740-8709):112–21.

279. Mertz W, Tsui JC, Judd JT, Reiser S, Hallfrisch J, Morris ER, et al. What are people really eating? The relation between energy intake derived from estimated diet records and intake determined to maintain body weight. Am J Clin Nutr. 1991 Aug 1;54(2):291–5.

280. Poslusna K, Ruprich J, de Vries JH, Jakubikova M, Van't VP. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. BrJNutr. Department of Food Safety and Nutrition, N; 2009 Jul 19;101 Suppl(1475-2662 (Electronic)):S73–85.

281. Lanigan JA, Wells JC, Lawson MS, Cole TJ, Lucas A. Number of days needed to assess energy and nutrient intake in infants and young children between 6 months and 2 years of age. EurJClinNutr. 2004 May;58(0954-3007 (Print)):745–50.

282. Beitz R, Mensink GBM, Hintzpeter B, Fischer B, Erbersdobler HF. Do users of dietary supplements differ from nonusers in their food consumption? Eur J Epidemiol. 2004 Jan;19(4):335–41.

283. Murphy SP, White KK, Park S-Y, Sharma S. Multivitamin-multimineral supplements' effect on total nutrient intake. Am J Clin Nutr. 2007 Jan;85(1):280S – 284S.

284. EURopean micronutrients RECcommendations Aligned., Hermoso M. Report on actual nutrient intake of infants in the European countries. [Internet]. Eurreca web page/Outputs/Reports. 2011 [cited 2015 Aug 1]. Available from: http://www.eurreca.org/everyone/8565/7/0/32

285. Räsänen M, Kronberg-Kippilä C, Ahonen S, Uusitalo L, Kautiainen S, Erkkola M, et al. Intake of vitamin D by Finnish children aged 3 months to 3 years in relation to sociodemographic factors. Eur J Clin Nutr. 2006 Nov;60(11):1317–22.

286. Lind T, Lönnerdal B, Persson L-A, Stenlund H, Tennefors C, Hernell O. Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: a randomized intervention in infants from 6 to 12 mo of age. Am J Clin Nutr. 2003 Jul;78(1):168–75.

287. Lind T, Hernell O, Lönnerdal B, Stenlund H, Domellöf M, Persson L-A. Dietary iron intake is positively associated with hemoglobin concentration during infancy but not during the second year of life. J Nutr. 2004 May;134(5):1064–70.

288. Hopkins D, Emmett P, Steer C, Rogers I, Noble S, Emond A. Infant feeding in the second 6 months of life related to iron status: an observational study. Arch Dis Child. 2007 Oct;92(10):850–4.

289. Noble S, Emmett P. Differences in weaning practice, food and nutrient intake between breast- and formula-fed 4-month-old infants in England. J Hum Nutr Diet. 2006 Aug;19(4):303–13.

290. Noble S, Emmett P. Food and nutrient intake in a cohort of 8-month-old infants in the south-west of England in 1993. Eur J Clin Nutr. 2001 Aug;55(8):698–707.

291. Serra-Majem L, Ribas-Barba L, Pérez-Rodrigo C, Bartrina JA. Nutrient adequacy in Spanish children and adolescents. Br J Nutr. 2006 Aug;96 Suppl 1:S49–57.

292. Gallagher CM, Black LJ, Oddy WH. Micronutrient intakes from food and supplements in Australian adolescents. Nutrients. 2014 Jan;6(1):342–54.

293. Bojar I, Owoc A, Humeniuk E, Wierzba W, Fronczak A. Inappropriate consumption of vitamins and minerals by pregnant women in Poland. Ann AgricEnvironMed. 2012;19(1898-2263 (Electronic)):263–6.

294. Amare B, Moges B, Moges F, Fantahun B, Admassu M, Mulu A, et al. Nutritional status and dietary intake of urban residents in Gondar, Northwest Ethiopia. BMC Public Health. 2012 Jan;12:752.

295. EFSA Panel on Dietetic Products Nutrition and Alergies. Scientific opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. EFSA J. 2013;11(10):3408.

296. Whiting SJ. Calcium: a nutrient deserving a special issue. Nutrients. 2010 Oct;2(10):1044–7.

297. Vatanparast H, Dolega-Cieszkowski JH, Whiting SJ. Many adult Canadians are not meeting current calcium recommendations from food and supplement intake. Appl Physiol Nutr Metab. 2009 Apr;34(2):191–6.

298. Grote V, Schiess SA, Closa-Monasterolo R, Escribano J, Giovannini M, Scaglioni S, et al. The introduction of solid food and growth in the first 2 y of life in formula-fed children: analysis of data from a European cohort study. Am J Clin Nutr. 2011 Dec;94(6 Suppl):1785S – 1793S.

299. Caulfield L, Black R. Zinc Deficiency. In: Ezzati M, Lopez A, Rodgers A, Murray C, editors. Comparative quantification of health risks Global and regional burden of disease atributable to selected major risk factors Volume I. Geneva, Switzerland: Worlds Health Organization; 2004. p. 257–80.

300. Brown KH, Wuehler SE, Peerson JM. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr Bull. 2001;22:113. – 125.

301. Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. Br J Nutr. 2008 Jun;99 Suppl 3:S14–23.

302. Andersson M, de Benoist B, Rogers L. Epidemiology of iodine deficiency: Salt iodisation and iodine status. Best Pract Res Clin Endocrinol Metab. 2010 Feb;24(1):1–11.

303. De Benoist B, McLean E, Andersson M, Rogers L. Iodine deficiency in 2007: global progress since 2003. Food Nutr Bull. 2008 Sep;29(3):195–202.

304. Koliaki C, Katsilambros N. Dietary sodium, potassium, and alcohol: key players in the pathophysiology, prevention, and treatment of human hypertension. Nutr Rev. 2013 Jun;71(6):402–11.

305. Nowson CA, Margerison C. Vitamin D intake and vitamin D status of Australians. Med J Aust. 2002 Aug 5;177(3):149–52.

306. Nielsen FH. Magnesium, inflammation, and obesity in chronic disease. Nutr Rev. 2010 Jun;68(6):333–40.

307. Tylavsky FA, Cowan PA, Terrell S, Hutson M, Velasquez-Mieyer P. Calcium intake and body composition in African-American children and adolescents at risk for overweight and obesity. Nutrients. 2010 Sep;2(9):950–64.

308. Lee WT, Leung SS, Lui SS, Lau J. Relationship between long-term calcium intake and bone mineral content of children aged from birth to 5 years. Br J Nutr. 1993 Jul;70(1):235–48.

309. Gabay C, Ruedin P, Slosman D, Bonjour JP, Leski M, Rizzoli R. Bone mineral density in patients with end-stage renal failure. Am J Nephrol. 1993 Jan;13(2):115–23.

310. Abrams SA. Calcium absorption in infants and small children: methods of determination and recent findings. Nutrients. 2010 Apr;2(4):474–80.

311. Jones G. Early life nutrition and bone development in children. Nestle Nutr Workshop Ser Pediatr Program. 2011 Jan;68:227–33; discussion 233–6.

Annexes

ANNEX I. FOOD RECORDS DESIGN

1. Food records from 0 to 3 months

Dear Chop-Participant!

To assess the eating habits of your child, it's necessary to conduct a food record over three consecutive days. Please record on **two week-days** and **one weekend-day** (i.e. Sunday, Monday, and Tuesday). It's important to accurately record everything that your child has eaten!
If it is not possible at certain times for you to fill in this form, please ask the person who takes care of your child at that time (i.e. grand-mother, day-care attendant) to record the exact amounts of milk meals or other foods consumed by the child.
If you will have any problems or questions with recording please call the CHOPIN-hotline *****.

How to fill out the record properly?

→ Please record breast-feeding and the intake of formula or other consumed fluids (like tea, juices..) in the provided charts
→ Note immediately the exact time when your child has a milk meal or drinks anything else.
→ Formula intake:
- Note type and brand of the formula.
- Note the amount of water and the number of measuring spoons which you have been using for bottle preparation.
- Please record the type and brand of cereals or other ingredients which you have been using for bottle preparation. Give the number of tea- or tablespoons, grams or millilitres.
- Note the amount of milk your child has been offered in the bottle and also the volume your child has drunk at each meal.
- Please also record the time taken (duration) for each meal.

→ Other fluid intake:
- Please record the type, the brand and the amount of fluid consumed (e.g. apple juice, brand, 50 ml).
- Note the age of your child, when it was given a certain fluid for the first time.

→ Solid foods:
- In case your child already gets solid foods, please use the extra table for recording (see separate instructions)

Many thanks for your participation and your active support!

Sincerely yours
The Chop-Team

Food record at the end of month |____|

Screening-Number: |__|__|__| Randomisation Number: |__|__|__|

Child Initials: |__|__|__|

Date: |__|__|/|__|__|/|__|__| (**day 1**)
month day year

Milk preparation and MILK INTAKE										
Time	Type of milk	Water	Powder	Cereal		Other		Amount offered	Volume consumed	Meal duration
	(type, brand)	(ml)	(number of spoons)	(type, brand)	(number of spoons)	(type, brand)	(g, ml or spoons)	(ml)	(ml)	(minutes)

OTHER FLUID INTAKE*				
Time	Type of fluid	Amount offered	Volume consumed	Age of first introduction
	(type, brand)	(ml)	(ml)	(week)

* Like tea, water, juices, water + sugar, cow's milk, soy milk, other...

Food record at the end of month |___|

Screening-Number: |__|__|__| Randomisation Number: |__|__|__|
Child Initials: |__|__|__|

Date: |__|__|/|__|__|/|__|__| (**day 1**)
month day year

SOLID FOOD INTAKE					
Time/ Meal	Where was meal eaten? (Home, day-care...)	Details of food consumed (incl. preparation, brand, type, characteristics)	Quantity offered	Quantity not consumed	Code (reserved for the dietitian)

Description of recipe: _____

Quantity	food items + ingredients (incl. preparation)

Description of recipe: _____

Quantity	food items + ingredients (incl. preparation)

2. Food records from 4 to 9 months

Dear Chop-Participant!

To assess the dietary habits of your child it`s necessary to conduct a food record over three consecutive days. Please record on **two week-days** and **one weekend-day** (i.e. Sunday, Monday, and Tuesday). It's important to accurately record everything that your child has eaten!
If it is not possible at certain times for you to fill in this form, please ask the person who takes care of your child at that time (i.e. grand-mother, day-care attendant) to record the exact amounts of milk meals or other foods consumed by the child.

How to fill out the record properly?
Please again record breast-feeding and the intake of formula, other consumed fluids and the consumption of solid foods in separate tables.

Column 1: Please note immediately at any time your child is eating or drinking the time and the kind of meal (i.e. 7:00 a.m., breakfast; 3:00 p.m. afternoon snack).

Column 2: Please record the site where your child is eating or drinking (i.e. home, grand-mother, shopping mall).

Column 3: Please give an exact description of all consumed foods and beverages. Give information about the preparation (like raw, cooked), about brand name, type and characteristics (i.e. low fat, light).

→ Commercial babyfood ready-to-eat : Do not just record cereal, but powder of "milk cereal fruits, brand name" or "Buttered carrots with potatoes and chicken, jar „brand name".
→ Brand name: Please always note the brand name where possible! Do not just note cookie, but i.e. "Butter cookies or fig cookies, brand name"
→ Fat content: Cow`s milk, 3,5% fat, cream cheese 60% fat i.d.m, curd 20% fat
→ Dietetic products: Juice multivitamin light (brand name), lemonade lemon light (brand name), margarine low-fat (brand name) etc.
→ Type: Mortadella, vienna sausage, ham cooked, meat pork lean fillet, cottage cheese, parmesan, olive oil, wheat bread (white or whole grain), butter cookies, whole-grain or fig cookies, cherry juice
→ Preparation/consumption: cooked, raw, breaded and fried, grilled, baked, with peel or without peel (i.e. banana without peel, chicken breast grilled without skin)
→ Convenience products: Please enclose empty packages or package labels with nutrient analysis to the food record.

Column 4: Please fill in the quantities which you have estimated before consumption or the amount which you child has really consumed.

→ Please weigh the food with a digital scale or use the following household measures:
1 teaspoon (tsp.) level or heaped, 1 tablespoon (tbsp.) level or heaped,
1 tea-cup small (about 150 ml).
1 glass/mug (about 200 ml)
1 bowl medium (about 200 g)
1 soup-plate (about 250 g)
1 slice (bread, sausage, cheese)
1 piece (i.e. apple small, potato medium)
1 jar of baby food (corresponding amount of grams)

→ For packaged foods please give quantities,
i.e. "Rice-Fruits-Cereal (jar 190 g, brand name)", "Banana and Peach in Apple, (jar 190 g, brand name)", "Apple-Carrot Juice (250 ml, brand name)", "Yoghurt, fruit-flavoured, 3,5% fat, 125 g"

→ Warm dishes/beverages:
Infant-Formula: Please note the amount of water, the measuring spoons of formula powder and the quantity of other ingredients you use for bottle preparation (see former food records!).
Commercial baby food ready-to-eat: "220 g Spaghetti in Sauce Bolognese (jar, brand name)".
Homemade dishes:
- for your child: Please note every single food item or ingredient separately or write down the recipe or enclose the recipe on a separate paper sheet. Note the consumed amount of each consumed food item or the amount of the consumed dish.
- for the whole family: Please also write down or enclose the recipe and note the consumed amount (i.e. 1 small bowl, 3 tbsp.).
→ Vegetables, rice, mashed potatoes, sauces: Please note in tablespoons. For potatoes record in terms of size - small/medium/large.
→ Meat, fish, cooked pasta: Please weigh the foods at the beginning to get a good estimate of the quantities.
→ Fried and/or breaded foods: Please also record the used amount of fat or flour for frying and breading.

Column 5: Please again estimate with the above mentioned household measures or weigh with a digital scale left-overs in the bowl, in the jar, on the plate, in the bottle and record the quantity.

Many thanks for your participation and your active support!

Sincerely yours
The Chop-Team

Example of food intake for a 7 month old child:

Child`s Screening Number: _____ Randomisation Number:_____ Date/day: 06.01.2002 Sunday (day 1)

MILK PREPARATION AND MILK INTAKE										
Time	Type of milk	Water	Powder	Cereal		Other		Amount offered	Volume consumed	Meal duration
	(type, brand)	(ml)	(number of spoons)	(type, brand)	(number of spoons)	(type, brand)	(g, ml or spoons)	(ml)	(ml)	(minutes)
7:00	Breast-feeding									20
10:00	Bledina... (infant formula)	130	4	rice flakes, brand	2 tsp. heaped			160	140	10
17:00	Bledina... (infant formula)	160	5		3 tsp. heaped			180	180	12
21:00	Breast-feeding									20

OTHER FLUID INTAKE			
Time	Type of fluid	Quantity consumed	Age of first introduction
	(type, brand)	(ml)	(week)
11:00	Herbal tea	100	28

SOLID FOOD INTAKE				
Time, Meal	Site	Food like consumed (incl. preparation, brand, type...)	Quantity offered	Quantity not consumed
13:00 lunch	at home	Chicken with rice and vegetables (jar, brand name)	190 g (1 jar)	2 tbsp. heaped
18:00 dinner	at home	Milk-cereal home made: Millet flakes	5 tbsp. heaped	
		Type of milk (formula or other)	200 ml	
		Apricots (jar, brand name)	2 tbsp. level	

Food record at the end of month 4 

Child`s Screening Number: _____ Randomisation Number:_____ Date/day:_____**(day 1)**

MILK PREPARATION AND MILK INTAKE										
Time	Type of milk	Water	Powder	Cereal		Other		Amount offered	Volume consumed	Meal duration
	(type, brand)	(ml)	(number of spoons)	(type, brand)	(number of spoons)	(type, brand)	(g, ml or spoons)	(ml)	(ml)	(minutes)

OTHER FLUID INTAKE*			
Time	Type of fluid	Quantity consumed	Age of first introduction
	(type, brand)	(ml)	(week)

* like tea, water, juices, water + sugar, cow`s milk, soy milk, other...

Food record at the end of month 4

Child`s Screening Number: _____ Randomisation Number:_____  Date/day:_____**(day 1)**

SOLID FOOD INTAKE					
Time, Meal	Site (home, day-care…)	Food like consumed (incl. preparation, brand, type, characteristics)	Quantity offered	Quantity not consumed	Code (reserved for the dietitian)

Food record at the end of month 4

Child`s Screening Number: __________ Randomisation Number:___________

Date/day:_________________**(day 1)**

Description of recipe: ______________________________

Quantity	food items + ingredients (incl. preparation)

Description of recipe: ______________________________

Quantity	food items + ingredients (incl. preparation)

Description of recipe: ______________________________

Quantity	food items + ingredients (incl. preparation)

Description of recipe: ______________________________

Quantity	food items + ingredients (incl. preparation)

3. Food records from 12 to 24 months

Food record at the end of month |___|

Child`s Screening Number:_____ Randomisation Number:_____ Date/day:_____ **(day 1)**

CONSUME OF FOODS					
Time, Meal	Site (home, day-care...)	Food like consumed (incl. preparation, brand, type, characteristics)	Quantity offered	Quantity not consumed	Code (reserved for the dietitian)

Food record at the end of month |____|

Child`s Screening Number: _____________ Randomisation Number:__________________ Date/day:________________**(day 1)**

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

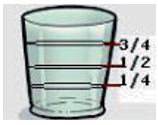
4. Food records from 36 to 96 months

Instruction to complete the three day food diary

Chose 3 days near your child's birthday and write down all the food and drinks (including water) your child eats during these three days (**2 week days and 1 weekend day**):

- During meals and between
- Annotate the way of cooking
- Annotate addition or not of fats (oil, butter, and margarine), sugar, sauces...
- Remember to write the amount consumed to the column prepared for

Please, when possible weight the food with the **scale**, in case that your child eats **out of home, please, use the food photos album**. You will see, that in the diary pages you have the possibility to write the amount of food in grams or with the number of photo from the album.



Thank you very much and do not hesitate to contact us for any doubt!!!

000000000 / 000000000

Sincerely yours
The Chop-Team

3 day food diary for the child. X years old

The diary has been fulfilled by: Mother □ Father □ Career □ Others □

Screening number _____ Age: _____ (months)  Date: _____ **(day 1)**

FOOD AND DRINKS INTAKE												
Time, meal	**Place** (Home, restaurant...)	**Foods + Drinks consumed** (include: trade mark, type, features)	**Offered amount** (g or ml or home measure or portion or photo number)	Amount or portion consumed comparing with the album's photos (g or ml or home measure or portion)								
				Consumed amount	or portion consumed (look at the album)							
					Photo number	¼p	½p	¾p	1p	1¼p	1½p	2p
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□
						□	□	□	□	□	□	□

3 day food diary for the child. X years old

Screening number __________ Age: _______ (months)  Date: ________________ **(day 1)**

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

Description of recipe: ____________________________

Quantity	food items + ingredients (explain preparation)

ANNEX II. DIETARY REFERENCE INTAKES FROM FAO/WHO/UNU AND FROM FNB-IOM

	FAO/WHO/UNU DRI				FNB-IOM DRI			
Age	EAR	units	RNI	units	EAR	units	RDA	units
Phosphorus								
1-3y	-		-		380	mg	460	mg
4-6y	-		-		405	mg	500	mg
7-9y	-		-		405	mg	500	mg
Calcium								
0-6m	240[1]-300[2]	mg	300[1]-400[2]	mg	-		-	
1-3y	440	mg	500	mg	500	mg	700	mg
4-6y	440	mg	600	mg	800	mg	1000	mg
7-9y	440	mg	700	mg	800	mg	1000	mg
Iron [5]								
5-11m	7.2	mg	-		6,9	mg	11	mg
1-3y	4.6	mg	6.5	mg	3	mg	7	mg
4-6y	5	mg	7.1	mg	4,1	mg	10	mg
7-9y	7.1[3]-12[4]	mg	10	mg	4,1	mg	10	mg
Zinc [5]								
0-3m	0.64[3]-0.57[4]	mg/kg	3.5	mg	-		-	
3-6m	0.25	mg/kg	3.5	mg	-		-	
1-3y	0.28	mg/kg	4.9	mg	2.5	mg	3	mg
4-6y	0.23	mg/kg	5.8	mg	4	mg	5	mg
7-9y	0.18	mg/kg	6.7	mg	4	mg	5	mg

(Continued on next page)

*(**Annex II.** Continued)*

Age	FAO/WHO/UNU DRI				FNB-IOM DRI			
	EAR		RNI		EAR		RDA	
		units		units		units		units
Magnesium								
0-6m	-		26[1]-36[2]	mg	-		-	
1-3y	-		60	mg	65	mg	80	mg
4-6y	-		76	mg	110	mg	130	mg
7-9y	-		100	mg	110	mg	130	mg
Iodine								
0-6m	-		90	µg	-		-	
1-3y	-		90	µg	65	µg	90	µg
4-6y	-		90	µg	65	µg	90	µg
7-9y	-		120	µg	65	µg	90	µg
Vitamin B_{12}								
0-6m	0,3	µg	0,4	µg	-		-	
1-3y	0,7	µg	0,9	µg	0.7	µg	0,9	µg
4-6y	1	µg	1,2	µg	1	µg	1.2	µg
7-9y	1,5	µg	1,8	µg	1	µg	1.2	µg
Vitamin B_9								
0-6m	65	µg	80	µg	-		-	
1-3y	120	µg	150	µg	120	µg	150	µg
4-6y	160	µg	200	µg	160	µg	200	µg
7-9y	250	µg	300	µg	160	µg	200	µg

(Continued on next page)

*(**Annex II.** Continued)*

Age	FAO/WHO/UNU DRI				FNB-IOM DRI			
	EAR		RNI		EAR		RDA	
		units		units		units		units
Vitamin A (retinol equivalents)								
0-6	180	µg	375	µg	-		-	
1-3y	200	µg	400	µg	210	µg	300	µg
4-6y	200	µg	450	µg	275	µg	400	µg
7-9y	250	µg	500	µg	275	µg	400	µg
Vitamin D								
0-6m	-		5	µg	-		-	
1-3y	-		5	µg	10	µg	15	µg
4-6y	-		5	µg	10	µg	15	µg
7-9y	-		5	µg	10	µg	15	µg

FAO/WHO/UNU DRI: Dietary Reference Intakes developed by the Food and Agriculture Organization of the United Nations, the World Health Organization and the United Nations University[31,274]. FNB-IOM DRI: Dietary Reference Intakes developed by the Food and Nutrition Board of the American Institute of Medicine[34,115,172,174,176]. EAR: Estimated Average Requirements. RNI: Recommended Nutrient Intake. RDA: Recommended Dietary Allowances. [1]: for Breastfed infants. [2]: for formula fed infants. [3]: for male. [4]: for female. 36 months: 3years. 48 months: 4 years. 72 months: 6 years. 84 months: 7 years. 108 months: 9 years. [5]: EAR and RNI for iron and zinc from FAO/WHO/UNU are provided as the mean value of different recommendations according to bioavailability.

ANNEX III. MICRONUTRIENTS INTAKE BY GENDER AND GENDER DIFFERENCES

1. Sodium intake by gender (mean ±SD).

	Boys			Girls		
	n	mg/day	mg/Kcal/day	n	mg/day	mg/Kcal/day
3 m	452	210.1 ±51.0	0.35 ±0.05	452	198.4 ±49.5***	0.35 ±0.05
6 m	412	315.5 ±102.5	0.44 ±0.13	427	304.7 ±115.9	0.45 ±0.14
12 m	386	550.6 ±265.5	0.62 ±0.29	436	566.1 ±294.5	0.67 ±0.34*
24 m	357	938.3 ±415. 7	0.84 ±0.33	388	983.1 ±422.9	0.90 ±0.36*
36 m	249	1150.6 ±441.2	0.92 ±0.32	278	1115.3 ±410.9	0.95 ±0.32
48 m	252	1263.0 ±479.6	0.93 ±0.31	251	1226.2 ±398.9	0.96 ±0.29
60 m	215	1391.9 ±476.1	0.98 ±0.29	230	1308.8 ±413.4	0.98 ±0.27
72 m	227	1444.3 ±489.7	0.97 ±0.31	241	1473.1 ±444.9	1.02 ±0.28
96 m	191	1786.8 ±575.8	1.07 ±0.30	205	1603.9 ±469.2***	1.06 ±0.26

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

2. Potassium intake by gender (mean ±SD).

	Boys			Girls		
	n	mg/day	mg/Kcal/day	n	mg/day	mg/Kcal/day
3 m	452	820.2 ±201.5	1.38 ±0.20	452	774.9 ±197.6***	1.38 ±0.21
6 m	412	1227.1 ±423.0	1.72 ±0.50	427	1192.5 ±401.4	1.75 ±0.49
12 m	386	1376.7 ±529.6	1.54 ±0.53	436	1384.4 ±514.4	1.61 ±0.49*
24 m	357	1566.3 ±481.7	1.40 ±0.30	388	1576.5 ±527.3	1.44 ±0.36
36 m	249	1679.4 ±439.2	1.35 ±0.28	278	1596.8 ±408.8*	1.36 ±0.28
48 m	252	1786.7 ±476.1	1.33 ±0.28	251	1703.4 ±446.7*	1.34 ±0.28
60 m	215	1852.4 ±487.3	1.31 ±0.26	230	1755.9 ±446.7*	1.30 ±0.26
72 m	227	1919.5 ±501.8	1.28 ±0.25	241	1866.1 ±425.3	1.30 ±0.25
96 m	191	2100.4 ±521.4	1.26 ±0.25	205	1915.5 ±506.8***	1.27 ±0.26

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

3. Calcium intake by gender (mean ±SD).

	Boys			Girls		
	n	mg/day	mg/Kcal/day	n	mg/day	mg/Kcal/day
3 m	452	646.1 ±168.8	1.09 ±0.19	452	609.0 ±167.5***	1.08 ±0.21
6 m	412	781.3 ±246.4	1.09 ±0.27	427	760.7 ±242.7	1.12 ±0.29
12 m	386	716.8 ±258.7	0.80 ±0.26	436	710.1 ±258.9	0.83 ±0.25
24 m	357	694.6 ±262.1	0.63 ±0.21	388	702.9 ±297.3	0.64 ±0.24
36 m	249	684.7 ±271.4	0.55 ±0.20	278	642.0 ±238.2	0.55 ±0.19
48 m	252	671.6 ±252.8	0.50 ±0.17	251	641.1 ±254.6	0.50 ±0.19
60 m	215	671.1 ±254.4	0.47 ±0.16	230	641.8 ±259.8	0.48 ±0.17
72 m	227	651.8 ±257.9	0.44 ±0.15	241	643.9 ±245.8	0.45 ±0.16
96 m	191	724.8 ±272.8	0.43 ±0.15	205	645.7 ±224.3**	0.43 ±0.13

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

4. Phosphorus intake by gender (mean ±SD).

	Boys			**Girls**		
	n	mg/day	mg/Kcal/day	n	mg/day	mg/Kcal/day
3 m	452	530.4 ±159.2	0.89 ±0.20	452	499.3 ±154.6**	0.88 ±0.21
6 m	412	620.6 ±190.6	0.87 ±0.21	427	602.9 ±182.7	0.88 ±0.22
12 m	386	659.2 ±214.5	0.74 ±0.20	436	653.2 ±218.4	0.76 ±0.20
24 m	357	766.2 ±242.1	0.69 ±0.16	388	776.9 ±249.9	0.71 ±0.18
36 m	249	810.9 ±237.4	0.65 ±0.15	278	773.3 ±214.8	0.66 ±0.15
48 m	252	844.3 ±237.5	0.63 ±0.14	251	803.2 ±224.8*	0.63 ±0.14
60 m	215	860.4 ±223.8	0.61 ±0.12	230	818.2 ±232.6	0.61 ±0.13
72 m	227	865.9 ±217.5	0.58 ±0.12	241	853.7 ±204.6	0.59 ±0.12
96 m	191	958.3 ±242.5	0.59 ±0.11	205	887.4 ±220.0***	0.59 ±0.11

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

5. Iron intake by gender (mean ±SD).

	Boys			Girls		
	n	mg/day	mg/100Kcal/day	n	mg/day	mg/100Kcal/day
3 m	452	6.64 ±2.27	1.11 ±0.32	452	6.27±2.25*	1.10 ±0.33
6 m	412	9.74 ±3.62	1.35 ±0.42	427	9.49 ±3.70	1.38 ±0.44
12 m	386	8.42 ±4.71	0.93 ±0.48	436	8.58 ±4.85	0.99 ±0.47
24 m	357	6.40 ±3.08	0.57 ±0.23	388	6.80 ±4.50	0.61 ±0.33
36 m	249	6.62 ±2.70	0.53 ±0.19	278	6.41 ±2.82	0.54 ±0.23
48 m	252	7.01 ±2.85	0.52 ±0.18	251	6.73 ±2.81	0.53 ±0.20
60 m	215	7.27 ±2.64	0.51 ±0.16	230	7.16 ±2.74	0.53 ±0.18
72 m	227	7.77 ±2.79	0.52 ±0.16	241	7.58 ±2.82	0.53 ±0.19
96 m	191	9.64 ±3.28	0.58 ±0.17	205	8.43 ±2.80***	0.56 ±0.16

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

6. Zinc intake by gender (mean ±SD).

	Boys			Girls		
	n	mg/day	mg/100Kcal/day	n	mg/day	mg/100Kcal/day
3 m	452	3.96 ±1.22	0.67 ±0.15	452	3.73 ±1.10**	0.66 ±0.14
6 m	412	4.52 ±1.74	0.63 ±0.19	427	4.49 ±2.10	0.66 ±0.27
12 m	386	6.73 ±16.55	0.74 ±1.71	436	5.00 ±3.04*	0.58 ±0.32
24 m	357	5.60 ±2.53	0.50 ±0.20	388	6.14 ±3.51*	0.56 ±0.28**
36 m	249	6.02 ±2.62	0.48 ±0.17	278	5.87 ±2.42	0.50 ±0.21
48 m	252	6.42 ±2.56	0.48 ±0.19	251	6.09 ±2.39	0.48 ±0.17
60 m	215	6.67 ±2.19	0.47 ±0.14	230	6.37 ±2.44	0.48 ±0.18
72 m	227	6.87 ±2.31	0.46 ±0.13	241	6.77 ±2.36	0.47 ±0.18
96 m	191	7.96 ±2.72	0.48 ±0.16	205	7.07 ±2.31***	0.47 ±0.15

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

7. Magnesium intake by gender (mean ±SD).

	Boys			Girls		
	n	mg/day	mg/100Kcal/day	n	mg/day	mg/100Kcal/day
3 m	452	54.3 ±19.2	9.08 ±2.75	452	51.4 ±18.8*	9.01 ±2.86
6 m	412	84.3 ±33.4	11.74 ±4.16	427	81.5 ±31.8	11.89 ±4.00
12 m	386	106.7 ±44.7	11.91 ±4.53	436	107.6 ±46.5	12.48 ±4.56
24 m	357	139.6 ±46.2	12.50 ±2.91	388	141.0 ±47.0	12.87 ±3.31
36 m	249	154.5 ±41.7	12.45 ±2.68	278	148.4 ±39.2	12.66 ±2.90
48 m	252	167.2 ±43.9	12.47 ±2.63	251	161.3 ±45.9	12.68 ±2.96
60 m	215	177.6 ±48.2	12.57 ±2.71	230	173.3 ±49.0	12.88 ±2.91
72 m	227	185.3 ±48.5	12.43 ±2.60	241	181.8 ±47.2	12.66 ±2.67
96 m	191	211.2 ±49.1	12.71 ±2.52	205	196.0 ±49.6**	13.05 ±2.89

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

8. Iodine intake by gender (mean ±SD).

	Boys			Girls		
	n	µg/day	µg/100Kcal/day	n	µg/day	µg/100Kcal/day
3 m	452	54.4 ±19.3	9.09 ±2.76	452	51.2 ±18.6*	9.01 ±2.89
6 m	412	69.3 ±25.7	9.70 ±3.24	427	68.7 ±31.5	10.09 ±4.17
12 m	386	62.2 ±31.5	6.98 ±3.52	436	61.7 ±30.1	7.28 ±3.28
24 m	357	57.1 ±47.4	5.19 ±4.13	388	59.3 ±49.4	5.44 ±3.88
36 m	249	54.1 ±27.1	4.44 ±2.31	278	62.9 ±51.5*	5.44 ±4.73**
48 m	252	57.9 ±43.4	4.36 ±3.30	251	58.1 ±43.1	04.58 ±3.43
60 m	215	60.9 ±28.8	4.32 ±1.89	230	60.3 ±46.6	4.53 ±3.40
72 m	227	62.1 ±51.1	4.23 ±3.45	241	62.0 ±36.5	4.35 ±2.38
96 m	191	65.9 ±44.9	3.95 ±2.27	205	61.0 ±39.7	4.11 ±2.75

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

9. Vitamin B_{12} intake by gender (mean ±SD).

	Boys			Girls		
	n	µg/day	µg/100Kcal/day	n	µg/day	µg/100Kcal/day
3 m	452	2.00 ±0.71	0.34 ±0.10	452	1.89 ±0.68*	0.33 ±0.11
6 m	412	1.85 ±0.76	0.26 ±0.09	427	1.85 ±0.88	0.27 ±0.11
12 m	386	2.17 ±3.80	0.24 ±0.40	436	2.16 ±3.41	0.24 ±0.31
24 m	357	2.62 ±1.78	0.23 ±0.15	388	2.71 ±1.98	0.25 ±0.16
36 m	249	2.66 ±1.54	0.21 ±0.12	278	2.92 ±2.34	0.25 ±0.19*
48 m	252	2.93 ±1.66	0.22 ±0.11	251	2.77 ±1.61	0.22 ±0.12
60 m	215	3.34 ±1.77	0.24 ±0.13	230	3.04 ±1.64	0.23 ±0.12
72 m	227	3.48 ±3.68	0.24 ±0.29	241	3.36 ±2.03	0.23 ±0.14
96 m	191	3.97 ±2.10	0.24 ±0.11	205	3.64 ±2.63	0.24 ±0.17

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

10. Vitamin B_9 intake by gender (mean ±SD).

	Boys			Girls		
	n	µg/day	µg/100Kcal/day	n	µg/day	µg/100Kcal/day
3 m	452	69.9 ±24.8	11.7 ±3.5	452	65.6 ±23.8**	11.5 ±3.6
6 m	412	111.8 ±45.8	15.6 ±5.7	427	109.9 ±47.5	16.1 ±6.2
12 m	386	111.1 ±53.9	12.4 ±5.5	436	111.7 ±54.1	13.0 ±5.6
24 m	357	106.8 ±40.6	9.6 ±3.0	388	113.1 ±54.0	10.3 ±4.1**
36 m	249	118.2 ±43.4	9.5 ±3.0	278	113.2 ±42.9	9.6 ±3.1
48 m	252	125.1 ±47.2	9.3 ±3.1	251	121.8 ±44.3	9.6 ±3.1
60 m	215	135.2 ±45.7	9.6 ±2.8	230	129.3 ±47.9	9.5 ±3.0
72 m	227	145.9 ±54.2	9.7 ±3.2	241	142.2 ±48.4	9.9 ±3.1
96 m	191	168.8 ±57.6	10.2 ±3.3	205	151.2 ±53.9**	10.0 ±3.1

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

11. Vitamin A intake by gender (mean ±SD).

	Boys			Girls		
	n	µg/day	µg/Kcal/day	n	µg/day	µg/Kcal/day
3 m	452	476.7 ±91.0	0.81 ±0.10	452	449.9 ±81.6***	0.81 ±0.09
6 m	412	855.0 ±792.4	1.21 ±1.16	427	864.8 ±859.9	1.28 ±1.26
12 m	386	984.8 ±853.3	1.11 ±0.98	436	1010.4 ±1621.9	1.16 ±1.64
24 m	357	781.5 ±902.5	0.71 ±0.82	388	850.3 ±1550.2	0.77 ±1.28
36 m	249	704.6 ±514.7	0.57 ±0.41	278	819.2 ±1633.6	0.70 ±1.43
48 m	252	780.4 ±693.3	0.58 ±0.47	251	793.5 ±652.3	0.62 ±0.50
60 m	215	845.4±881.4	0.60 ±0.62	230	798.1± 884.5	0.59 ±0.63
72 m	227	924.8 ±949.4	0.63 ±0.72	241	871.9 ±961.0	0.61 ±0.66
96 m	191	972.6 ±1049.0	0.59 ±0.61	205	842.6 ±808.2	0.56 ±0.56

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

12. Vitamin D intake by gender (mean ±SD).

	Boys			Girls		
	n	µg/day	µg/100Kcal/day	n	µg/day	µg/100Kcal/day
3 m	452	7.75 ±2.75	1.30 ±0.40	452	7.30 ±2.64*	1.28 ±0.40
6 m	412	9.37 ±3.76	1.31 ±0.47	427	9.30 ±4.08	1.36 ±0.53
12 m	386	6.47 ±5.37	0.71 ±0.57	436	6.71 ±4.80	0.78 ±0.51
24 m	357	2.49 ±2.71	0.22 ±0.24	388	3.18±4.03**	0.28 ±0.34**
36 m	249	2.08 ±2.25	0.17 ±0.19	278	1.88 ±1.76	0.16 ±0.16
48 m	252	1.59 ±1.60	0.12 ±0.12	251	1.69 ±1.66	0.13 ±0.13
60 m	215	1.53 ±1.32	0.11 ±0.09	230	1.70 ±1.99	0.13 ±0.20
72 m	227	1.59 ±1.28	0.11 ±0.08	241	1.75 ±1.78	0.12 ±0.13
96 m	191	2.28 ±2.25	0.14 ±0.13	205	1.88 ±1.30*	0.13 ±0.09

m: months. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. male, according to T-Test between genders.

ANNEX IV. ENERGY AND MACRONUTRIENTS INTAKE (TOTAL AND BY GENDER) AND GENDER DIFFERENCES.

Energy and macronutrients intake (mean ±SD)						
		N	Energy (kcal/day)	Protein (g/day)	Fat (g/day)	Carbo-hydrates (g/day)
3 m	All	904	574.1 ±108.0	13.1 ±4.4	29.6 ±6.0	63.7 ±12.4
	Boys	452	589.4 ±106.8	13.5 ±4.4	30.3 ±6.2	65.1 ±12.0
	Girls	452	558.9 ±107.1***	12.6 ±4.3**	28.9 ±5.8***	62.0 ±12.6***
6 m	All	839	696.3 ±133.9	19.5 ±6.8	28.2 ±7.0	90.9 ±21.3
	Boys	412	713.1 ±133.5	20.1 ±6.8	28.6 ±7.2	93.6 ±20.9
	Girls	427	680.0 ±132.4***	18.9 ±6.7*	27.8 ±6.7	88.3 ±21.4***
12 m	All	822	874.6 ±171.5	32.2 ±9.6	31.9 ±8.1	114.5 ±26.8
	Boys	386	892.9 ±171.5	32.8 ±9.5	32.5 ±8.3	117.0 ±27.0
	Girls	436	858.3 ±170.0**	31.6 ±9.8	31.3 ±8.0*	112.3 ±26.6*
24 m	All	745	1104.0 ±229.5	44.9 ±12.8	42.0 ±11.8	136.6 ±32.6
	Boys	357	1109.8 ±228.9	45.1 ±13.0	41.9 ±11.7	138.2 ±33.0
	Girls	388	1098.6 ±230.2	44.7 ±12.6	42.1 ±11.8	135.1 ±32.3

m: months. *: p<0.05 male vs. female, **: p<0.01 male vs. female and ***: p<0.001 male vs. female, and ł p= 0.051 according to T-test between genders.

(Continued on next page)

*(**ANNEX IV.** Continued)*

Energy and macronutrients intake (mean ±SD)						
		N	Energy (kcal/day)	Protein (g/day)	Fat (g/day)	Carbo-hydrates (g/day)
36 m	All	527	121.1 ±233.2	46.7 ±11.8	46.8 ±12.6	151.6 ±35.2
	Boys	249	1248.3 ±240.6	48.1 ±11.9	48.2 ±13.2	156.1 ±37.2
	Girls	278	1179.6 ±221.8***	45.4 ±11.6**	45.5 ±11.9*	147.5 ±32.8**
48 m	All	503	1310.6 ±236.5	49.7 ±12.6	51.5 ±13.5	163.0 ±33.9
	Boys	252	1344.6 ±246.3	50.8 ±12.9	53.0 ±14.2	167.3 ±34.3
	Girls	251	1276.4 ±221.6***	48.6 ±12.1ł	50.1 ±12.6*	158.7 ±32.9**
60 m	All	445	1384.1 ±258.2	51.7 ±13.3	54.2 ±14.1	173.4 ±39.6
	Boys	215	1419.9 ±264.6	53.3 ±13.0	55.1 ±14.5	178.9 ±40.8
	Girls	230	1350.7 ±248.0**	50.2 ±13.4*	53.4 ±13.8	168.2 ±37.8**
72 m	All	468	1467.4 ±244.1	54.7 ±12.4	57.6 ±14.0	183.8 ±38.4
	Boys	227	1492.6 ±251.5	55.3 ±12.6	58.0 ±13.9	188.5 ±39.6
	Girls	241	1443.7 ±235.1*	54.2 ±12.2	57.1 ±14.2	179.3 ±36.8**
96 m	All	396	1590.3 ±284.9	60.7 ±15.0	64.9 ±16.9	191.9 ±40.6
	Boys	191	1673.3 ±273.6	64.2 ±15.0	68.6 ±16.6	201.3 ±39.9
	Girls	205	1513.0 ±273.8***	57.5 ±14.2***	61.5 ±16.5***	183.2 ±39.3***

m: months. *: p<0.05 male vs. female, **: p<0.01 male vs. female and ***: p<0.001 male vs. female, and ł p= 0.051 according to T-test between genders.

ANNEX V. ENERGY AND MACRONUTRIENTS INTAKE BY COUNTRY AND COUNTRY DIFFERENCES.

1. Energy and macronutrients intake by country

Energy and macronutrients intake (mean ±SD)						
		N	Energy (kcal/day)	Protein (g/day)	Fat (g/day)	Carbo-hydrates (g/day)
3 m	GE	138	557.2 ±89.3	13.0 ±3.9	29.2±4.9	60.8 ±10.4
	BE	93	573.7 ±92.2	13.2 ±3.9	29.6 ±4.5	63.8 ±12.5
	IT	273	536.8 ±105.5	11.6 ±4.3	27.2 ±6.4	60.6 ±11.7
	PO	159	577.6 ±120.7	13.2 ±4.3	29.5 ±6.3	65.1 ±14.3
	SP	241	624.0 ±98.0	14.7 ±4.4	32.6 ±5.2	68.0 ±1.5
6 m	GE	131	630.8 ±102.1	19.0 ±6.0	27.0 ±6.5	78.1 ±16.1
	BE	96	667.8 ±95.2	18.2 ±5.9	26.1 ±6.3	88.9 ±17.9
	IT	248	695.3 ±151.8	20.6 ±7.5	29.5 ±7.7	86.4 ±20.2
	PO	145	723.6 ±141.2	21.1 ±6.6	29.1 ±7.5	94.6 ±21.4
	SP	219	730. 9 ±121.7	18.1 ±6.4	27.7 ±5.8	102.0±20.7
12 m	GE	132	769.7 ±145.2	23.6 ±6.4	27.5 ±7.8	106.0 ±22.7
	BE	92	836.6 ±150.6	28.8 ±7.1	28.0 ±7.1	115.5 ±23.6
	IT	250	865.7 ±151.1	33.7 ±7.7	34.5 ±7.6	105.3 ±23.7
	PO	148	900.6 ±181.3	30.7 ±8.2	32.4 ±8.3	121.1 ±28.7
	SP	200	953.0 ±171.7	38.6 ±10.5	32.9 ±7.7	126.1 ±27.3
24 m	GE	120	984.7 ±206.3	35.2 ±8.7	35.7 ±10.6	128.3 ±32.3
	BE	83	1037.6 ±187.1	40.6 ±8.9	38.4 ±10.5	130.0 ±27.5
	IT	224	1054.5 ±174.3	43.6 ±9.2	40.7 ±9.5	130.2 ±27.2
	PO	138	1183.9 ±262.6	42.7 ±10.8	43.4 ±11.9	153.9 ±38.7
	SP	180	1214.4 ±228.2	56.6 ±13.6	48.4 ±12.2	139.8 ±30.9
36 m	GE	120	1069.8 ±208.7	37.0 ±9.5	38.1 ±10.7	141.8 ±32.7
	BE	83	1195.0 ±217.3	42.8 ±7.9	45.6 ±12.2	150.5 ±34.0
	IT	224	1176.9 ±205.5	46.3 ±10.3	44.1 ±10.2	151.9 ±35.7
	PO	138	1360.9 ±228.3	47.0 ±9.5	52.3 ±11.3	173.4 ±36.4
	SP	180	1258.1 ±228.3	54.8 ±12.3	52.9 ±13.1	143.2 ±29.3
48 m	GE	67	1253.9 ±210.9	41.5 ±8.8	46.9±12.1	163.2 ±32.7
	BE	52	1302.5 ±241.1	46.6 ±11.0	47.0 ±14.1	170. 0 ±33.1
	IT	162	1255. 7 ±229.7	47.3 ±10.8	46.9 ±12.1	164.9 ±34.6
	PO	79	1395.5 ±237.2	47.8 ±10.0	55.4 ±11.8	173.8 ±34.9
	SP	143	1355.4 ±233.6	58.4 ±13.1	58.4 ±12.9	152.2 ±30.6

(Continued on next page)

*(**ANNEX V – 1.** Energy and macronutrients intake by country – Continued)*

Energy and macronutrients intake (mean ±SD)						
		N	Energy (kcal/day)	Protein (g/day)	Fat (g/day)	Carbo-hydrates (g/day)
60 m	GE	52	1256.7 ±285.9	40.3 ±10.2	45.6 ±12.6	167.8 ±47.7
	BE	45	1299.0 ±237.6	46.2 ±10.0	47.1 ±12.4	169.4 ±36.2
	IT	152	1396.5 ±231.7	50.7 ±10.7	51.8 ±11.8	185.3 ±37.3
	PO	73	1467.1 ±199.2	51.1 ±9.3	56.9 ±8.6	186.2 ±35.5
	SP	123	1404.6 ±289.7	60.2 ±15.2	61.9 ±16.3	154.9 ±34.4
72 m	GE	56	1327.6 ±230.5	42.2 ±8.7	48.2 ±9.4	177.6 ±40.4
	BE	46	1349.2 ±224.9	46.9 ±9.5	50.4 ±13.7	173.8 ±41.3
	IT	145	1486.6 ±217.4	54.0 ±9.2	54.6 ±11.0	199.0 ±36.1
	PO	95	1527.4 ±227.3	54.6 ±9.3	60.0 ±11.5	190.1 ±35.9
	SP	126	1505.3 ±263.2	64.0 ±12.9	65.9 ±15.7	167.9 ±33.3
96 m	GE	60	1535.8 ±333.3	49.7 ±12.8	56.9 ±16.1	202.4 ±49.6
	BE	30	1463.0 ±317.9	48.4 ±10.0	59.4 ±18.6	181.1 ±43.4
	IT	72	1549.3 ±237.1	57.2 ±9.7	57.0 ±11.7	205.7 ±42.4
	PO	83	1635.4 ±172.9	59.9 ±9.3	66.5 ±9.4	197.2 ±30.3
	SP	151	1632.0 ±315.3	69.8 ±15.7	72.2 ±18.8	180.4 ±36.7

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

2. Energy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	0.582	0.875	<0.001	0.029	1.000	0.001	0.001	<0.001	<0.001
6 m	0.340	<0.001	<0.001	<0.001	0.776	0.011	0.001	0.370	0.031	1.000
12 m	0.023	<0.001	<0.001	<0.001	1.000	0.028	<0.001	0.373	<0.001	0.028
24 m	0.823	0.038	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	<0.001	1.000
36 m	0.009	0.003	<0.001	<0.001	1.000	<0.001	0.678	<0.001	0.014	0.007
48 m	1.000	1.000	0.002	0.031	1.000	0.245	1.000	<0.001	0.0012	1.000
60 m	1.000	0.006	<0.001	0.004	0.229	0.005	0.164	0.492	1.000	0.934
72 m	1.000	<0.001	<0.001	<0.001	0.007	<0.001	0.001	1.000	1.000	1.000
96 m	1.000	1.000	0.369	0.253	1.000	0.042	0.028	0.577	0.405	1.000

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

3. Protein country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	0.019	1.000	0.002	0.028	1.000	0.032	0.004	<0.001	0.004
6 m	1.000	0.294	0.087	1.000	0.031	0.009	1.000	1.000	0.001	<0.001
12 m	<0.001	<0.001	<0.001	<0.001	<0.001	0.856	<0.001	0.006	<0.001	<0.001
24 m	0.003	<0.001	<0.001	<0.001	0.290	1.000	<0.001	1.00	<0.001	<0.001
36 m	0.012	<0.001	<0.001	<0.001	0.253	0.173	<0.001	1.000	<0.001	<0.001
48 m	0.140	0.003	0.007	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
60 m	0.142	<0.001	<0.001	<0.001	0.250	0.296	<0.001	1.000	<0.001	<0.001
72 m	0.225	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	1.000	<0.001	<0.001
96 m	1.000	0.007	<0.001	<0.001	0.014	<0.001	<0.001	1.000	<0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

4. Fat country differences

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	0.008	1.000	<0.001	0.005	1.000	<0.001	0.001	<0.001	<0.001
6 m	1.000	0.009	0.120	1.000	0.001	0.012	0.610	1.000	0.060	0.639
12 m	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.072	0.254	1.000
24 m	0.804	<0.001	<0.001	<0.001	0.980	0.012	<0.001	0.283	<0.001	0.001
36 m	0.002	0.001	<0.001	<0.001	1.000	0.007	0.001	<0.001	<0.001	1.000
48 m	1.000	1.000	0.001	<0.001	1.000	0.002	<0.001	<0.001	<0.001	0.862
60 m	1.000	0.029	<0.001	<0.001	0.307	0.001	<0.001	0.062	<0.001	0.095
72 m	1.000	0.014	<0.001	<0.001	0.551	<0.001	<0.001	0.011	<0.001	0.007
96 m	1.000	1.000	0.004	<0.001	1.000	0.338	0.001	0.002	<0.001	0.077

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

5. Carbohydrates country differences

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	0.681	1.000	0.025	<0.001	0.300	1.000	0.039	0.002	<0.001	0.165
6 m	<0.001	0.001	<0.001	<0.001	1.000	0.297	<0.001	0.001	<0.001	0.005
12 m	0.036	1.000	<0.001	<0.001	0.011	0.956	0.009	<0.001	<0.001	0.695
24 m	1.000	1.000	<0.001	0.019	1.000	<0.001	0.186	<0.001	0.023	0.001
36 m	1.000	0.256	<0.001	1.000	1.000	0.001	1.000	<0.001	0.255	<0.001
48 m	1.000	1.00	0.557	0.259	1.000	1.000	0.010	0.520	0.009	<0.001
60 m	1.000	0.039	0.070	0.379	0.132	0.185	0.266	1.000	<0.001	<0.001
72 m	1.000	0.002	0.429	0.964	0.001	0.151	1.000	0.650	<0.001	<0.001
96 m	0.159	1.000	1.000	0.003	0.041	0.550	1.000	1.000	<0.001	0.019

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

ANNEX VI. MICRONUTRIENTS INTAKE BY COUNTRY AND COUNTRY DIFFERENCES.

1. Sodium and potassium intake by country

			Sodium intake (mean ±SD)		Potassium intake (mean ±SD)	
		N	mg/day	mg/kcal/day	mg/day	mg/kcal/day
3 m	GE	138	207.6 ±38.5	0.37 ±0.04	805.9 ±137.4	1.45 ±0.07
	BE	93	211.8 ±33.5	0.37 ±0.03	825.6 ±128.1	1.44 ±0.06
	IT	273	175.2 ±59.3	0.32 ±0.08	679.1 ±243.1	1.24 ±0.32
	PO	159	207.2 ±43.4	0.36 ±0.02	814.2 ±171.6	1.41 ±0.07
	SP	241	230.3 ±38.1	0.37 ±0.03	905.2 ±141.6	1.45 ±0.06
6 m	GE	131	350.1 ±78.5	0.56 ±0.10	1233.1 ±263.6	1.96 ±0.32
	BE	96	294.3 ±95.3	0.44 ±0.13	1439.8 ±402.9	2.15 ±0.47
	IT	248	303.2 ±129.1	0.43 ±0.13	937.6 ±401.3	1.33 ±0.46
	PO	145	369.2 ±92.1	0.51 ±0.10	1430.9 ±296.7	1.98 ±0.21
	SP	219	261.5 ±90.5	0.36 ±0.10	1255.6 ±397.5	1.71 ±0.42
12 m	GE	132	646.7 ±256.2	0.84 ±0.31	1177.9 ±360.0	1.53 ±0.36
	BE	92	689.3 ±450.8	0.82 ±0.52	1578.9 ±457.5	1.90 ±0.49
	IT	250	435.3 ±167.6	0.50 ±0.16	1055.4 ±336.8	1.22 ±0.34
	PO	148	578.1 ±217.5	0.65 ±0.22	1376.2 ±387.6	1.53 ±0.32
	SP	200	580.9 ±294.8	0.62 ±0.32	1833.7 ±550.0	1.94 ±0.56

(Continued on next page)

*(**ANNEX VI – 1.** Sodium and potassium intake by country – Continued)*

			Sodium intake (mean ±SD)		Potassium intake (mean ±SD)	
		N	mg/day	mg/kcal/day	mg/day	mg/kcal/day
24 m	GE	120	977.6 ±316.1	1.00 ±0.28	1353.5 ±401.1	1.39 ±0.34
	BE	83	1182.7 ±541.1	1.14 ±0.48	1546.9 ±451.7	1.49 ±0.36
	IT	224	711.9 ±291.8	0.67 ±0.26	1371.4 ±361.9	1.30 ±0.26
	PO	138	1007.2 ±371.8	0.85 ±0.26	1672.9 ±535.2	1.41 ±0.29
	SP	180	1124.8 ±436.7	0.93 ±0.33	1900.0 ±530.4	1.57 ±0.35
36 m	GE	120	977.6 ±316.1	1.02 ±0.29	1460.3 ±377.9	1.38 ±0.29
	BE	83	1182.7 ±541.1	1.11 ±0.32	1594.5 ±335.0	1.36 ±0.30
	IT	224	711.9 ±291.8	0.77 ±0.28	1476.8 ±369.3	1.26 ±0.25
	PO	138	1007.2 ±371.8	0.88 ±0.24	1854.4 ±408.0	1.37 ±0.22
	SP	180	1124.8 ±436.7	1.06 ±0.31	1833.2 ±427.9	1.47 ±0.29
48 m	GE	67	1274.4 ±406.5	1.02 ±0.29	1571.2 ±371.9	1.27 ±0.29
	BE	52	1342.0 ±420.9	1.04 ±0.30	1695.3 ±468.2	1.31 ±0.32
	IT	162	1003.6 ±363.4	0.80 ±0.25	1564.9 ±432.6	1.25 ±0.27
	PO	79	1311.5 ±371.8	0.94 ±0.24	1907.9 ±480.1	1.37 ±0.25
	SP	143	1431.3 ±462.7	1.06 ±0.31	1959.0 ±404.0	1.46 ±0.23

(Continued on next page)

*(**ANNEX VI – 1.** Sodium and potassium intake by country – Continued)*

			Sodium intake (mean ±SD)		Potassium intake (mean ±SD)	
		N	mg/day	mg/kcal/day	mg/day	mg/kcal/day
60 m	GE	52	1260.4 ±458.2	1.01 ±0.29	1565.3 ±439.8	1.27 ±0.28
	BE	45	1391.9 ±318.8	1.08 ±0.22	1679.2 ±424.4	1.31 ±0.32
	IT	152	1212.4 ±426.4	0.87 ±0.26	1713.3 ±434.5	1.23 ±0.25
	PO	73	1343.7 ±375.8	0.92 ±0.24	1933.5 ±423.2	1.31 ±0.19
	SP	123	1542.7 ±476.1	1.11 ±0.28	1980.5 ±482.3	1.42 ±0.23
72 m	GE	56	1278.3 ±371.8	0.97 ±0.25	1610.4 ±365.2	1.23 ±0.25
	BE	46	1393.5 ±369.1	1.04 ±0.23	1643.2 ±381.1	1.23 ±0.29
	IT	145	1294.4 ±403.2	0.87 ±0.25	1821.4 ±408.2	1.22 ±0.20
	PO	95	1469.6 ±395.1	0.96 ±0.23	2023.6 ±474.9	1.32 ±0.22
	SP	126	1745.0 ±519.5	1.17 ±0.33	2089.7 ±465.2	1.40 ±0.26
96 m	GE	60	1606.2 ±553.8	1.05 ±0.27	1773.0 ±548.7	1.16 ±0.25
	BE	30	1750.0 ±467.3	1.21 ±0.30	1703.5 ±373.8	1.19 ±0.25
	IT	72	1489.8 ±407.5	0.97 ±0.25	1879.0 ±493.4	1.21 ±0.24
	PO	83	1659.7 ±412.1	1.01 ±0.22	2142.5 ±385.3	1.31 ±0.20
	SP	151	1828.9 ±603.6	1.12 ±0.30	2140.8 ±545.8	1.32 ±0.27

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

1.1. Sodium intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.010	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	0.152	1.000	<0.001	0.794	0.000	<0.001	<0.001	0.373
6 m	raw	0.001	<0.001	1.000	<0.001	1.000	<0.001	0.093	<0.001	<0.001	<0.001
	/kcal	<0.001	<0.001	0.015	<0.001	1.000	<0.001	<0.001	<0.001	<0.001	<0.001
12 m	raw	1.000	<0.001	0.322	0.285	<0.001	0.018	0.013	<0.001	<0.001	1.000
	/kcal	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	1.000
24 m	raw	0.002	<0.001	1.000	0.011	<0.001	0.010	1.000	<0.001	<0.001	0.066
	/kcal	0.019	<0.001	0.001	0.501	<0.001	<0.001	<0.001	<0.001	<0.001	0.275
36 m	raw	0.010	0.006	1.000	<0.001	<0.001	0.540	1.000	<0.001	<0.001	0.143
	/kcal	0.883	<0.001	0.016	1.000	<0.001	<0.001	1.000	0.040	<0.001	<0.001
48 m	raw	1.000	<0.001	1.000	0.094	<0.001	1.000	1.000	<0.001	<0.001	0.362
	/kcal	1.000	<0.001	1.000	1.000	<0.001	0.504	1.000	0.001	<0.001	0.033
60 m	raw	1.000	1.000	1.000	0.001	0.137	1.000	0.436	0.316	<0.001	0.017
	/kcal	1.000	0.012	0.733	0.224	<0.001	0.011	1.000	1.000	<0.001	<0.001
72 m	raw	1.000	1.000	0.085	<0.001	1.000	1.000	<0.001	0.021	<0.001	<0.001
	/kcal	1.000	0.271	1.000	<0.001	0.004	1.000	0.039	0.116	<0.001	<0.001
96 m	raw	1.000	1.000	1.000	0.051	0.212	1.000	1.000	0.422	<0.001	0.172
	/kcal	0.065	0.972	1.000	0.780	<0.001	0.006	0.880	1.000	0.001	0.043

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

1.2. Potassium intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.003	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	0.986	1.000	<0.001	1.000	1.000	<0.001	<0.001	0.307
6 m	raw	<0.001	<0.001	<0.001	1.000	<0.001	1.000	<0.001	<0.001	<0.001	<0.001
	/kcal	0.005	<0.001	1.000	<0.001	<0.001	0.020	<0.001	<0.001	<0.001	<0.001
12 m	raw	<0.001	0.073	0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
	/kcal	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001
24 m	raw	0.032	1.000	<0.001	<0.001	0.029	0.476	<0.001	<0.001	<0.001	<0.001
	/kcal	0.166	0.166	1.000	<0.001	<0.001	0.438	0.571	0.021	<0.001	<0.001
36 m	raw	0.458	1.000	<0.001	<0.001	0.478	0.001	0.001	<0.001	<0.001	1.000
	/kcal	1.000	0.014	1.000	0.155	0.182	1.000	0.100	0.032	<0.001	0.063
48 m	raw	1.000	1.000	<0.001	<0.001	0.569	0.057	0.002	<0.001	<0.001	1.000
	/kcal	1.000	1.000	0.281	<0.001	1.000	1.000	0.009	0.012	<0.001	0.192
60 m	raw	1.000	0.396	<0.001	<0.001	1.000	0.028	0.001	0.006	<0.001	1.000
	/kcal	1.000	1.000	1.000	0.002	0.581	1.000	0.106	0.156	<0.001	0.043
72 m	raw	1.000	0.020	<0.001	<0.001	0.150	<0.001	<0.001	0.004	<0.001	1.000
	/kcal	1.000	1.000	0.216	<0.001	1.000	0.467	0.001	0.027	<0.001	0.175
96 m	raw	1.000	1.000	<0.001	<0.001	1.000	<0.001	<0.001	0.010	0.003	1.000
	/kcal	1.000	1.000	0.003	<0.001	1.000	0.197	0.074	0.097	0.015	1.000

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

2. Calcium and phosphorus intake by country

			Calcium intake (mean ±SD)		Phosphorus intake (mean ±SD)	
		N	mg/day	mg/kcal/day	mg/day	mg/kcal/day
3 m	GE	138	637.8 ±105.1	1.15 ±0.06	529.6 ±90.0	0.95 ±0.05
	BE	93	654.1 ±99.8	1.14 ±0.06	540.5 ±83.9	0.94 ±0.05
	IT	273	525.5 ±214.9	0.96 ±0.31	415.3 ±208.6	0.75 ±0.33
	PO	159	643.2 ±135.9	1.12 ±0.07	534.8 ±115.4	0.93 ±0.07
	SP	241	716.6 ±112.2	1.15 ±0.05	596.0 ±97.7	0.96 ±0.05
6 m	GE	131	801.0 ±192.5	1.27 ±0.26	621.2 ±148.5	0.99 ±0.20
	BE	96	715.2 ±196.8	1.07 ±0.26	581.3 ±150.6	0.87 ±0.19
	IT	248	709.0 ±283.0	1.00 ±0.32	561.2 ±231. 4	0.79 ±0.27
	PO	145	883.8 ±213.5	1.22 ±0.17	699.4 ±157.4	0.97 ±0.13
	SP	219	772.2 ±235.3	1.06 ±0.25	617.9 ±159.3	0.85 ±0.17
12 m	GE	132	549.6 ±206.1	0.72 ±0.24	499.6 ±168.1	0.25 ±0.19
	BE	92	639.9 ±141.2	0.78 ±0.17	591.5 ±141.0	0.71 ±0.15
	IT	250	741.7 ±200.3	0.86 ±0.18	645.4 ±157.0	0.75 ±0.15
	PO	148	694.4 ±209.5	0.77 ±0.19	646.3 ±180.2	0.72 ±0.14
	SP	200	833.5 ±346.8	0.88 ±0.37	809.4 ±262.2	0.85 ±0.26

(Continued on next page)

*(**ANNEX VI – 2.** Calcium and phosphorus intake by country – Continued)*

			Calcium intake (mean ±SD)		Phosphorus intake (mean ±SD)	
		N	mg/day	mg/kcal/day	mg/day	mg/kcal/day
24 m	GE	120	527.1 ±227.0	0.54 ±0.23	615.2 ±194.3	0.63 ±0.18
	BE	83	703.9 ±218.2	0.69 ±0.19	712.9 ±181.7	0.69 ±0.14
	IT	224	726.6 ±236.4	0.69 ±0.20	756.3 ±161.9	0.72 ±0.12
	PO	138	584.0 ±239.6	0.49 ±0.16	679.6 ±196.1	0.57 ±0.10
	SP	180	864.9 ±315.4	0.72 ±0.23	993.3 ±274.0	0.82 ±0.18
36 m	GE	120	531.5 ±209.7	0.49 ±0.15	653.2 ±185.7	0.61 ±0.12
	BE	83	673.7 ±229.0	0.58 ±0.21	727.7 ±166.1	0.62 ±0.16
	IT	224	663.1 ±252.0	0.57 ±0.20	775.0 ±193.8	0.66 ±0.14
	PO	138	567.2 ±200.5	0.42 ±0.03	730.8 ±156.9	0.54 ±0.09
	SP	180	802.1 ±260.2	0.64 ±0.18	968.5 ±247.6	077 ±0.15
48 m	GE	67	528.8 ±187.9	0.42 ±0.14	692.7 ±176.1	0.56 ±0.11
	BE	52	682.4 ±255.0	0.52 ±0.17	779.2 ±210.5	0.60 ±0.12
	IT	162	612.8 ±259.5	0.49 ±0.19	779.6 ±206.0	0.62 ±0.12
	PO	79	549.7 ±198.0	0.39 ±.013	737.9 ±185.5	0.53 ±0.09
	SP	143	814.9 ±220.3	0.61 ±0.16	999.0 ±216.2	0.74 ±0.12

(Continued on next page)

*(**ANNEX VI – 2.** Calcium and phosphorus intake by country – Continued)*

			Calcium intake (mean ±SD)		Phosphorus intake (mean ±SD)	
		N	mg/day	mg/kcal/day	mg/day	mg/kcal/day
60 m	GE	52	515.6 ±203.6	0.42 ±0.15	683.0 ±189.2	0.55 ±0.12
	BE	45	591.2 ±251.5	0.45 ±0.18	757.6 ±201.8	0.59 ±0.14
	IT	152	609.6 ±234.6	0.44 ±0.15	814.4 ±191.7	0.58 ±0.10
	PO	73	565.7 ±185.1	0.39 ±0.11	770.0 ±160.5	0.52 ±0.08
	SP	123	849.8 ±239.0	0.61 ±0.14	1004.7 ±238.0	0.72 ±0.10
72 m	GE	56	515.3 ±160.9	0.39 ±0.11	698.2 ±157.9	0.53 ±0.10
	BE	46	604.7 ±240.3	0.45 ±0.17	764.7 ±201.6	0.57 ±0.15
	IT	145	542.3 ±188.6	0.37 ±0.13	826.3 ±155.6	0.56 ±0.09
	PO	95	611.6 ±191.6	0.40 ±0.11	822.3 ±157.4	0.54 ±0.08
	SP	126	871.1 ±250.2	0.58 ±0.14	1032.8 ±214.7	0.69 ±0.10
96 m	GE	60	586.6 ±257.0	0.38 ±0.13	823.5 ±231.0	0.54 ±0.10
	BE	30	555.3 ±200.2	0.38 ±0.13	751.7 ±185.3	0.52 ±0.11
	IT	72	566.5 ±156.9	0.37 ±0.09	837.5 ±164.2	0.54 ±0.08
	PO	83	587.2 ±176.2	0.36 ±0.10	878.4 ±141.0	0.54 ±0.06
	SP	151	857.1 ±235.4	0.53 ±0.13	1092.2 ±229.0	0.67 ±0.10

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

2.1. Calcium intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.008	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	0.585
6 m	raw	0.072	0.003	0.039	1.000	1.000	<0.001	0.499	<0.001	0.041	<0.001
	/kcal	<0.001	<0.001	1.000	<0.001	0.267	<0.001	1.000	<0.001	0.304	<0.001
12 m	raw	0.061	<0.001	<0.001	<0.001	0.006	0.900	<0.001	0.592	0001	<0.001
	/kcal	0.677	<0.001	0.532	<0.001	0.087	1.000	0.010	0.012	1.000	0.001
24 m	raw	<0.001	<0.001	0.745	<0.001	1.000	0.008	<0.001	<0.001	<0.001	<0.001
	/kcal	<0.001	<0.001	0.495	<0.001	1.000	<0.001	1.000	<0.001	1.000	<0.001
36 m	raw	0.006	<0.001	1.000	<0.001	1.000	0.090	0.007	0.024	<0.001	<0.001
	/kcal	0.069	0.021	0.067	<0.001	1.000	<0.001	0.247	<0.001	0.004	<0.001
48 m	raw	0.003	0.124	1.000	<0.001	0.585	0.013	0.004	0.464	<0.001	<0.001
	/kcal	0.010	0.060	1.000	<0.001	1.000	<0.001	0.018	<0.001	<0.001	<0.001
60 m	raw	1.000	0.102	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	1.000	0.143	<0.001	0.130	<0.001	<0.001
72 m	raw	0.329	1.000	0.067	<0.001	0.798	1.000	<0.001	0.128	<0.001	<0.001
	/kcal	0.248	1.000	1.000	<0.001	0.002	0.421	<0.001	0.497	<0.001	<0.001
96 m	raw	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

2.2. Phosphorus intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.013	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	raw	1.000	0.022	0.004	1.000	1.000	<0.001	0.996	<0.001	0.008	<0.001
	/kcal	<0.001	<0.001	1.000	<0.001	0.010	0.003	1.000	<0.001	0.032	<0.001
12 m	raw	0.004	<0.001	<0.001	<0.001	0.215	0.316	<0.001	1.000	<0.001	<0.001
	/kcal	0.107	<0.001	0.023	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
24 m	raw	0.010	<0.001	0.129	<0.001	1.000	1.000	<0.001	0.007	<0.001	<0.001
	/kcal	0.053	<0.001	0.014	<0.001	1.000	<0.001	<0.001	<0.001	<0.001	<0.001
36 m	raw	0.308	<0.001	0.120	<0.001	1.000	1.000	<0.001	0.938	<0.001	<0.001
	/kcal	1.000	0.039	0.006	<0.001	0.492	0.003	<0.001	<0.001	<0.001	<0.001
48 m	raw	0.214	0.033	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	0.435	0.001	1.000	<0.001	1.000	0.007	<0.001	<0.001	<0.001	<0.001
60 m	raw	0.704	0.001	0.180	<0.001	0.982	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	0.975	0.628	1.000	<0.001	1.000	0.015	<0.001	0.001	<0.001	<0.001
72 m	raw	0.621	<0.001	<0.001	<0.001	0.419	0.731	<0.001	1.000	<0.001	<0.001
	/kcal	0.350	0.584	1.000	<0.001	1.000	0.698	<0.001	1.000	<0.001	<0.001
96 m	raw	1.000	1.000	1.000	<0.001	0.485	0.030	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

3. Iron and zinc intake by country

			Iron intake (mean ±SD)		Zinc intake (mean ±SD)	
		N	mg/day	mg/100kcal/day	mg/day	mg/100kcal/day
3 m	GE	138	6.69 ±1.17	1.20 ±0.08	3.95 ±0.69	0.71 ±0.05
	BE	93	6.82 ±1.16	1.19 ±0.11	3.99 ±0.57	0.70 ±0.03
	IT	273	4.97 ±3.03	0.89 ±0.51	3.13 ±1.46	0.57 ±0.22
	PO	159	6.76 ±1.47	1.17 ±0.09	3.98 ±0.82	0.69 ±0.03
	SP	241	7.65 ±1.45	1.22 ±0.10	4.45 ±0.92	0.71 ±0.09
6 m	GE	131	9.11 ±2.21	1.45 ±0.30	4.25 ±1.17	0.68 ±0.17
	BE	96	9.05 ±2.10	1.36 ±0.26	4.78 ±2.87	0.72 ±0.40
	IT	248	8.15 ±4.29	1.14 ±0.53	4.05 ±1.54	0.57 ±0.19
	PO	145	10.55 ±2.38	1.46 ±0.19	4.95 ±1.11	0.69 ±0.10
	SP	219	11.20 ±4.03	1.52 ±0.43	4.74 ±2.43	0.65 ±0.27
12 m	GE	132	6.29 ±2.44	0.82 ±0.29	3.57 ±1.81	0.46 ±0.21
	BE	92	8.94 ±2.27	1.09 ±0.26	5.35 ±1.98	0.65 ±0.24
	IT	250	5.68 ±2.67	0.65 ±0.27	6.67 ±20.35	0.74 ±2.10
	PO	148	8.27 ±2.66	0.92 ±0.24	4.62 ±1.27	0.52 ±0.12
	SP	200	13.45 ±6.00	0.41 ±0.61	7.31 ±4.71	0.78 ±0.53

(Continued on next page)

*(**ANNEX VI – 3.** Iron and zinc intake by country – Continued)*

			Iron intake (mean ±SD)		Zinc intake (mean ±SD)	
		N	mg/day	mg/100kcal/day	mg/day	mg/100kcal/day
24 m	GE	120	4.95 ±1.81	0.50 ±0.15	4.51 ±1.44	0.46 ±0.13
	BE	83	7.73 ±3.10	0.75 ±0.28	6.46 ±2.59	0.62 ±0.22
	IT	224	4.72 ±1.64	0.45 ±0.14	4.85 ±1.95	0.46 ±0.16
	PO	138	6.32 ±2.38	0.54 ±0.18	5.42 ±2.14	0.46 ±0.18
	SP	180	9.77 ±5.61	0.80 ±0.40	8.15 ±4.34	0.68 ±0.35
36 m	GE	120	4.91 ±1.60	0.46 ±0.11	4.62 ±1.31	0.43 ±0.09
	BE	83	7.15 ±2.20	0.61 ±0.19	6.42 ±2.35	0.55 ±0.24
	IT	224	4.96 ±1.56	0.42 ±0.11	5.18 ±1.42	0.44 ±0.10
	PO	138	7.20 ±2.14	0.53 ±0.15	5.81 ±1.49	0.43 ±0.11
	SP	180	8.86 ±3.21	0.71 ±0.27	7.68 ±3.64	0.62 ±0.27
48 m	GE	67	5.63 ±1.27	0.45 ±0.09	5.13 ±1.30	0.41 ±0.09
	BE	52	6.68 ±2.21	0.52 ±0.15	6.24 ±2.33	0.48 ±0.17
	IT	162	5.30 ±1.56	0.42 ±0.10	5.40 ±1.56	0.43 ±0.10
	PO	79	6.97 ±2.04	0.50 ±0.12	5.77 ±1.52	0.41 ±0.09
	SP	143	9.25 ±3.39	0.68 ±0.23	8.00 ±3.22	0.60 ±0.25

(Continued on next page)

*(**ANNEX VI – 3.** Iron and zinc intake by country – Continued)*

			Iron intake (mean ±SD)		Zinc intake (mean ±SD)	
		N	mg/day	mg/100kcal/day	mg/day	mg/100kcal/day
60 m	GE	52	6.08 ±2.24	0.48 ±0.11	5.34 ±1.61	0.43 ±0.12
	BE	45	6.77 ±1.59	0.53 ±0.14	5.97 ±1.72	0.47 ±0.16
	IT	152	6.08 ±2.16	0.44 ±0.15	6.04 ±1.47	0.43 ±0.09
	PO	73	7.31 ±2.24	0.50 ±0.13	6.28 ±1.53	0.43 ±0.08
	SP	123	9.22 ±2.89	0.66 ±0.17	7.94 ±3.23	0.57 ±0.22
72 m	GE	56	6.30 ±2.37	0.47 ±0.15	5.36 ±1.37	0.41 ±0.10
	BE	46	6.91 ±1.86	0.52 ±0.14	5.95 ±1.74	0.44 ±0.11
	IT	145	6.47 ±1.64	0.44 ±0.10	6.59 ±1.46	0.44 ±0.08
	PO	95	7.62 ±1.87	0.50 ±0.11	6.45 ±1.37	0.42 ±0.07
	SP	126	9.97 ±3.45	0.67 ±0.22	8.33 ±3.30	0.56 ±0.24
96 m	GE	60	7.65 ±2.68	0.50 ±0.13	6.16 ±1.76	0.40 ±0.09
	BE	30	7.19 ±1.87	0.50 ±0.15	6.41 ±1.79	0.46 ±0.18
	IT	72	7.64 ±2.46	0.49 ±0.14	6.94 ±1.48	0.45 ±0.09
	PO	83	9.05 ±2.18	0.55 ±0.12	7.33 ±1.86	0.45 ±0.11
	SP	151	10.55 ±3.40	0.65 ±0.18	8.61 ±3.17	0.53 ±0.20

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

3.1. Iron intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.009	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	0.708
6 m	raw	1.000	0.101	0.006	<0.001	0.298	0.010	<0.001	<0.001	<0.001	0.791
	/kcal	0.911	<0.001	1.000	0.981	<0.001	0.535	0.008	<0.001	<0.001	1.000
12 m	raw	<0.001	1.000	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	<0.001
	/kcal	<0.001	<0.001	0.287	<0.001	<0.001	0.009	<0.001	<0.001	<0.001	<0.001
24 m	raw	<0.001	1.000	0.010	<0.001	<0.001	0.024	<0.001	<0.001	<0.001	<0.001
	/kcal	<0.001	0.499	1.000	<0.001	<0.001	<0.001	1.000	0.009	<0.001	<0.001
36 m	raw	<0.001	1.000	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	<0.001
	/kcal	<0.001	1.000	0.054	<0.001	<0.001	0.168	0.002	<0.001	<0.001	<0.001
48 m	raw	0.153	1.000	0.006	<0.001	0.002	1.000	<0.001	<0.001	<0.001	<0.001
	/kcal	0.260	1.000	0.754	<0.001	0.002	1.000	<0.001	0.005	<0.001	<0.001
60 m	raw	1.000	1.000	0.043	<0.001	0.843	1.000	<0.001	0.003	<0.001	<0.001
	/kcal	0.766	0.663	1.000	<0.001	0.001	1.000	<0.001	0.056	<0.001	<0.001
72 m	raw	1.000	1.000	0.012	<0.001	1.000	1.000	<0.001	0.003	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	0.014	1.000	<0.001	0.018	<0.001	<0.001
96 m	raw	1.000	1.000	0.035	<0.001	1.000	0.020	<0.001	0.019	<0.001	0.001
	/kcal	1.000	1.000	0.364	<0.001	1.000	1.000	<0.001	0.146	<0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

3.2. Zinc intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.003	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	0.816
6 m	raw	0.397	1.000	0.025	0.216	0.014	1.000	1.000	<0.001	0.001	1.000
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	0.110	<0.001	0.009	0.954
12 m	raw	1.000	0.126	1.000	0.039	1.000	1.000	1.000	0.869	1.000	0.315
	/kcal	1.000	0.298	1.000	0.186	1.000	1.000	1.000	0.671	1.000	0.425
24 m	raw	<0.001	1.000	0.079	<0.001	<0.001	0.069	<0.001	0.561	<0.001	<0.001
	/kcal	<0.001	1.000	1.000	<0.001	<0.001	<0.001	0.937	1.000	<0.001	<0.001
36 m	raw	<0.001	0.683	0.007	<0.001	0.004	1.000	0.005	0.344	<0.001	<0.001
	/kcal	0.001	1.000	1.000	<0.001	<0.001	0.001	0.188	1.000	<0.001	<0.001
48 m	raw	0.068	1.000	0.824	<0.001	0.176	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	0.247	1.000	1.000	<0.001	0.490	0.234	<0.001	1.000	<0.001	<0.001
60 m	raw	1.000	0.449	0.166	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	1.000	1.000	0.001	1.000	<0.001	<0.001
72 m	raw	1.000	0.003	0.024	<0.001	0.790	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
96 m	raw	1.000	0.618	0.042	<0.001	1.000	0.720	<0.001	1.000	<0.001	0.001
	/kcal	1.000	0.782	0.836	<0.001	1.000	1.000	0.107	1.000	0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

4. Magnesium and iodine intake by country

			Magnesium intake (mean ±SD)		Iodine intake (mean ±SD)	
		N	mg/day	mg/100kcal/day	µg/day	µg/100kcal/day
3 m	GE	138	55.3 ±9.2	9.93 ±0.47	55.6 ±9.3	9.98 ±0.52
	BE	93	57.4 ±9.8	10.00 ±0.39	56.4 ±10.3	9.87 ±1.28
	IT	273	39.8 ±26.3	7.06 ±4.47	40.0 ±26.2	7.10 ±4.46
	PO	159	56.0 ±11.6	9.69 ±0.39	56.5 ±12.0	9.78 ±0.56
	SP	241	62.4 ±9.9	10.00 ±0.30	62.0 ±10.2	9.94 ±0.58
6 m	GE	131	75.2 ±21.4	11.96 ±3.05	79.2 ±19.4	12.58 ±2.58
	BE	96	98.3 ±28.1	14.62 ±3.20	66.6 ±22.4	10.01 ±3.22
	IT	248	63.4 ±33.5	8.88 ±4.35	58.3 ±29.8	8.19 ±3.98
	PO	145	95.6 ±19.6	13.27 ±1.57	81.8 ±20.0	11.32 ±1.67
	SP	219	94.3 ±33.7	12.87 ±3.87	67.4 ±34.3	9.23 ±4.01
12 m	GE	132	84.3 ±34.8	11.02 ±4.46	64.7 ±25.3	8.40 ±2.94
	BE	92	121.1 ±37.3	14.46 ±3.53	62.9 ±24.7	7.76 ±3.35
	IT	250	83.3 ±27.7	9.62 ±2.79	50.4 ±22.7	5.84 ±2.56
	PO	148	98.9 ±27.8	11.00 ±2.34	62.0 ±20.7	6.91 ±1.98
	SP	200	151.9 ±49.2	16.11 ±4.99	74.1 ±43.6	7.81 ±4.67

(Continued on next page)

*(**ANNEX VI – 4.** Magnesium and iodine intake by country – Continued)*

			Magnesium intake (mean ±SD)		Iodine intake (mean ±SD)	
		N	mg/day	mg/100kcal/day	µg/day	µg/100kcal/day
24 m	GE	120	130.7 ±41.5	13.37 ±3.41	62.4 ±28.3	6.43 ±2.77
	BE	83	142.1 ±43.1	13.63 ±2.85	67.6 ±40.0	6.60 ±3.88
	IT	224	118.4 ±32.0	11.23 ±2.43	52.0 ±33.6	4.93 ±3.23
	PO	138	137.1 ±45.4	11.53 ±2.64	42.6 ±21.7	3.57 ±1.58
	SP	180	175.7 ±47.4	14.52 ±2.95	71.0 ±79.1	5.81 ±5.93
36 m	GE	120	144.4 ±39.8	13.61 ±3.31	60.2 ±20.5	5.74 ±1.96
	BE	83	152.9 ±36.3	13.00 ±3.13	68.0 ±46.7	6.18 ±6.55
	IT	224	132.7 ±35.0	11.30 ±2.35	57.0 ±34.6	4.85 ±2.84
	PO	138	156.5 ±33.8	11.52 ±1.77	45.2 ±17.8	3.31 ±1.11
	SP	180	176.3 ±39.9	14.09 ±2.35	65.1 ±63.2	5.20 ±4.89
48 m	GE	67	155.8 ±36.3	12.56 ±2.71	66.0 ±34.9	5.33 ±3.00
	BE	52	161.9 ±39.5	12.54 ±2.55	68.3 ±43.3	5.23 ±2.99
	IT	162	142.3 ±36.2	11.37 ±2.21	56.9 ±31.5	4.49 ±2.23
	PO	79	164.3 ±50.9	11.76 ±2.95	42.9 ±12.9	3.08 ±0.81
	SP	143	193.9 ±39.9	14.41 ±2.48	60.0 ±62.7	4.53 ±4.99

(Continued on next page)

*(**ANNEX VI – 4.** Magnesium and iodine intake by country – Continued)*

			Magnesium intake (mean ±SD)		Iodine intake (mean ±SD)	
		N	mg/day	mg/100kcal/day	µg/day	µg/100kcal/day
60 m	GE	52	163.2 ±50.0	13.11 ±2.86	62.8 ±25.4	5.21 ±2.57
	BE	45	159.5 ±36.6	12.44 ±2.90	64.4 ±30.8	5.19 ±3.30
	IT	152	160.9 ±40.0	11.52 ±2.20	65.6 ±32.6	4.69 ±2.17
	PO	73	168.2 ±39.3	11.43 ±1.95	44.9 ±17.7	3.03 ±0.90
	SP	123	208.4 ±51.3	14.93 ±2.52	61.3 ±56.9	4.32 ±3.64
72 m	GE	56	166.2 ±44.8	12.64 ±3.19	67.1 ±22.5	5.17±1.89
	BE	46	164.5 ±44.2	12.28 ±2.80	61.4 ±27.0	4.69 ±2.69
	IT	145	167.8 ±36.7	11.29 ±1.86	65.6 ±37.1	4.45 ±2.62
	PO	95	176.5 ±39.2	11.53 ±1.82	48.2 ±14.6	3.16 ±.0.84
	SP	126	221.5 ±46.9	14.82 ±2.55	66.3 ±69.8	4.43 ±4.29
96 m	GE	60	182.6± 50.6	11.99 ±2.63	74.7 ±34.0	4.92 ±1.86
	BE	30	183.9 ±44.0	12.80 ±2.86	67.7 ±77.2	4.70 ±5.53
	IT	72	183.6 ±41.9	11.86 ±2.01	68.4 ±35.6	4.50 ±2.41
	PO	83	192.7 ±35.2	11.80 ±1.93	51.7 ±16.4	3.18±1.05
	SP	151	230.7 ±49.1	14.34 ±2.77	61.9 ±47.1	3.79 ±2.29

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

4.1. Magnesium intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	0.001	<0.001	1.000	0.146	<0.001	<0.001	0.002
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	raw	<0.001	0.002	<0.001	<0.001	<0.001	1.000	1.000	<0.001	<0.001	1.000
	/kcal	<0.001	<0.001	0.023	0.201	<0.001	0.039	0.001	<0.001	<0.001	1.000
12 m	raw	<0.001	1.000	0.008	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	/kcal	<0.001	0005	1.000	<0.001	<0.001	<0.001	0.005	0.004	<0.001	<0.001
24 m	raw	0.532	0.091	1.000	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	<0.001	0.006	<0.001	<0.001	0.174	1.000	<0.001	<0.001
36 m	raw	1.000	0.184	0.341	<0.001	0.004	1.000	0.001	<0.001	<0.001	0.001
	/kcal	1.000	<0.001	<0.001	1.000	<0.001	0.006	0.074	1.000	<0.001	<0.001
48 m	raw	1.000	0.212	1.000	<0.001	0.023	1.000	<0.001	0.001	<0.001	<0.001
	/kcal	1.000	0.011	0.564	<0.001	0.036	0.851	<0.001	1.000	<0.001	<0.001
60 m	raw	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	0.001	0.001	<0.001	0.256	0.279	<0.001	1.000	<0.001	<0.001
72 m	raw	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	0.003	0.050	<0.001	0.127	0.731	<0.001	1.000	<0.001	<0.001
96 m	raw	1.000	1.000	1.000	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
	/kcal	1.000	1.000	1.000	<0.001	0.810	0.600	0.019	1.000	<0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

4.2. Iodine intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	0.004	<0.001	1.000	0.068	<0.001	<0.001	0.015
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	raw	0.007	<0.001	1.000	0.001	0.121	<0.001	1.000	<0.001	0.004	<0.001
	/kcal	<0.001	<0.001	0.022	<0.001	<0.001	0.036	0.622	<0.001	0.011	<0.001
12 m	raw	1.000	<0.001	1.000	0.044	0.005	1.000	0.027	0.002	<0.001	0.002
	/kcal	1.000	<0.001	0.001	1.000	<0.001	0.503	1.000	0.016	<0.001	0.113
24 m	raw	1.000	0.518	0.008	1.000	0.106	0.002	1.000	0.680	0.001	<0.001
	/kcal	1.000	0.007	<0.001	1.000	0.008	<0.001	1.000	0.012	0.249	<0.001
36 m	raw	1.000	1.000	0.192	1.000	0.831	0.013	1.000	0.309	0.963	0.006
	/kcal	1000	0.750	<0.001	1.000	0.202	<0.001	1.000	0.019	1.000	0.003
48 m	raw	1.000	1.000	0.012	1.000	0.941	0.009	1.000	0.174	1.000	0.043
	/kcal	1.000	0.816	<0.001	1.000	1.000	0.003	1.000	0.020	1.000	0.019
60 m	raw	1.000	1.000	0.108	1.000	1.000	0.077	1.000	0.002	1.000	0.042
	/kcal	1.000	1.000	<0.001	0.474	1.000	<0.001	0.664	<0.001	1.000	0.012
72 m	raw	1.000	1.000	0.109	1.000	1.000	0.944	1.000	0.027	1.000	0.025
	/kcal	1.000	1.000	<0.001	1.000	1.000	0.035	1.000	0.008	1.000	0.016
96 m	raw	1.000	1.000	0.013	0.462	1.000	0.736	1.000	0.134	1.000	0.735
	/kcal	1.000	1.000	<0.001	0.029	1.000	0.040	0.657	0.010	0.456	0.705

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

5. Vitamin B_{12} and vitamin B_9 intake by country

			Vitamin B_{12} intake (mean ±SD)		Vitamin B_9 intake (mean ±SD)	
		N	µg/day	µg/100kcal/day	µg/day	µg/100kcal/day
3 m	GE	138	2.04 ±0.34	0.37 ±0.02	70.9 ±11.6	12.72 ±0.51
	BE	93	2.06 ±0.30	0.36 ±0.02	72.0 ±10.4	12.59 ±0.69
	IT	273	1.48 ±0.98	0.26 ±0.17	51.2 ±33.8	9.08 ±5.74
	PO	159	2.06 ±0.43	0.36 ±0.02	71.9 ±15.0	12.45 ±0.53
	SP	241	2.30 ±0.36	0.37±0.01	80.4 ±13.2	12.88 ±0.63
6 m	GE	131	1.88 ±0.57	0.30 ±0.08	110.8 ±31.4	17.63 ±4.37
	BE	96	1.80 ±0.54	0.27 ±0.07	130.5 ±42.8	19.41 ±5.42
	IT	248	1.41 ±0.85	0.20 ±0.12	83.4 ±47.7	11.64 ±6.31
	PO	145	2.19 ±0.57	0.30 ±0.05	131.4 ±30.9	18.17 ±2.49
	SP	219	2.14 ±0.92	0.29 ±0.11	119.7 ±48.6	13.31 ±5.48
12 m	GE	132	1.56 ±1.42	0.20 ±0.18	86.6 ±36.2	11.27 ±2.30
	BE	92	2.48 ±5.44	0.29 ±0.54	151.6 ±62.6	18.26 ±6.82
	IT	250	1.46 ±1.06	0.17 ±0.12	75.2 ±31.4	8.65 ±3.26
	PO	148	2.00 ±0.68	0.22 ±0.06	115.7 ±35.3	12.89 ±3.22
	SP	200	3.44 ±5.84	0.25 ±0.57	151.4 ±52.7	15.97 ±5.24

(Continued on next page)

*(**ANNEX VI – 5.** Vitamin B_{12} and vitamin B_9 intake by country – Continued)*

			Vitamin B_{12} intake (mean ±SD)		Vitamin B_9 intake (mean ±SD)	
		N	µg/day	µg/100kcal/day	µg/day	µg/100kcal/day
24 m	GE	120	2.14 ±0.96	0.22 ±0.10	92.6 ±31.4	9.53 ±2.85
	BE	83	3.14 ±1.49	0.31 ±0.16	122.0 ±46.6	11.80 ±3.98
	IT	224	2.07 ±1.80	0.19 ±0.15	87.7 ±37.1	8.27 ±3.02
	PO	138	2.76 ±2.38	0.23 ±0.18	129.0 ±53.0	10.94 ±3.93
	SP	180	3.46±1.85	0.29 ±0.15	129.4 ±50.4	10.67 ±3.51
36 m	GE	120	2.22 ±0.88	0.21±0.08	107.6 ±40.1	10.00 ±3.01
	BE	83	3.33 ±1.78	0.28 ±0.14	108.8 ±33.9	9.31 ±3.12
	IT	224	2.30 ±1.99	0.19 ±0.16	99.6 ±36.5	8.47 ±2.81
	PO	138	2.70 ±2.08	0.20 ±0.15	150.9 ±50.5	11.09 ±3.22
	SP	180	3.66 ±2.25	0.29 ±0.19	121.3 ±37.0	9.73 ±2.79
48 m	GE	67	2.38 ±0.98	0.19 ±0.08	122.1 ±35.5	9.89 ±2.84
	BE	52	3.21 ±1.55	0.24 ±0.09	111.0 ±38.5	8.62 ±2.96
	IT	162	2.16 ±1.54	0.17 ±0.11	105.3 ±42.1	8.34 ±2.93
	PO	79	2.41 ±0.95	0.17 ±0.07	151.0 ±51.1	10.83 ±3.12
	SP	143	3.96 ±1.72	0.29 ±0.12	134.0 ±43.3	9.95 ±3.01

(Continued on next page)

*(**ANNEX VI – 5.** Vitamin B_{12} and vitamin B_9 intake by country – Continued)*

			Vitamin B_{12} intake (mean ±SD)		Vitamin B_9 intake (mean ±SD)	
		N	µg/day	µg/100kcal/day	µg/day	µg/100kcal/day
60 m	GE	52	2.50 ±1.17	0.20 ±0.10	117.7 ±53.0	9.31 ±2.84
	BE	45	3.96 ±2.35	0.31 ±0.19	11.8 ±39.8	8.67 ±2.96
	IT	152	2.72 ±1.63	0.20 ±0.11	126.3 ±44.1	9.09 ±3.09
	PO	73	2.58 ±0.86	0.18 ±0.06	159.7 ±45.3	10.86 ±2.65
	SP	123	4.12 ±1.60	0.30 ±0.11	136.6 ±43.3	9.77 ±2.55
72 m	GE	56	3.20 ±5.99	0.24 ±0.46	128.4 ±50.9	9.61 ±2.97
	BE	46	4.37 ±3.69	0.34 ±0.38	106.2 ±33.3	8.13 ±3.18
	IT	145	2.82 ±2.07	0.19 ±0.12	140.8 ±49.8	9.51 ±3.26
	PO	95	2.77 ±0.93	0.18 ±0.06	169.7 ±47.9	11.07 ±2.59
	SP	126	4.35 ±2.03	0.29 ±0.13	148.9 ±50.3	9.94 ±3.21
96 m	GE	60	2.73 ±1.18	0.18 ±0.07	138.4 ±52.7	9.03 ±2.93
	BE	30	3.70 ±2.38	0.26 ±0.18	116.5 ±38.5	8.09 ±2.54
	IT	72	2.68 ±1.04	0.18 ±0.07	163.8 ±60.9	10.52 ±3.54
	PO	83	3.86 ±3.24	0.24 ±0.19	189.4 ±46.8	11.59 ±2.62
	SP	151	4.75 ±2.29	0.29 ±0.13	158.5 ±54.4	9.81 ±3.09

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

5.1. Vitamin B_{12} intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	0.001	<0.001	1.000	0.019	<0.001	<0.001	0.002
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	raw	1.000	<0.001	0.008	0.025	<0.001	0.001	0.003	<0.001	<0.001	1.000
	/kcal	0.205	<0.001	1.000	1.000	<0.001	0.090	0.580	<0.001	<0.001	1.000
12 m	raw	0.543	1.000	1.000	<0.001	0.169	1.000	0.300	1.000	<0.001	0.002
	/kcal	0.706	1.000	1.000	0.001	0.048	1.000	1.000	1.000	<0.001	0.005
24 m	raw	0.001	1.000	0.060	<0.001	<0.001	1.000	1.000	0.004	<0.001	0.006
	/kcal	<0.001	1.000	1.000	0.002	<0.001	0.002	1.000	0.196	<0.001	0.012
36 m	raw	0.009	1.000	1.000	<0.001	0.005	0.578	1.000	1.000	<0.001	0.004
	/kcal	0.054	1.000	1.000	0.002	0.002	0.018	1.000	1.000	<0.001	<0.001
48 m	raw	0.021	1.000	1.000	<0.001	<0.001	0.022	0.016	1.000	<0.001	<0.001
	/kcal	0.062	1.000	1.000	<0.001	<0.001	0.002	0.039	1.000	<0.001	<0.001
60 m	raw	<0.001	1.000	1.000	<0.001	<0.001	<0.001	1.000	1.000	<0.001	<0.001
	/kcal	<0.001	1.000	1.000	<0.001	<0.001	<0.001	1.000	1.000	<0.001	<0.001
72 m	raw	0.403	1.000	1.000	0.122	0.015	0.020	1.000	1.000	<0.001	0.001
	/kcal	0.227	1.000	1.000	1.000	<0.001	0.001	1.000	1.000	0.002	0.004
96 m	raw	0.546	1.000	0.033	<0.001	0.380	1.000	0.200	0.013	<0.001	0.038
	/kcal	0.053	1.000	0.145	<0.001	0.027	1.000	1.000	0.061	<0.001	0.022

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

5.2. Vitamin B_9 intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.014	<0.001	<0.001	0.001
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	raw	0.006	<0.001	0.001	0.602	<0.001	1.000	0.394	<0.001	<0.001	0.110
	/kcal	0.109	<0.001	1.000	0.226	<0.001	0.699	<0.001	<0.001	<0.001	0009
12 m	raw	<0.001	0.140	<0.001	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001
	/kcal	<0.001	<0.001	0.026	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
24 m	raw	<0.001	1.000	<0.001	<0.001	<0.001	1.000	1.000	<0.001	<0.001	1.000
	/kcal	<0.001	0.012	0.010	0.048	<0.001	0.703	0.128	<0.001	<0.001	1.000
36 m	raw	1.000	1.000	<0.001	0.148	1.000	<0.001	0.484	<0.001	<0.001	<0.001
	/kcal	1.000	0.001	0.169	1.000	0.623	0.004	1.000	<0.001	0.003	0.009
48 m	raw	1.000	0.073	0.001	0.598	1.000	<0.001	0.010	<0.001	<0.001	0.050
	/kcal	0.219	0.004	0.563	1.000	1.000	<0.001	0.059	<0.001	<0.001	0.0357
60 m	raw	1.000	1.000	<0.001	0.112	0.579	<0.001	0.016	<0.001	0.592	0.005
	/kcal	1.000	1.000	0.028	1.000	1.000	0.001	0.265	<0.001	0.507	0.094
72 m	raw	0.216	1.000	<0.001	0.086	<0.001	<0.001	<0.001	<0.001	1.000	0.016
	/kcal	0.158	1.000	0.051	1.000	0.081	<0.001	0.007	0.001	1.000	0.072
96 m	raw	0.642	0.063	<0.001	0.135	<0.001	<0.001	0.001	0.029	1.000	<0.001
	/kcal	1.000	0.053	<0.001	0.924	0.0003	<0.001	0.047	0.279	1.000	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

6. Vitamin A and Vitamin D intake by country

			Vitamin A intake (mean ±SD)		Vitamin D intake (mean ±SD)	
		N	µg/day	µg/kcal/day	µg/day	µg/100kcal/day
3 m	GE	138	438.1 ±70.9	0.79 ±0.02	7.88 ±1.30	1.42 ±0.46
	BE	93	439.5 ±64.2	0.77±0.04	7.98 ±1.16	1.40 ±0.07
	IT	273	463.5 ±90.0	0.88±0.14	5.69 ±3.75	1.01 ±0.64
	PO	159	458.2 ±102.0	0.79 ±0.03	7.98 ±1.71	1.38 ±0.08
	SP	241	489.9 ±83.4	0.79 ±0.05	8.91 ±1.48	1.43 ±0.07
6 m	GE	131	483.2 ±162.1	0.77 ±0.24	9.92 ±2.46	1.58 ±0.33
	BE	96	976.3 ±599.7	1.48 ±0.92	9.03 ±3.23	1.36 ±0.46
	IT	248	685.4 ±296.1	0.98 ±0.32	6.89 ±4.07	0.96 ±0.55
	PO	145	1786.3 ±1514.7	2.58 ±2.28	11.02 ±2.79	1.52 ±0.24
	SP	219	618.7 ±294.1	0.84 ±0.33	10.77 ±4.02	1.47 ±0.44
12 m	GE	132	638.0 ±783.4	0.82 ±0.94	5.75 ±4.06	0.75 ±0.56
	BE	92	1257.1 ±3143.1	1.45 ±3.12	6.43 ±2.46	0.80 ±0.33
	IT	250	842.3 ±511.3	0.97 ±0.54	3.25 ±2.60	0.37 ±0.29
	PO	148	1464.9 ±1389.5	1.64 ±1.53	7.87 ±3.03	0.87 ±0.30
	SP	200	966.9 ±417.0	1.03 ±0.46	10.47 ±6.82	1.10 ±0.68

(Continued on next page)

*(**ANNEX VI – 6.** Vitamin A and vitamin D intake by country – Continued)*

			Vitamin A intake (mean ±SD)		Vitamin D intake (mean ±SD)	
		N	µg/day	µg/kcal/day	µg/day	µg/100kcal/day
24 m	GE	120	590.8 ±456.5	0.61 ±0.48	92.59 ±31.38	0.21 ±0.32
	BE	83	1068.7 ±1237.8	1.03 ±1.21	122.00 ±46.56	0.41 ±0.33
	IT	224	809.5 ±1867.6	0.75 ±1.55	87.68 ±37.13	0.10 ±0.10
	PO	138	1069.3 ±1302.0	0.91±1.06	129.05 ±53.02	0.30 ±0.30
	SP	180	669.0 ±449.7	0.56 ±0.36	129.43 ±50.42	0.36 ±0.33
36 m	GE	120	628.8 ±498.5	0.59 ±0.44	60.22 ±20.47	0.12 ±0.14
	BE	83	1004.7 ±1380.0	0.84 ±1.02	68.04 ±46.72	0.22 ±0.26
	IT	224	681.9 ±587.0	0.58 ±0.48	57.03 ±34.60	0.08 ±0.09
	PO	138	991.5 ±806.8	0.76 ±0.72	45.17 ±17.77	0.18 ±0.16
	SP	180	706.2 ±2083.1	0.58 ±1.88	65.07 ±63.17	0.26 ±0.17
48 m	GE	67	802.4 ±723.7	0.65 ±0.56	1.10 ±0.71	0.09 ±0.06
	BE	52	1080.0 ±1190.3	0.81 ±0.79	1.50 ±1.99	0.12 ±0.16
	IT	162	747.8 ±639.5	0.60 ±0.50	0.84 ±0.74	0.07 ±0.06
	PO	79	993.8 ±563.0	0.70 ±0.35	1.90 ±1.60	0.14 ±0.11
	SP	143	603.3 ±343.1	0.45 ±0.25	2.70 ±1.92	0.20 ±0.15

(Continued on next page)

*(**ANNEX VI – 6.** Vitamin A and vitamin D intake by country – Continued)*

			Vitamin A intake (mean ±SD)		Vitamin D intake (mean ±SD)	
		N	µg/day	µg/kcal/day	µg/day	µg/100kcal/day
60 m	GE	52	753.9 ±740.4	0.60 ±0.55	0.93 ±0.54	0.07 ±0.04
	BE	45	1681.0 ±1816.1	1.29 ±1.33	1.46 ±3.39	0.13 ±0.40
	IT	152	699.9 ±603.7	0.50 ±0.42	1.04 ±0.89	0.08 ±0.07
	PO	73	980.3 ±924.5	0.65 ±0.53	1.69 ±1.00	0.11 ±0.06
	SP	123	589.8 ±311.4	0.43 ±0.23	2.63 ±1.76	0.18 ±0.11
72 m	GE	56	796.0 ±838.0	0.60 ±062	1.35 ±1.50	0.11 ±0.15
	BE	46	1663.3 ±1919.9	1.30 ±1.54	0.99 ±0.68	0.07 ±0.05
	IT	145	826.4 ±801.7	0.55 ±0.49	1.05 ±0.89	0.07 ±0.06
	PO	95	1019.9 ±792.7	0.66 ±0.48	1.69 ±0.97	0.11 ±0.06
	SP	126	652.7 ±508.6	0.43 ±0.33	2.75 ±2.11	0.18 ±0.14
96 m	GE	60	888.0 ±627.2	0.59 ±0.43	1.45 ±1.27	0.10 ±0.09
	BE	30	1696.8 ±1902.9	1.15 ±1.29	1.04 ±0.69	0.07 ±0.05
	IT	72	859.5 ±490.3	0.55 ±0.30	1.33 ±0.90	0.09 ±0.06
	PO	83	1079.0 ±563.5	0.67 ±0.37	2.14 ±1.17	0.13 ±0.07
	SP	151	681.3 ±978.4	0.41 ±0.52	2.85 ±2.40	0.18 ±0.14

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

6.1. Vitamin A intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	0.046	0.441	<0.001	0.195	0.940	<0.001	1.000	0.005	0.003
	/kcal	1.000	<0.001	1.000	1.000	<0.001	0.325	1.000	<0.001	<0.001	1.000
6 m	raw	<0.001	0.076	<0.001	0.799	0.006	<0.001	<0.001	<0.001	1.000	<0.001
	/kcal	<0.001	0.526	<0.001	1.000	0.001	<0.001	<0.001	<0.001	1.000	<0.001
12 m	raw	0.004	1.000	<0.001	0.234	0.086	1.000	0.748	<0.001	1.000	0.004
	/kcal	0.005	1.000	<0.001	1.000	0.034	1.000	0.124	<0.001	1.000	<0.001
24 m	raw	0.087	1.000	0.027	1.000	1.000	1.000	0.181	0.594	1.000	0.055
	/kcal	0.055	1.000	0.234	1.000	0.418	1.000	0.008	1.000	0.687	0.036
36 m	raw	0.782	1.000	0.577	1.000	0.877	1.000	1.000	0.581	1.000	0.977
	/kcal	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
48 m	raw	0.224	1.000	0.794	0.407	0.016	1.000	<0.001	0.065	0.552	<0.001
	/kcal	0.590	1.00	1.000	0.042	0.049	1.000	<0.001	1.000	0.048	0.001
60 m	raw	<0.001	1.000	1.000	1.000	<0.001	<0.001	<0.001	0.178	1.000	0.015
	/kcal	<0.001	1.000	1.000	0.744	<0.001	<0.001	<0.001	0.784	1.000	0.102
72 m	raw	<0.001	1.000	1.000	1.000	<0.001	0.001	<0.001	1.000	1.000	0.033
	/kcal	<0.001	1.000	1.000	1.000	<0.001	<0.001	<0.001	1.000	1.000	0.098
96 m	raw	0.001	1.000	1.000	1.000	<0.001	0.013	<0.001	1.000	1.000	0.013
	/kcal	<0.001	1.000	1.000	0.285	<0.001	0.001	<0.001	1.000	0.713	0.006

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

6.2. Vitamin D intake country differences (p-value)

		GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	raw	1.000	<0.001	1.000	0.001	<0.001	1.000	0.016	<0.001	<0.001	0.002
	/kcal	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	raw	0.922	<0.001	0.106	0.307	<0.001	<0.001	0.001	<0.001	<0.001	1.000
	/kcal	0.003	<0.001	1.000	0.276	<0.001	0.057	0.445	<0.001	<0.001	1.000
12 m	raw	1.000	<0.001	<0.001	<0.001	<0.001	0.114	<0.001	<0.001	<0.001	<0.001
	/kcal	1.000	<0.001	0.304	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	<0.001
24 m	raw	<0.001	0.050	0.004	<0.001	<0.001	1.000	1.000	<0.001	<0.001	0.124
	/kcal	<0.001	0.005	0.089	<0.001	<0.001	0.035	1.000	<0.001	<0.001	0.378
36 m	raw	<0.001	1.000	<0.001	<0.001	<0.001	1.000	0.083	<0.001	<0.001	0.005
	/kcal	0.001	1.000	0.090	<0.001	<0.001	0.971	0.972	<0.001	<0.001	0.001
48 m	raw	1.000	1.000	0.010	<0.001	0.046	1.000	<0.001	<0.001	<0.001	0.001
	/kcal	1.000	1.000	0.094	<0.001	0.060	1.000	<0.001	<0.001	<0.001	<0.001
60 m	raw	0.998	1.000	0.082	<0.001	1.000	1.000	<0.001	0.041	<0.001	0.001
	/kcal	0.502	1.000	1.000	<0.001	0.212	1.000	0.453	0.680	<0.001	0.012
72 m	raw	1.000	1.000	1.000	<0.001	1.000	0.056	<0.001	0.005	<0.001	<0.001
	/kcal	1.000	0.241	1.000	<0.001	1.000	0.526	<0.001	0.040	<0.001	<0.001
96 m	raw	1.000	1.000	0.185	<0.001	1.000	0.027	<0.001	0.036	<0.001	0.024
	/kcal	1.000	1.000	0.513	<0.001	1.000	0.077	<0.001	0.099	<0.001	0.022

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

ANNEX VII. MICRONUTRIENTS PROBABILITY OF ADEQUATE INTAKE AT INDIVIDUAL LEVEL BY COUNTRY AND COUNTRY DIFFERENCES

1. Micronutrients probability of adequate intake by country

	N	Calcium (mean ±SD)	Phosphorus (mean ±SD)	Iron (mean ±SD)	Zinc (mean ±SD)	Magnesium (mean ±SD)	Iodine (mean ±SD)	Vitamin B_{12} (mean ±SD)	Vitamin B_9 (mean ±SD)
3 months									
GE	138	98.9 ±2.8	-	-	64.4 ±25.0	-	-	99.9 ±0.5	61.5 ±22.7
BE	93	99.1 ±3.5	-	-	66.7 ±24.4	-	-	99.9 ±0.3	64.7 ±20.3
IT	273	78.0 ±34.7	-	-	44.7 ±35.6	-	-	78.4 ±33.9	45.2 ±34.0
PO	159	98.6 ±4.7	-	-	59.3 ±27.5	-	-	99.9 ±0.3	61.3 ±25.9
SP	241	99.6 ±1.9	-	-	79.4 ±20.7	-	-	100.0 ±0.3	77.7 ±19.2
6 months									
GE	131	98.0 ±6.3	-	72.7 ±24.9	*	-	-	98.3 ±4.6	86.0 ±20.3
BE	96	95.2 ±11.8	-	71.7 ±24.1	*	-	-	97.9 ±6.1	90.0 ±16.3
IT	248	88.2 ±23.1	-	59.2 ±35.8	*	-	-	85.2 ±26.3	65.0 ±36.6
PO	145	99.3 ±3.4	-	85.4 ±18.2	*	-	-	99.7 ±1.3	95.2 ±9.6
SP	219	98.1 ±4.6	-	83.3 ±20.8	*	-	-	99.5 ±2.4	87.5 ±14.9
12 months									
GE	132	66.5 ±30.3	71.3 ±27.8	67.0 ±23.5	52.6 ±4.8	65.9 ±28.9	48.3 ±29.8	*	23.4 ±24.6
BE	92	82.4 ±18.7	85.9 ±19.0	87.8 ±15.0	57.7 ±5.3	88.9 ±18.3	47.7 ±29.3	*	65.4 ±30.7
IT	250	88.3 ±17.5	90.0 ±16.0	59.6 ±26.1	54.7 ±7.7	67.2 ±25.5	28.9 ±28.4	*	17.2 ±20.7
PO	148	83.4 ±23.2	88.2 ±19.6	83.1 ±18.7	55.3 ±3.8	81.0 ±21.1	45.7 ±29.4	*	46.3 ±28.9
SP	200	92.5 ±13.7	96.8 ±9.36	95.8 ±11.1	61.8 ±9.5	97.5 ±5.6	55.3 ±29.5	*	70.2 ±27.3

(Continued on next page)

*(**ANNEX VII – 1.** Micronutrients probability of adequate intake by country – Continued)*

	N	Calcium (mean ±SD)	Phosphorus (mean ±SD)	Iron (mean ±SD)	Zinc (mean ±SD)	Magnesium (mean ±SD)	Iodine (mean ±SD)	Vitamin B_{12} (mean ±SD)	Vitamin B_9 (mean ±SD)
24 months									
GE	120	60.2 ±30.6	83.2 ±21.0	52.9 ±23.1	55.6 ±7.1	91.9 ±15.2	*	83.5 ±15.6	24.3 ±24.1
BE	83	82.6 ±20.2	91.7 ±14.6	77.9 ±25.1	63.6 ±9.6	94.2 ±10.9	*	92.1 ±11.1	47.9 ±31.8
IT	224	83.8 ±22.0	95.0 ±10.7	50.4 ±20.5	56.4 ±9.0	89.6 ±14.2	*	78.5 ±19.9	21.8 ±24.5
PO	138	67.6 ±29.0	89.1 ±17.4	68.4 ±24.0	58.8 ±9.2	93.1 ±13.9	*	88.1 ±12.8	51.4 ±34.3
SP	180	93.3 ±11.9	99.1 ±3.2	87.9 ±16.2	69.9 ±12.0	98.8 ±4.7	*	94.6 ±8.8	53.7 ±31.1
36 months									
GE	82	61.7 ±30.5	88.3 ±17.1	54.0 ±26.3	55.4 ±13.3	96.9 ±7.0	42.8 ±25.6	*	37.2 ±33.3
BE	57	79.0 ±22.5	93.5 ±12.5	80.4 ±21.4	70.5 ±15.1	98.3 ±3.5	46.5 ±29.9	*	37.8 ±30.2
IT	173	76.4 ±26.9	95.1 ±10.6	55.2 ±24.6	60.5 ±14.3	94.5 ±11.9	38.4 ±32.0	*	31.2 ±29.7
PO	86	68.3 ±27.7	95.4 ±8.9	83.9 ±18.1	66.1 ±13.6	98.9 ±4.6	23.3 ±18.4	*	68.7 ±32.9
SP	129	89.7 ±17.8	98.8 ±4.6	90.6 ±16.9	77.8 ±13.9	99.3 ±2.9	34.6 ±28.8	*	48.5 ±30.9
48 months									
GE	67	62.8 ±29.9	91.0 ±11.3	61.4 ±22.9	73.0 ±17.0	83.6 ±20.0	*	84.9 ±14.2	18.7 ±24.1
BE	52	77.4 ±26.8	92.2 ±14.0	71.2 ±20.3	82.9 ±18.5	84.9 ±19.4	*	89.6 ±13.0	16.4 ±23.7
IT	162	70.5 ±30.1	94.4 ±13.3	53.6 ±23.6	75.5 ±21.0	76.4 ±24.1	*	74.2 ±24.0	15.2 ±24.4
PO	79	66.1 ±29.1	93.6 ±10.8	77.3 ±22.0	79.0 ±20.5	87.6 ±17.0	*	85.8 ±14.3	38.9 ±33.5
SP	143	92.6 ±15.9	98.1 ±3.5	91.2 ±1.1	93.2 ±11.9	96.1 ±9.6	*	96.2 ±7.8	27.7 ±30.3

(Continued on next page)

*(**ANNEX VII – 1.** Micronutrients probability of adequate intake by country – Continued)*

	N	Calcium (mean ±SD)	Phosphorus (mean ±SD)	Iron (mean ±SD)	Zinc (mean ±SD)	Magnesium (mean ±SD)	Iodine (mean ±SD)	Vitamin B_{12} (mean ±SD)	Vitamin B_9 (mean ±SD)
60 months									
GE	52	58.7 ±29.4	90.1 ±12.8	63.5 ±28.3	69.3 ±25.7	83.4 ±19.7	43.8 ±29.1	84.0 ±15.4	18.0 ±29.4
BE	45	68.5 ±31.3	90.5 ±15.2	77.1 ±17.6	75.9 ±25.2	83.0 ±23.6	43.1 ±25.4	92.8 ±11.1	18.5 ±24.9
IT	152	71.5 ±28.0	96.2 ±8.6	64.8 ±26.3	76.4 ±22.9	85.1 ±19.7	47.6 ±32.0	85.2 ±16.4	26.2 ±30.2
PO	73	68.3 ±25.9	96.5 ±7.6	80.9 ±21.2	78.2 ±25.4	89.5 ±14.9	22.1 ±19.5	88.8 ±11.5	45.9 ±31.4
SP	123	92.9 ±14.0	99.1 ±2.77	91.4 ±14.7	87.9 ±19.4	95.9 ±10.1	35.7 ±26.0	96.8 ±6.5	32.3 ±31.7
72 months									
GE	56	62.3 ±26.9	92.9 ±12.6	65.7 ±25.6	59.2 ±25.4	86.3 ±19.1	50.8 ±24.2	*	25.5 ±31.7
BE	46	70.9 ±27.2	94.5 ±11.2	77.2 ±23.0	70.0 ±25.9	86.3 ±15.8	42.4 ±21.9	*	13.6 ±17.3
IT	145	65.4 ±28.0	98.4 ±4.1	72.2 ±20.7	73.7 ±22.7	89.6 ±15.1	45.6 ±31.2	*	34.7 ±33.1
PO	95	75.2 ±25.5	97.9 ±5.5	85.4 ±18.4	71.8 ±25.3	91.2 ±15.2	28.2 ±17.0	*	55.3 ±33.3
SP	126	93.3 ±1.0	99.2 ±4.5	94.6 ±11.4	85.8 ±18.1	97.9 ±7.0	39.9 ±23.5	*	41.0 ±33.8
96 months									
GE	60	66.6 ±29.6	95.7 ±11.6	30.5 ±35.9	66.5 ±23.2	90.8 ±15.8	54.5 ±26.2	74.5 ±18.4	5.3 ±14.8
BE	30	66.2 ±31.2	91.9 ±12.3	15.8 ±25.7	72.7 ±21.3	90.4 ±14.4	37.2 ±30.5	78.2 ±18.9	1.3 ±2.8
IT	72	70.9 ±24.8	98.0 ±5.7	34.0 ±36.2	74.5 ±21.8	93.6 ±13.1	47.4 ±33.2	74.6 ±18.5	11.8 ±22.3
PO	83	73.5 ±25.4	99.2 ±2.2	47.6 ±38.4	75.7 ±24.2	96.6 ±6.1	30.5 ±16.5	85.7 ±16.1	16.5 ±23.9
SP	151	94.5 ±11.8	99.7 ±1.0	55.1 ±40.0	84.0 ±19.6	98.8 ±3.8	38.8 ±25.0	92.7 ±9.7	8.9 ±18.1

GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain.

2. Calcium adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	1.000	<0.001	1.000	1.000	<0.001	0.232	0.865	<0.001	<0.001	1.000
12 m	<0.001	<0.001	<0.001	<0.001	0.175	1.000	0.001	0.209	0.326	<0.001
24 m	<0.001	<0.001	0.103	<0.001	1.000	<0.001	0.005	<0.001	<0.001	<0.001
36 m	0.001	<0.001	0.925	<0.001	1.000	0.133	0.079	0.148	<0.001	<0.001
48 m	0.029	0.456	1.000	<0.001	0.999	0.170	0.004	1.000	<0.001	<0.001
60 m	0.570	0.016	0.354	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001
72 m	0.729	1.000	0.016	<0.001	1.000	1.000	<0.001	0.021	<0.001	<0.001
96 m	1.000	1.000	0.725	<0.001	1.000	1.000	<0.001	1.000	<0.001	<0.001

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

3. Phosphorus adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
12 m	<0.001	<0.001	<0.001	<0.001	0.623	1.000	<0.001	1.000	0.001	<0.001
24 m	<0.001	<0.001	0.005	<0.001	0.629	1.000	0.001	0.001	0.031	<0.001
36 m	0.055	<0.001	<0.001	<0.001	1.000	1.000	0.025	1.000	0.036	0.281
48 m	1.000	0.310	1.000	<0.001	1.000	1.000	0.001	1.000	0.001	0.003
60 m	1.000	<0.001	0.001	<0.001	0.002	0.005	<0.001	1.000	0.080	0.471
72 m	1.000	<0.001	<0.001	<0.001	0.008	0.067	0.001	1.000	1.000	1.000
96 m	0.059	0.336	0.012	<0.001	<0.001	<0.001	<0.001	1.000	0.596	1.000

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

4. Iron adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
6 m	1.000	<0.001	0.001	0.003	0.001	0.001	0.004	<0.001	<0.001	1.000
12 m	<0.001	0.007	<0.001	<0.001	<0.001	0.853	0.018	<0.001	<0.001	<0.001
24 m	<0.001	1.000	<0.001	<0.001	<0.001	0.013	0.005	<0.001	<0.001	<0.001
36 m	<0.001	1.000	<0.001	<0.001	<0.001	1.000	0.036	<0.001	<0.001	0.301
48 m	0.102	0.087	<0.001	<0.001	<0.001	0.971	<0.001	<0.001	<0.001	<0.001
60 m	0.028	1.000	<0.001	<0.001	0.012	1.000	0.002	<0.001	<0.001	0.015
72 m	0.028	0.308	<0.001	<0.001	1.000	0.167	<0.001	<0.001	<0.001	0.005
96 m	0.808	1.000	0.073	<0.001	0.262	0.001	<0.001	0.245	0.001	1.000

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

5. Zinc adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	<0.001	1.000	<0.001	<0.001	0.0432	0.002	<0.001	<0.001	<0.001
12 m	<0.001	0.063	0.17	<0.001	0.006	0.117	<0.001	1.000	<0.001	<0.001
24 m	<0.001	1.000	0.059	<0.001	<0.001	0.003	<0.001	0.191	<0.001	<0.001
36 m	<0.001	0.083	<0.001	<0.001	<0.001	0.719	0.012	0.029	<0.001	<0.001
48 m	0.044	1.000	0.586	<0.001	0.103	1.000	0.005	1.000	<0.001	<0.001
60 m	1.000	0.625	0.395	<0.001	1.000	1.000	0.028	1.000	<0.001	0.046
72 m	0.201	0.001	0.014	<0.001	1.000	1.000	0.001	1.000	<0.001	<0.001
96 m	1.000	0.369	0.134	<0.001	1.000	1.000	0.099	1.000	0.027	0.056

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

6. Magnesium adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
12 m	<0.001	1.000	<0.001	<0.001	<0.001	0.055	0.015	<0.001	<0.001	<0.001
24 m	1.000	1.000	1.000	<0.001	0.040	1.000	0.048	0.093	<0.001	<0.001
36 m	1.000	0.203	1.000	0.319	0.014	1.000	1.000	<0.001	<0.001	1.000
48 m	1.000	0.079	1.000	<0.001	0.044	1.000	0.002	<0.001	<0.001	0.012
60 m	1.000	1.000	0.522	<0.001	1.000	0.471	<0.001	0.717	<0.001	0.123
72 m	1.000	1.000	0.367	<0.001	1.000	0.537	<0.001	1.000	<0.001	0.005
96 m	1.000	1.000	0.006	<0.001	1.000	0.034	<0.001	0.617	0.003	0.988

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

7. Iodine adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
12 m	1.000	<0.001	1.000	0.317	<0.001	1.000	0.373	<0.001	<0.001	0.024
36 m	1.000	1.000	<0.001	0.386	0.591	<0.001	0.078	0.001	1.000	0.044
60 m	1.000	1.000	<0.001	0.780	1.000	<0.001	0.515	<0.001	0.004	0.009
72 m	0.936	1.000	<0.001	0.068	1.000	0.016	1.000	<0.001	0.588	0.006
96 m	0.029	1.000	<0.001	0.001	0.678	1.000	1.000	0.001	0.196	0.194

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

8. Vitamin B_{12} adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	<0.001	1.000	1.00	<0.001	1.000	1.000	<0.001	<0.001	1.000
6 m	1.000	<0.001	1.000	1.000	<0.001	1.000	1.000	<0.001	<0.001	1.000
24 m	<0.001	0.023	0.121	<0.001	<0.001	0.442	1.000	<0.001	<0.001	0.001
48 m	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.159	<0.001	<0.001	<0.001
60 m	0.009	1.000	0.396	<0.001	0.006	1.000	0.723	0.477	<0.001	<0.001
96 m	1.000	1.000	<0.001	<0.001	1.000	0.206	<0.001	<0.001	<0.001	0.009

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.

9. Vitamin B_9 adequacy country differences (p-value)

	GE vs BE	GE vs IT	GE vs PO	GE vs SP	BE vs IT	BE vs PO	BE vs SP	IT vs PO	IT vs SP	PO vs SP
3 m	1.000	<0.001	1.000	<0.001	<0.001	1.000	0.001	<0.001	<0.001	<0.001
6 m	1.000	<0.001	0.013	1.000	<0.001	0.963	1.000	<0.001	<0.001	0.025
12 m	<0.001	0.245	<0.001	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001
24 m	<0.001	1.000	<0.001	<0.001	<0.001	1.000	1.000	<0.001	<0.001	1.000
36 m	1.000	1.000	<0.001	0.107	1.000	<0.001	0.314	<0.001	<0.001	<0.001
48 m	1.000	1.000	<0.001	0.281	1.000	<0.001	0.120	<0.001	0.001	0.039
60 m	1.000	0.923	<0.001	0.045	1.000	<0.001	0.095	<0.001	0.986	0.024
72 m	0.635	0.667	<0.001	0.026	0.001	<0.001	<0.001	<0.001	1.000	0.011
96 m	1.000	0.524	0.006	1.000	0.121	0.002	0.500	1.000	1.000	0.038

m: months. GE: Germany. BE: Belgium. IT: Italy. PO: Poland. SP: Spain. Country differences analysed with ANOVA and Bonferroni post hoc analyses.