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OXIDATIVE STRESS AND FATTY ACIDS PROFILE IN INFANTS: INFLUENCE OF GENETIC FACTORS AND DIETARY PATTERNS

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la gioia del domani
e l'amore per sempre

A SOFIA E MATTIA

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LIST OF ABBREVIATIONS

AAP	American Academy of Paediatrics
AI	Adequate Intake
ALA	Alfa-linolenic acid
ANC	AnteNatal Care
AND	Academy of Nutrition and Dietetics
AR	Avarage Requirement
ARA	Arachidonic Acid
BFHI	Baby-friendly Hospital Initiative
BMI	Body Mass Index
CF	Complementary Feeding
DHA	DocosaHexanoic acid
DRV	Dietary Reference Value
EPA	EicosaPentaenoic acid
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FA	Fatty acid
GWG	Gestational Weight Gain
HIC	High Income Country
HM	Human Milk
HMOs	Human milk Oligosaccharides
IOM	Institute of Medicine
LA	Linoleic Acid
LMIC	Low-Middle Income Country
MUFA	MonoUnsaturated Fatty Acids
PHIME	Public Health Impact of long-term, low-level Mixed Exposure in susceptible population strata
PRI	Population Reference Intake
PUFA	Polyunsaturated Fatty Acids
RI	Reference Intake for macronutrients
SFA	Saturated Fatty Acids
SINU	Società Italiana di Nutrizione Umana
STD	Suggested Dietary Target
TFA	Trans Fatty Acids
UNICEF	United NatIons ChildrEn's Fund

WHA	World Health Assembly
WHO	World Health Organization
PUFA ω3	PolyUnsaturated Fatty Acids Omega 3
PUFA ω6	PolyUnsaturated Fatty Acids Omega 6

ABSTRACT

The 1000 days between a women's pregnancy and her child's 2nd birthday is a period of both tremendous potential and enormous vulnerability and offer a unique window of opportunity to build healthier and more prosperous futures. The adoption of good nutrition practices during this period is important in order to provide the essential building blocks for child's brain development, health growth and strong immune system and to set the foundations for lifelong health, including the predispositions to obesity and certain chronic diseases.

The aim of my PhD project was to investigate the relationship of different typology of epidemiological data (dietary and biological data) collected and already available, obtained from the Italian component of PHIME study, a prospective mother-child cohort study.

Maternal dietary data were collected using a food frequency questionnaire administered at 30-32 weeks of gestation (n = 646) whereas children dietary data were gathered using a 7-day dietary record (food diary) at 18 months of age (n = 389). These nutritional data were used to estimate energy and nutrients intake for the evaluation of adherence to Italian dietary recommendations and to assess the different eating behaviours in term of foods and food groups. Lipidomic analysis was carried out on human milk samples (n = 61) collected one month after birth. Correlation analysis between fatty acids (FAs) profile and indexes from maternal dietary intake, human milk samples, maternal age, maternal pre-pregnancy BMI was performed using Spearman rank test.

Pregnant women showed a low compliance with dietary recommendations with an excessive intake of total fats, saturated fatty acids (SFAs) and sugars and insufficient intake of essential fatty acids (linoleic acid - LA, eicosapentaenoic acid- EPA and docosahexaenoic acid - DHA) iron, folate and vitamin D. This was probably due to a low varied diet, characterized by a high consumption of milk and dairy products and meat and cured meat whereas the eating of fish, pulses and nuts were negligible. As well, children diet at 18 months of age was unbalanced with an excessive intake of proteins, mainly from animal sources (milk and dairy products and meat and cured meat) and a scarce intake of polyunsaturated fatty acids (PUFAs), in particular essential FAs (EPA and DHA) and vitamin D. Moreover, most of children consumed a high amount of sweets and dessert reflecting in an excessive intake of soluble carbohydrates while the consumption of fish and pulses were scarce. Human milk FAs profile was characterized by higher levels of total SFAs, in particular palmitic acid, and total monounsaturated fatty acids (MUFAs) even if the most abundant fatty acid was LA. The content of PUFAs ω3 series was very low. A negative correlation was found between arachidonic acid in human milk samples and maternal dietary intake of total MUFA, oleic acid, LA and ALA ($p<0.005$).

Understanding how eating patterns change during this sensitive period (first 1000 days) is important in order to identify possible critical aspects, which should be monitored and addressed in accordance with national and international recommendations.

1. INTRODUCTION

1.1. FIRST 1000 DAYS

The 1.000 days between a woman's pregnancy and her child's 2nd birthday, is a period of tremendous potential and enormous vulnerability and represents a unique window of opportunity to build healthier and more prosperous futures. How well or how poorly mothers and children are nourished and cared for has a profound impact on a child's ability to grow, learn and thrive. This is because during the first 1.000 days, a neurodevelopment of the child occurs and the foundations for their lifelong are built [(1)]. In 2015, during the World Health Organization (WHO) European Ministerial Conference in Minsk, entitled "The Life-course Approach in the Context of Health 2020", the Member States of the European Union (EU) agreed to affirm that the earliest years of life set the tone for the whole of the lifespan. Moreover, they affirmed that the life-course approach requires whole-of-government commitment to early, appropriate, timely and collective action to promote and protect health and well-being through various developmental phases and critical transitions in life [(2)].

Research in the fields of neuroscience, biology and early childhood development provide powerful insights into how nutrition, human relationships, and environments shape future outcomes [(1)]. In particular, the adoption of good nutrition practices provides the essential building blocks for brain development, healthy growth, and a strong immune system while poor nutrition could cause irreversible damage principally related to child's growing brain. It could also set the stage for later obesity, diabetes, and other chronic diseases, which could lead to a lifetime of health problems [(1)]. There are three crucial stages in the first 1000 days: **pregnancy** (280 days), **infancy** (365 days) and **toddlerhood** (365 days) and, at each stage, the role of nutrition is fundamental to develop an adequate child's physical growth, to protect them from illness and chronic disease and to build health eating habits [(1)].

The Lancet Nutrition Series [(3)], the Global Nutrition Report [(4)], and the post Millennium Development Goals set by the World Health Assembly (WHA) [(5)] highlight the need to address the global burden of maternal and childhood under- and over-nutrition and to scale up nutrition action through proven nutrition-specific and nutrition sensitive interventions in order to achieve commitments and nutrition targets, set by the agenda 2025-2035 [(1)]. Based on a literature review of existing scientific evidence, along with recommendations from the WHO and the American Academy of Pediatrics (AAP), in 2016, it was published a chart contains a set of 10 "building blocks" for good nutrition in the first 1.000 days **(Fig. 1)** [(6)].

Figure 1. The 10 building blocks for good nutrition during the first 1.000 days [6]

Finally, the Italian Ministry of Health has recently published a policy document addressed to parents, health workers and decision makers in order to protect and promote child's health concerning the quality of prenatal care, childbirth, and post-natal care. This document contains the first results of the Italian Child Surveillance 0-2 year (*Sistema di Sorveglianza sui determinanti della salute nella prima infanzia*), started in 2014 and ended in 2016 and promoted and financed by the National Centre for Disease Prevention and Control (*Centro nazionale per la prevenzione ed il Controllo delle Malattie* – CCM) of the Italian Ministry of Health. This surveillance was included in in the National Programme "*GenitoriPiù*" aimed to evaluate different health determinants during the first 1000 days: assumption of folic acid before and during pregnancy, abstention from tobacco and alcohol during pregnancy and lactation, breastfeeding, infant sleep position, vaccination attitude, and early reading[7].

1.1.1. PREGNANCY

1.1.1.1. Nutrition: general aspects and health impact

The central role of nutrition and metabolism in pregnancy for health and well-being of pregnant women, pregnancy outcomes, and long-term health and development of the offspring has been generally recognized and supported by the most recent scientific literature [8]. In particular, current researches underline that specific maternal conditions prior and during gestational period, such as excessive maternal prepregnancy body mass index (BMI) and inadequate gestational weight gain (GWG) could affect the immediate and long-term health of the child, and may predispose the mother to complication during pregnancy and delivery. Obesity (prepregnancy BMI $\geq$ 30) represents a major risk factor for obstetric complications including miscarriage, gestational hypertension, gestational diabetes, caesarean delivery, pre-eclampsia, as well as foetal macrosomia, birth defects, congenital heart disease, and multiple other congenital anomalies. Moreover, it increases the risk for preterm birth and later obesity, cardio-metabolic disease, type 2 diabetes in adulthood [8]. Inadequate GWG, rather outside the Institute of Medicine (IOM) recommendations [9], has been associated with a range of potentially adverse outcomes including preterm birth, large for gestational age infants, increased rates of caesarean delivery and postpartum weight retention and represents a risk factor for gestational hypertension and pre-eclampsia as well as foetal macrosomia [8]. On the other hand, an underweight pre-pregnancy status and an insufficient GWG during pregnancy might be accompanied by preterm birth, small for gestational age infants, and failure to initiate breastfeeding [1]**.** It is therefore important for mothers to adopt appropriate dietary behaviours, based on rational criteria that consider not only the health effects, but also the implications of the mother's diet on the child's lifelong eating habits, since it is in this period that babies start to develop food preferences [10].

1.1.1.2. Nutritional recommendations

Unless pre-pregnancy nutrition is sub-optimal, macronutrients balance in the diet does not need to change during this vulnerably period but it is important that women consume a varied and balanced diet with foods rich in critical nutrients, rather than eating more [11]. In **Table 1.** are reported the Italian Dietary Reference Values (DRVs) for energy, macro and micronutrients intake during pregnancy, published in 2014 by *Società Italiana di Nutrizione Umana* (SINU) [12].

The energy requirements for healthy, normal weight women undergoes a moderate increase during pregnancy (depend on pregnancy stage) following the synthesis of new tissues in the foetus and mother (mammary gland, womb and afterbirth), the deposition of mother's fat stores and the increase of metabolic demands need for mother and foetus growing up [11]. Among the

macronutrients, proteins requirement increases during this period, in order to support protein synthesis for maintaining maternal tissues and foetal growth while concern fats, it is important to improve the relative proportion of omega-3 long-chain polyunsaturated fatty acids (ω-3 LC-PUFAs) than to increase their total amount. In fact, adequate intakes of alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential for the growth and development of brain and retina as well as to reduce the risk of early preterm birth (< 34 weeks of gestation). ALA is present in high concentrations in some vegetables oils (palm oil, soya oil, and rape oil) while EPA and DHA are contained in high concentration in fatty fish living in cold seas (mackerel, anchovies, salmon, and sardines) [12].

The available data from recent researchers indicate that the intake of selected micronutrients are often insufficient in particular after the first trimester of pregnancy when the requirements markedly increase; whereas an increased intake from conception or even before is recommended for folate (folic acid), iodine, and iron [11,13]. It is necessary to achieve adequate blood folate concentration in all women of childbearing age in order to prevent neural tube defects, to support proper development of the placenta and to reduce the risk of congenital heart disease. For these reasons, maternal supplementation with folic acid is well recommended, should begin two months before conceiving and should reach 800 μg/day [13]. However, the Italian DRV during this period increases by 50% for pregnant as compared with non-pregnant women of childbearing age (Population Reference Intake – PRI equal to 600 μg/day vs. 400 μg/day) [12]. Regarding iodine intake, national and international scientific societies are agreed to affirm that, average daily requirement should be increase about 50% in order to allow the production of foetal and maternal thyroid hormones, to prevent spontaneous abortion and perinatal mortality and to reduce the risk of birth defects and neurological disorders [13]. Moreover, during gestational period, iron requirement progressively increases starting from 1.2 mg/day in first trimester, 4.7 mg/day in second trimester to 5.6 mg/day in third trimester [14]. An inadequate intake could affect growth and development of the foetus, increase the risk of preterm delivery, low birth weight, post-partum haemorrhages and cardiovascular diseases from the offspring to the adulthood [13]. However, the use of supplements contain iron should be decided based on individual clinical assessment [12]. An adequate intake of vitamin D is essential for maintaining maternal calcium homeostasis, for the development of foetal bone and for reducing the risk of developing pre-eclampsia and gestational diabetes mellitus. Impaired skeletal development (rickets and osteopenia), low birth weight, respiratory infections and allergic diseases during infancy are often associated with inadequate contribution of vitamin D from maternal diet and maternal insufficient sun exposure [11]. Despite, there is no homogenous consensus on recommended intake concern this vitamin, a recent consensus document published in 2018 by the Societies of Pediatrics

emphasizes the high prevalence of vitamin D deficiency and the importance of prophylaxis starting already at the beginning of gestational period [12]. Finally, the requirement of calcium increases due to the mobilization from the maternal skeleton, the greater efficiency of intestinal absorption and the increased renal retention, during the third trimester of gestation. The Italian DRV increases by 50% for pregnant women as compared with non-pregnant women of childbearing age [13].

It is important to highlight that particular attention should be paid to women who exclude whole categories of foods from their diet, both for health or ethical reasons (e.g. vegetarian and vegan diets, gluten-free diet) in order to reduce the risk of major nutritional deficiencies, in particular concern iron, calcium, vitamin B group, folate and ω-3 LC-PUFAs. Moreover, specific cases requiring clinical examinations and targeted interventions in the perinatal period, include women with weight problems, smokers, adolescents, mothers who have had multiple or close pregnancies, and those with previous unfavourable pregnancy outcomes [13].

Table 1. Italian Dietary Reference Values (DRVs) of energy, macro, and micronutrients intake during pregnancy (expressed as daily intake) [12]

NUTRIENT	AI	AR	PRI	RI	STD
Energy* (1st trimester) (kcal)		(+) 69			
Energy* (2nd trimester) (kcal)		(+) 266			
Energy* (3rd trimester) (kcal)		(+) 496			
Total protein (1st trimester) (g)		(+) 0.5	(+) 1		
Total protein (2nd trimester) (g)		(+) 7.2	(+) 8		
Total protein (3rd trimester) (g)		(+) 23	(+) 26		
Total fat (E %)				20–35	
SFA (E %)					< 10
PUFA (E %)				5-10	
PUFA ω6 (E %)				4-8	
PUFA ω3 (E %)				0.5-2	
EPA + DHA (mg)	250 (+) 100–200 of DHA				
TFA (mg)					Less possible
Cholesterol (mg)					< 300
Available carbohydrates (E %)				45-60	
Soluble carbohydrates (E %)					< 15
Fiber (mg)					25
Calcium (mg)		1000	1200		
Iodine (µg)	200				
Iron (mg)		22	27		
Magnesium (mg)		170	240		
Phosphorus (mg)		580	700		
Potassium (g)	3.9				
Sodium (g)	1.5				2
Zinc (mg)		9	11		
Thiamin (vitamin B1) (mg)		1.2	1.4		
Riboflavin (vitamin B2) (mg)		1.4	1.7		
Niacin (vitamin B3 o PP) (mg)		17	22		
Pantothenic acid (vitamin B5) (mg)	6				
Vitamin B6 (mg)		1.6	1.9		
Biotin (vitamin B7) (µg)	35				
Cobalamin (vitamin B12) (µg)		2.2	2.6		
Vitamin C (mg)		70	100		
Folate (µg)		520	600		
Vitamin A (µg retinol equivalent)		500	700		
Vitamin D (µg)		10	15		
Vitamin E (mg α-tocopherol equivalent)	12				
Vitamin K (µg)	140				

Abbreviation: AI, adequate intake; AR, average requirement; PRI, population reference intake; RI, reference intake range for macronutrients; STD, suggested dietary target; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic; TFA, trans fatty acids

*Energy is calculated using Schofield equation (basal metabolic rate) [15] and Physical activity level (PAF) [12]

1.1.1.3. Scientific position

In 2016, the WHO published the *"*WHO recommendations on antenatal care for a positive pregnancy experience", a comprehensive guideline on antenatal care (ANC) for pregnant women and adolescent girls. Moreprecisley, this document is intended to reflect and respond to the complex nature of the issues surrounding the practice and the delivery of ANC, and to prioritize person-centred health and well-being, not only for the prevention of death and morbidity. Furthermore, it stresses the need to achieve positive pregnancy experience, that is defined as maintaining physical and sociocultural normality, maintaining a healthy pregnancy for mothers and infants (including preventing or treating risks, illness and death), having an effective transition to positive labour and birth, and achieving positive motherhood (including maternal self-esteem, competence and autonomy) [(16)]. Nevertheless, all international organisations and scientific societies agreed to state that pregnancy is a critical period during which maternal nutrition and lifestyle choices, already from periconceptional period, are major influences on mother and child health. As reported in the latest version (2014) of the position statement of the Academy of Nutrition and Dietetics (AND) , the components leading to healthy pregnancy outcome include healthy prepregnancy weight, appropriate GWG and physical activity, consumption of a wide variety of foods, appropriate vitamin and mineral supplementation, avoidance of alcohol, tobacco and other harmful substances, and safe food handling [(17)]. Authors suggest that women should have a normal BMI when they conceive and strive to gain within ranges recommended by the IOM recommendations to improve maternal and child health outcomes. Moreover, all women, particularly that in overweight and obese status, should have access to nutrition education and counselling regarding the potential maternal and foetal complications that could accompany an excessive weight before and during pregnancy. These important concepts have also been reported in the document entitle "*Linee guida sulla gravidanza fisiologica*" published in 2011 by the Italian Institute of Health, on mandate from Ministry of Health. In particular, the guidelines summarize the available information based on efficacy study allowing health practitioners to offer efficacy care pathway and pregnant women to choose most appropriate treatment, in terms of maternal and neonatal screening, health disorders, lifestyle, foetal growth and well-being [(18)]. Finally, the Italian Food-based Dietary Guidelines, which latest version was published in December 2019, devote a special issue to healthy nutritional practices and correct lifestyle behaviours in particular concern adequate prepregnancy BMI and GWG [(19)].

1.1.2. BREASTFEEDING AND FORMULA FEEDING

1.1.2.1. Breastfeeding: general aspects and health impact

Breastfeeding represents one of the most effective way to ensure child health and survival but unfortunately its importance is well recognised in low and middle-income countries (LMICs) rather than in high-income countries (HICs) where the assumption that human milk could be replaced with artificial products without detrimental consequences is widespread [20]. Since 1981, the paediatrician Bo Vahlquist wrote that "*In all mammalian species the reproductive cycle comprises both pregnancy and breast-feeding: in the absence of latter, none of these species, man included, could have survived"*. His principal objective was to emphasize and encourage this practice [21]. In 2016, the Lancet Breastfeeding Series Group published two documents (Series papers) draw on a large body of published and unpublished worldwide data from 28 systematic reviews and meta-analyses. The First Series entitled "Breastfeeding in the 21st century: epidemiology, mechanisms and lifelong effect" is concerned with breastfeeding trends worldwide and its implications on short and long-term health effects for the child and mother. While the Second Series entitled "Why invest, and what it will take to improve breastfeeding practices?" looks at determinants of breastfeeding and the effectiveness of interventions to promote it [20,22]. The first review emphasises how important breastfeeding is for all women and children. It highlights that appropriate breastfeeding practice prevents children mortality and morbidity due to diarrhoea, respiratory infections, and otitis media while the protect role against a child's later risk of overweight and type 2 diabetes is well not established. Regarding immune-mediated diseases (allergy, celiac disease, asthma, eczema), the available evidences do not allow to define a protective effect certantly, but it is known the potential mechanisms implicated in increase tolerance induction such as the presence of antigens and tolerogenic factors in human milk and the effects on gut microbiota and permeability. Breastfeeding is consistently associated with higher performance in intelligence tests and attained schooling. Finally, breastfeeding has positive effects on mother's health as improve birth spacing through longer periods of amenorrhoea and reduce the risk of type 2 diabetes, breast, and ovarian cancer. The only harmful consequences that this Series detected is an increase in tooth decay in children breastfed for more than 12 months but the authors are agreed to say that this observation should not lead to discontinuation of breastfeeding but rather to improved oral hygiene [20]. Breastfed babies have also a great capacity for self-regulation, allowing them to control the amount of milk that consume and this capacity remains with them in later life, thus prevent the onset of adult and childhood overweight and obesity [23]. Despite countless and recognized health benefits previously reported, the First Lancet Series shows that more than 80% of newborn receive human milk in nearly all countries but only about half begin this practice within the

first hour of life, as recommended by WHO [20,24]. In most countries, rates of exclusive breastfeeding are well below 50%, and the correlation with the duration of any breastfeeding is only moderate (Pearson's r = 0.54). In particular, HICs have shorter breastfeeding duration than LMICs: the prevalence of breastfeeding at 12 months of age are lower than 20% vs. 37%, respectively [20]. Finally, the authors report that the scaling up of breastfeeding practices to almost universal levels is estimated to prevent 823000 annual deaths or 13.8% of all deaths of children under 2 years of age in the 75 Countdown to 2015 countries [25]. Moreover, increase breastfeeding duration from presents levels to 12 months of child's age in HICs and 24 months of child's age in LMICs could save 22216 lives from breast cancer risks of death [20].

1.1.2.2. Nutritional recommendations

Maternal diet represents probably the most important factor that could influence human milk composition but the mechanisms behind are complex and could involve several intertwined metabolic pathways that produce direct and indirect effects. More precisely, scientific literature suggests that some metabolic pathways modulate the concentration and quality of certain human milk components such as fatty acids (FA) and/or fat- and water-soluble vitamins (vitamin A, C, B_6 and B_{12}) while the mineral content is less related [26]. For this reason, it is important to investigate human milk content and the factors contributing to its composition in order to improve the growth and development of infant's breastfeeding and to determine lactating mother's nutritional requirements [27]. In general, as suggested for gestational period, breastfeeding women should consume a varied and balanced diet providing adequate nutrients intake and promoting reduction of post-partum weight retention [11]. The Italian DRVs indicate an increase of energy, macro, and micronutrients requirement in line with what happens during pregnancy, as reported in **Table 2**. These recommendations are only referred at first semester of breastfeeding (exclusive breastfeeding), except for proteins and so none indications are reported concern mothers who continue to breastfeed their babies after the six months of age in combination of complementary foods, as recommended [28].

Table 2. Italian Dietary Reference Values (DRVs) of energy, macro, and micronutrients intake during exclusive breastfeeding (expressed as daily intake) [12]

NUTRIENT	AI	AR	PRI	RI	STD
Energy* (kcal)		(+) 500			
Total protein (1st semester) (g)		(+) 17	(+) 21		
Total protein (2nd semester) (g)		(+) 11	(+) 14		
Total fat (E %)				20–35	
SFA (E %)					< 10
PUFA (E %)				5-10	
PUFA ω6 (E %)				4-8	
PUFA ω3 (E %)				0.5-2	
EPA + DHA (mg)	250 (+) 100–200 of DHA				
TFA (mg)					Less possible
Cholesterol (mg)					< 300
Available carbohydrates (E %)				45-60	
Soluble carbohydrates (E %)					< 15
Fiber (mg)					25
Calcium (mg)		800	1000		
Iodine (µg)	200				
Iron (mg)		8	11		
Magnesium (mg)		170	240		
Phosphorus (mg)		580	700		
Potassium (g)	3.9				
Sodium (g)	1.5				2
Zinc (mg)		10	12		
Thiamin (vitamin B1) (mg)		1.2	1.4		
Riboflavin (vitamin B2) (mg)		1.5	1.8		
Niacin (vitamin B3 o PP) (mg)		17	22		
Pantothenic acid (vitamin B5) (mg)	7				
Vitamin B6 (mg)		1.7	2		
Biotin (vitamin B7) (µg)	35				
Cobalamin (vitamin B12) (µg)		2.4	2.8		
Vitamin C (mg)		90	130		
Folate (µg)		450	500		
Vitamin A (µg retinol equivalent)		800	1000		
Vitamin D (µg)		10	15		
Vitamin E (mg α-tocopherol equivalent)	15				
Vitamin K (µg)	140				

Abbreviation: AI, adequate intake; AR, average requirement; PRI, population reference intake; RI, reference intake range for macronutrients; STD, suggested dietary target; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TFA, trans fatty acids

*Energy is calculated using Schofield equation (basal metabolic rate) [15] and Physical activity level (PAF) [12]

1.1.2.3. Human milk composition

The several health benefits associated with breastfeeding and previous describe are driven by the combined action of the nutritional and bioactive compounds in human milk and the magnitude of the majority of the ascertained biological effects is directly dependent on breastfeeding duration [29].

Human milk is an individual-specific biofluid, which composition changes dynamically in response to many factor intra and extra subjects, matching the infant's requirements according to its age and other characteristics [30]. Its composition is influenced by genetic and environmental factors, infant health status, maternal lifestyle (dietary habits, smoking and alcohol attitude) [26]. However, subtle changes do occur over the course of lactation, even if same day and feed. For example, foremilk (milk at the beginning of feeding) differs from hindmilk (milk at the end of the feeding); colostrum (first milk produced) is strikingly different from transitional (milk produced after 5 days of delivery) and mature milk (milk produced by 4th to the 6th week of age of infants); malnourished mothers produce less quantity of milk with different micronutrients composition (e.g. less content of vitamin D, vitamin A, iodine and fatty acids) compared to well-nourished mothers [29].

Human milk is a complex matrix that contains energy nutrients (proteins, fats, and carbohydrates), water, minerals, vitamins, digestive enzymes, hormones, immune cells (macrophages), stem cells, and numerous other bioactive compounds. Proteins represent the third abundant solids in human milk (1.0 %) even so their mean content gradually decreases from the 2nd to the 6-7th month of lactation and stabilizes thereafter. Human milk proteins could be grouped into three major classes: caseins that are assembled in micelles, whey proteins that are presented in solution and mucins that are incorporated into the membrane of the milk fat globule. Caseins include α-, β- and k-casein while the main whey proteins are α-lactalbumin, lactoferrin, lysozyme, secretory IgA. They have several functional properties such as allowing infants' healthy growth, transport other nutrients (lactoferrin transports iron), promote gut development (lactoferrin), promote nutrients absorption (lactoferrin allows iron absorption, alpha-lactalbumin is essential for binding of calcium and zinc ions), and possess immune and antimicrobial activity allowing to reduce the risk of morbidities particularly infections (lactoferrin, secretory IgA, lysozyme). Moreover, non-protein nitrogen (urea, creatinine, nucleotides, free amino acids, peptides) comprises 25% of the total amount of human milk nitrogen and its content remains relatively stable during lactation period [31]. They play an important role in several cellular functions, acting as metabolic modulator, modulating enzymatic activities and promoting the development and maturation of the gastrointestinal and immunological systems [32]. The principal carbohydrate of human milk is lactose, whose content is constant in mature milk (~ 6.7 g/100 mL) thus ensuring its regular osmotic pressure. It also promotes the calcium absorption, supplies energy for growth and allows the development of central nervous system. The

other significant carbohydrates are represented by oligosaccharides such as Lactose-N-tetraose and its derivatives monofucosylated and their concentration decreases from 15-23 g/L in colostrum to 1-10 g/L in mature milk. However, human milk oligosaccharides (HMOs) specific composition is markedly affected by maternal genetic factors. They possess anti-infective properties against pathogens in the infant gastrointestinal tract, such as Salmonella, Listeria, and Campylobacter. They also play a vital role in the development of a diverse and balanced microbiota including Bifid bacterium and Bactericides, essential for appropriate innate and adaptive immune responses, and help colonize up to 90% of the infant biome (prebiotic role) [33]. Fats concentration in human milk increase throughout lactation (3.5% - 4.5%) according to the stage of nursing process, being most represented at the end of the feed, and to the time of the day. Moreover, the fat fraction appears to be the most sensible to maternal status (BMI) and diet in particular concern the amount and the type of FAs; it is known that human milk contains more than 200 FAs. Most fats secreted are triglycerides, contributing towards 98% of the fat fraction while the remainder (2%) predominantly consists of diacylglycerides, monoacylglycerides, free FAs (oleic acid, palmitic acid, and linoleic acid), phospholipids and cholesterol. Fats are the major sources of energy (44% of total energy provided by human milk) and are an important source of essential nutrients such as lipid soluble vitamins (A, D, E, and K), prostaglandins, and bioactive compounds (glycerophospholipids, sphingolipids, sphingomyelin, glycolipids, glycosylated, and proteins). Moreover, milk fats are a carrier of taste and aroma. The LCPUFAs are represented by arachidonic acid (ARA) and DHA and exert several major biological effects, particularly on membrane functions, on eicosanoid and docosanoid production and related physiological processes including growth and immune response. In addition, LCPUFAs positively affects retinal and brain cortical functional development in infants. Finally, short chain fatty acids, in particular palmitic acid, represent an important source of energy, contribute to the gastrointestinal tract maturation and allow a better absorption of calcium [13,31]. The minerals and vitamins content vary in relation to dietary intake and nutritional status of the mother, age and duration of lactation and environmental conditions and if not adequate, they could cause important health problems (e.g. rickets, anaemia). For example, the vitamin D and its metabolites concentration is between 0.1-1.0 mg/L with lower levels in the case of vegan mother and/or mother and infant with limited sun exposure while vitamin A content in human milk of well-nourished women is about 1.7 µmol/L and decreases with prolonged lactation, particularly in countries with endemic vitamin A deficiency. Minerals contribute to a variety of physiological functions, for example, they be part of many enzyme molecules. Their content varies during lactation period; for example the iron and zinc contents decrease rapidly between colostrum (0.4-0.8 mg/L of iron and 4-5 mg/L of zinc) and mature milk (0.2-0.4 mg/L of iron and 1.1 mg/L of zinc) with scarce influence from the maternal diet [31].

In addition to the nutritional components, human milk includes a wide range of bioactive constituents like microRNAs, immune and stem cells, hormones (insulin, leptin, adiponectin, and ghrelin). MicroRNAs are involved in the regulation of gene expression at the post-transcriptional, and in the modulation of several cell functions such as cell cycle, proliferation, differentiation, apoptosis and immune response. The bioactive constituents offer, also, protection in the gastrointestinal tract and, some of these, in the respiratory tract against invasive pathogens and they enhance digestive functions [32].

1.1.2.4. Formula feeding general aspects and legislation

When breastfeeding is not feasible in whole or in part, the only efficient alternative of human milk is represented by infant formulae. This impossibility could be due to a mother's choice or adverse health conditions. The latter are represented by maternal human immunodeficiency virus infection (HIV), maternal human T-cell lymphotropic virus (HTLV) type I-or-II positive infection, breast herpes simplex lesions, mother who receive diagnostic or therapeutic radioactive isotopes, mother who has had exposure to radioactive materials, infant with metabolic disorders (galactosemia, congenital lactase deficiency and LCPUFA oxidation disorder) [34].

Infant formula and follow-on formulae are usual industrially produced principally from cow's milk properly modified to get closer the composition of human milk. The manufacturing process is highly regulated and monitored to meet national and international quality criteria. More precisely, these products belong to the category of "baby foods" and they are regulated by the Regulation (EU) 2013/609 that has already been integrated with Regulation (EU) 2016/127, which transferred the provisions of Directive (EC) 2006/141.

In particular, the Regulation defines:

- *infant formulae*: foodstuffs intended for particular nutritional use by infants during the first months of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary foods.
- *follow-on formulae*: foodstuffs intended for particular nutritional use by infants when appropriate complementary feeding is started and constituting the principal liquid element in progressively diversified diet

At international level, the Food and Agriculture Organization of the United Nations (FAO) and the WHO (Codex Alimentarius Commission) published a Codex Standards (latest version in 2016)

which represents a collection of standards, guidelines and codes concern infant formulae and formulas for special medical purposes intended for infants [35].

1.1.2.5. Scientific position

Since 2002, WHO and United Nations Children's Fund (UNICEF) recommend initiation of breastfeeding within one hour of birth, exclusive breastfeeding for 6 months and continued breastfeeding, thereafter, with the gradual introduction of other foods and liquids, up to 2 years of age and beyond [36]. Moreprecisley, they defined exclusive breastfeeding a feeding practice characterized by the fact that infant receives only human milk and no other liquids or solids except for drops or syrups consisting of vitamins, mineral supplements, or medicines; thus infants who receive infant formulae are considered to have start complementary feeding (CF), even if this is from birth. The inclusion of infant formula as complementary food is intended to emphasize and encourage breastfeeding [36]. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) supports exclusive breastfeeding for around 6 months as a desirable goal, but partial breastfeeding as well as breastfeeding for shorter periods are also valuable. Continuation of breastfeeding after the introduction of CF is to be encouraged as long as mutually desired by mother and child [37]. Moreover, the AAP recommends exclusive breastfeeding for about 6 months, with continuation of this practice for 1 years or longer as mutually desired by mother and infant. Finally, many governments in Europe, including the Italian one, have issued national polices and guidelines that comply with WHO recommendations, at least in part. The Italian Ministry of Health, by the document entitle "*Linee di indirizzo nazionali sulla protezione, la promozione ed il sostegno dell'allattamento al seno"* published in 2007, recommends that children are breastfed exclusively for up to six months and that breastfeeding continues, with adequate CF until the mother and child desire.

All these international and national governments and the most important scientific societies are agreed to affirm that human milk is the best food and safest option to ensure good health and ideal growth for young children. Immediate and uninterrupted skin-to-skin contact and initiation of breastfeeding within the first hour after birth are important for the establishment of breastfeeding, and for neonatal and child survival and development. Exclusive breastfeeding for 6 months provides the nurturing, nutrients and energy needed for physical and neurological growth and development. Beyond 6 months, breastfeeding continues to provide energy and high-quality nutrients that, jointly with safe and adequate CF, help prevent hunger, undernutrition and obesity [38]. Finally, human milk could has an important effect also for the health of lactating mothers, ranging from the reduction of cardiovascular risk and hip fractures in post-menopause, to the protection against breast and ovarian cancers. In this regard, the World Cancer Research Fund (WCRF) includes breastfeeding among its

10 recommendations aimed at the cancer prevention [39]. However, data collected in Europe and worldwide highlight that breastfeeding rates fall short of those considered desirably by many professional organisations and scientific societies (59% of infants under 6 months of age are not exclusive breastfed worldwide).

Women need support to optimize their chances of breastfeeding in line with national and international recommendations. In 1991, WHO and UNICEF launched the Baby-friendly Hospital Initiative (BFHI) in order to help motivate facilities providing maternity and newborn services worldwide and to implement the "Ten Steps to Successful Breastfeeding" [40]. These ten steps were first published in a WHO/UNICEF joint statement (Protecting, promoting and supporting breastfeeding: the special role of maternity services) in 1989 and summarize a package of policies and procedures that facilities providing maternity a newborn services, should implement to support breastfeeding [41]. One of this step reported the compliance to the "International Code of Marketing of Breast-milk Substitutes" (NetCode), that was adopted by the WHA in 1981, in order to promote safe and adequate nutrition for infants by the protection and promotion of breastfeeding and by ensuring that human-milk substitutes are not marketed inappropriately [42]. The BFHI publication was updated in 2006 after extensive user survey, and relaunched in 2012 [24]. Moreover, in 2012, the WHA 65.6 endorsed a "Comprehensive implementation plan on maternal, infant and young child nutrition", specifying six global nutrition targets for agenda 2025, one of which is to increase the rate of exclusive breastfeeding in the first 6 months up to at least 50% [43]. In the same year, in Italy, it is established the scientific group "*Tavolo tecnico operativo interdisciplinare per la promozione dell'allattamento al seno"* aimed at endorsing the protection, promotion and support of breastfeeding as important natural practice with maternal and child's health benefits. In 2015, this working group, together with five medical and scientific societies, published an Italian Position Statement on Breastfeeding and the use of human milk, in which they also emphasize the importance of exclusive breastfeeding for at least six months continued breastfeeding, thereafter, in combination with CF, up to 2 years of age and beyond [44]. Finally, the promotion of breastfeeding is one of the main objectives of the *Piano della Prevenzione Nazionale 2014-2018* (extended to 2020) also in order to acquire information at regional level useful for planning any awareness programs and related strategies [45].

1.1.3. COMPLEMENTARY FEEDING

1.1.3.1. Nutrition: general aspects and health impact

Complementary feeding, as defined by WHO, is the process that begins when the human milk alone is no longer sufficient to meet the nutritional requirements of infants so that other foods and liquids are needed, along with human milk [46]. It is a period characterized by rapid growth and development when infants are susceptible to nutrients deficiency and excess (malnutrition phenomena), and during which marked changes in the diet with exposures to new foods, tastes, and feeding experiences take place [47]. In fact, data concern infants' dietary intake from a different European countries suggest that the intakes of energy, protein, sodium and potassium are generally higher that recommended while the intakes of ω-3 LCPUFAs, vitamin D, iron and iodine are critical and some subgroups in this population may be at risk of inadequacy [48]. It is therefore fundamental that its conduct be built on a rational basis that consider the short and long-term health effects and food tastes influence [49]. In fact, inadequate CF, as well as maternal malnutrition and inappropriate breastfeeding, can have direct and indirect negative consequences on child health, such as inadequate growth velocity, increased risk of infections, obesity, cardiovascular diseases, autoimmune diseases (celiac disease and type I diabetes) and atopic disorders [50].

Nevertheless, in contrast to the large literature of breast and formula feeding, less attention has been paid to CF period, especially to the type of foods given, or whether this period of significant dietary changes influences later health, development, or behaviour [47]. The more limited scientific evidence base is reflected in considerable variation in CF recommendations and practices between and within countries, also in relation to different cultural factors and food habits [51]. In fact, despite WHO recommendation to exclusive breastfeeding for 6 months, followed by the introduction of CF alongside breastfeeding is strongly supported by health policy-makers, the main source of discussion concerns the timing for introduction of CF with a wide variability between LMICs and HICs. [51]. There is a growing evidence that early food habits (< 4 months) may have a programming effect with an important impact on child's growth and body composition reflecting in the increase of long-term obesity risk by shaping infant appetite, food preferences, and metabolism [51]. Scientific literature are agree to affirm that the accumulation of excessive fat in the body of infants has adverse consequences on health, increasing the risk of adult type II diabetes, hypertension, dyslipidaemia, some cancers, and fatty liver diseases, besides psychosocial consequences [50]. Nevertheless, data used to evaluate the effects of age at introduction of CF and growth or obesity come almost exclusively from observational studies and so their interpretation is complicated by the fact that infant feeding practices may themselves be influenced by infant growth and energy intake [47]. Moreover, many HICs have

observed rising rates of food allergy both for infants in general population and for those with family history of atopy, despite the advice to restrict and delay exposure to potentially allergenic foods such as cow's milk, egg, fish, gluten, peanut and seeds. The hypothesis is based on early and repeated exposure to an antigen during critical windows such as CF leads to optimal development of immunotolerance [47,51]. Furthermore, most national organization and scientific societies recommend delaying the introduction of cow's milk as beverage after 12 months of age in order to prevent iron deficiency (cow's milk contains poor quantity of iron), childhood and adult obesity, hypertension and gastrointestinal bleeding even if the evidence are not well convincing. Recent studies have suggested that more prolonged exclusive breastfeeding may be associated with a reduce risk of respiratory infections and hospitalization for infection [52]. Thus evidence are consistent with the results from the UK Millennium Birth Cohort Study, which reports that the introduction of solids alongside breastfeeding may not result in an increased risk of infectious, with the exception of upper respiratory tract infection [53]. However, the relation between age of introduction of CFs and the risk of infections is already unclear. Finally, as previously reported, the CF age also influences food preferences in fact early exposure to a variety of flavours from CFs and human milk have a positive effect on the acceptance of new foods even if there are children's genetic predispositions. In fact, it is largely known that infants have innate preferences for sweet and salty tastes and the rejection of sour and bitter tastes which are likely to be a disadvantage in current obesogenic environments typically from HICs [54]. Overall, the timing of introduction of complementary foods needs to be decided on an individual basis considering that the age at which exclusive breastfeeding provides insufficient energy and nutrients cannot be defined by available data. However, national and international organizations are agreed to affirm that exclusive breastfeeding by well-nourished mothers could meet the energy and nutrients requirement (apart vitamin K and vitamin D; both of which can be addressed by supplementation) of most healthy infants for approximately 6 months [31,36,47].

In addition to the timing and the content of the CF diet, it is likely that the way chosen to give new food and the parent-infant relationship could have an important role in the development of dietary preferences and appetite regulation. For this reason, over the last 10-15 years, the Baby-Led Weaning (BLW) method has spread as alternative of traditionally CF approach in which infants are sharing family foods and mealtimes and feeding themselves with hand-held foods [55]. This method may provide a range of benefits to the infants such as greater infant autonomy and control over his intake, a more responsive parenting concern eating patterns and a reduction of the risk of child and adult overweight and obesity [51]. However, BLW method needs that infant's capacity fulfils the "Banana Challenge" proposed by United Kingdom National Health Service. It provides that infants are be able to stay in a sitting position holding their head steady, coordinate their eyes, hands and mouth so they

can look at their food, pick it up and put it in their mouth and swallow food. Finally, there is scientific evidence that parent-infant relationship, in term of attitudes and behaviours, could influence the infant's feeding practices acquisition with a strong correlation between optimal nutrition education of caregivers and parents and lower risk of overweight and obesity in later life [47].

1.1.3.2. Nutritional recommendations

Based on data from observational studies in combination with factorial approach, nutrients requirement during CF in healthy full-term infants must be calculated as the difference between the nutrients provided by human milk and the estimated total requirement. It was used an average composition and average quantity of human milk from literature [56]. However, this approach has a limitation because most infants, especially in HICs, do not receive human milk during CF period and so, it is important to known and consider the infant's main source of milk in order to determine the adequate amount of nutrients require for this period [47]. In **Table 3** and **Table 4** are reported the Italian DRVs for energy, macro and micronutrients during CF, considering the following age range: 6-12 months and 1-3 years, respectively [12].

In general, during the first two years of life, infants have a greater energy needs per body mass unit than adults (~70–120 kcal/kg b.w for infant versus ~30–40 kcal/kg b.w for adult) in response to the higher basal metabolic rate (BMR), the rapid growth, the neurophysiological and motor development and the deposition of new tissues [57]. In recent years, the emphasis has focused mainly on the protein's requirement, because high-protein intake is considered a risk factor for anticipation of adiposity rebound and, therefore, for overweight and obesity in childhood. In fact, a systematic review concerns the protein intake from 0 to 18 years of infants age and its relation to health, conducted in the 5th Nordic Nutrition Recommendations, concluded that there was convincing (grade 1) evidence that higher protein intake in infancy and early childhood are associated with increased growth and higher BMI in childhood. The authors also evaluated if different protein sources have similar effects on growth and adiposity, concluding that, dairy proteins have a stronger effect on insulin-like growth factor 1 (sIGF-1) compared with meat proteins [58]. Fat content in a diet is an important determinant of energy supply, and energy requirements remain high throughout the first two years of life (RI equal to 35-40 E %). However, high-fat intake is associated with an increased risk of overweight and obesity [12,59]. Particular attention must be paid to fat quality in particular concern the intake of LCPUFAs ω6, which have a positive effects on cognitive development and immune functions while the intake of SFA and trans fatty acids (TFA) should be reduced or even avoided [60]. Scientific societies and national and international organizations are agreed that too rapid reduction of fat consumption may cause reduction of the intake of total energy, essential fatty acids

and fat-soluble vitamins, change in the myelination process of nervous system and it does not have any protective effect against children and adult overweight and obesity [47]. Furthermore, there is no evidence that an excess of carbohydrates (>50 E%) is associated with an increased risk of children obesity while a high sugar intake could cause diarrhoea (especially if there is an excess consumption of fruit juices enriched with fructose) and the formation of dental caries even to increase the risk of obesity in children [60]. Micronutrients requirement is not met by human milk after 6 months of exclusive breastfeeding considering iron, zinc and vitamin A, D and K. In particular, the recommended iron assumption take into account the use of infants endogenous iron stores and the need for exogenous iron increases rapidly as the physiological requirement per kg body weight becomes greater than later in life [47]. Chronic iron deficiency could cause anaemia, psychomotor and cognitive disorders, irreversible mental retardation, and immune deficiency. The Italian DRV suggests a PRI value equal to 11 mg/day at 6-12 months and 8 mg/day at 1-3 years, considering that, until the first year of life, infants consume principally cereals-based products characterized by a low iron bioavailability (10%) while, subsequently, the consumption of meat and fish increases and also the iron bioavailability (15%) [12]. Concern vitamin D intake, there is no homogenous consensus between recommendations. The Italian DRV reports that "*the low vitamin D content of human milk in combination to the infants insufficient exposure to sunlight, carry a substantial risk of vitamin D deficiency and so the supplementation is strongly recommended, almost until 1 year"* [12] while WHO declare that *"that vitamin D supplements may be effective for infants and children who may be at higher risk due to limited sun exposure or those with darker skin pigmentation, however further research is needed before specific recommendations can be made"* [61].

Finally, special attention should be given to vegetarian and vegan diets especially during the first 2 years of life as infants and children may become deficient in essential nutrients including iron, zinc, calcium, DHA, protein, and vitamins A, D, B_2, and B_{12}. Current recommendations suggest that CF based on a vegan diet in infants and young children should be avoid or done under medical and nutritional supervision providing appropriate supplementation for critical nutrients [51].

Table 3. Italian Dietary Reference Values (DRVs) of energy, macro, and micronutrients intake at age range 6-12 months (expressed as daily intake) [12]

NUTRIENT	AI	AR	PRI	RI	STD
Energy* (6 months) (kcal)		620 (M) 570 (F)			
Energy* (12 months) (kcal)		760 (M) 690 (F)			
Total protein (g)		9	11		
Total fat (E %)	40				
SFA (E %)					< 10
PUFA (E %)				5-10	
PUFA ω6 (E %)				4-8	
PUFA ω3 (E %)				0.5-2	
EPA + DHA (mg)	250 (+) 100 of DHA				
TFA (mg)					Less possible
Available carbohydrates (E %)				45-60	
Soluble carbohydrates (E %)					< 15
Fiber (g)	8.4/1000 kcal				
Calcium (mg)	260				
Iodine (µg)	70				
Iron (mg)		7	11		
Magnesium (mg)	80				
Phosphorus (mg)	280				
Potassium (g)	0.7				
Sodium (g)	0.4				
Zinc (mg)		2	3		
Thiamine (vitamin B1) (mg)	0.3				
Riboflavin (vitamin B2) (mg)	0.4				
Niacin (vitamin B3 o PP) (mg)	5				
Pantothenic acid (vitamin B5) (mg)	2				
Vitamin B6 (mg)	0.4				
Biotin (vitamin B7) (µg)	7				
Cobalamin (vitamin B12) (µg)	0.7				
Vitamin C (mg)	35				
Folate (µg)	110				
Vitamin A (µg retinol equivalent)	450				
Vitamin D (µg)	10				
Vitamin E (mg α-tocopherol equivalent)	4				
Vitamin K (µg)	10				

Abbreviation: AI, adequate intake; AR, average requirement; PRI, population reference intake; RI, reference intake range for macronutrients; STD, suggested dietary target; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TFA, trans fatty acids; M, male; F, female

*Energy is calculated using Butte equation (basal metabolic rate) [57] and Energy deposition in new tissues [12]

Table 4. Italian Dietary Reference Values (DRVs) of energy, macro, and micronutrients intake at range 1-3 years (expressed as daily intake) [12]

NUTRIENT	AI	AR	PRI	RI	STD
Energy* (1 year) (kcal)		870 (M) 790 (F)			
Energy* (3 years) (kcal)		1390 (M) 1280 (F)			
Total protein (g)		11	14		
Total fat (E %)				35-40	
SFA (E %)					< 10
PUFA (E %)				5-10	
PUFA ω6 (E %)				4-8	
PUFA ω3 (E %)				0.5-2	
EPA + DHA (mg)	250 (+) 100 of DHA				
TFA (mg)					Less possible
Available carbohydrates (E %)				45-60	
Soluble carbohydrates (E %)					< 15
Fiber (g)	8.4/1000 kcal				
Calcium (mg)		450	600		
Iodine (µg)	100				
Iron (mg)		4	8		
Magnesium (mg)		65	80		
Phosphorus (mg)		380	460		
Potassium (g)	1.7				
Sodium (g)	0.7				0.9
Zinc (mg)		4	5		
Thiamin (vitamin B1) (mg)		0.3	0.4		
Riboflavin (vitamin B2) (mg)		0.4	0.5		
Niacin (vitamin B3 o PP) (mg)		5	7		
Pantothenic acid (vitamin B5) (mg)	2				
Vitamin B6 (mg)		0.4	0.5		
Biotin (vitamin B7) (µg)	10				
Cobalamin (vitamin B12) (µg)		0.7	0.9		
Vitamin C (mg)		25	35		
Folate (µg)		110	140		
Vitamin A (µg retinol equivalent)		200	300		
Vitamin D (µg)		10	15		
Vitamin E (mg α-tocopherol equivalent)	5				
Vitamin K (µg)	50				

Abbreviation: AI, adequate intake; AR, average requirement; PRI, population reference intake; RI, reference intake range for macronutrients; STD, suggested dietary target; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TFA, trans fatty acids; M, male; F, female

*Energy is calculated using Schofield equation (basal metabolic rate) [15] and Physical activity level (PAF) [12]

1.1.3.3. Scientific Position

In 2003, the WHO and UNICEF published a document entitled “Global Strategy for Infant and Young Child Feeding” that stresses such as inappropriate CF associated with a lack of exclusive breastfeeding in the first half of year is a major risk factor for childhood morbidity and mortality, with an estimated about 100.000 deaths/year in children younger than 5 years could preventable if conducted properly [62]. It further indicates that inadequate knowledge about appropriate foods and feeding practices is often a greater determinant of malnutrition than actual lack of food. Later, in 2008 and then in 2017 (latest version), ESPGHAN Committee published a position paper concern the introduction of foods other than human milk in order to review different recommendations and practices between countries, summarize evidence for nutritional aspects and short and long-term health effects of timing and composition of CF, provide advice to health care providers, and identify areas for future research [47].

As report previously, in its document, the WHO decided to include each type of formula (infant formula and follow-on formula) as CF in order to emphasize and encourage breastfeeding while ESPGHAN decided to not consider human milk substitutes as complementary food, because unhelpful and even confusing [47,62]. Properly for this reason, determine the optimal age for introduction of CFs in formula-fed infants has a matter of debate between scientific organisations. Regarding WHO recommendations, that are based on a consideration of the optimal duration of exclusive breastfeeding, the latest WHO systematic review and expert consultation allows to recommend that CFs could be introduce from 6 months of age alongside breastfeeding. It is also stated that the recommendation applies to populations rather than individuals, and it is recognized that some mothers would be unable to, or would choose not to, follow its and that these mothers should also be supported to optimise their infant's nutrition [36]. In fact, in 2005, WHO published a document entitled “Guiding principles for feeding non-breastfed children 6-24 months of age”, that includes a nine guiding principles and gives examples of diets from different parts of the world in order to meet energy and nutrients need of infants and young children after 6 months of age who are not breastfed [63]. The AAP and IOM have reconfirmed the indication to exclusively breastfeeding up to the 6 months of age, when CFs are introduced, and to continue this feeding practice up 1 year of age or older, as mother and child desire [64,65]. In Italy, main paediatrics scientific organizations such as Italian Society of Paediatrics (SIP) and Cultural Paediatrics Association (ACP) and Italian Ministry of Health are agreed with WHO recommendations [44,66].

Since some EU Member States and stakeholders have suggested that the Directive (EC) 2006/125 (Directive on processed cereal-based foods and baby foods for infants and young children) should be reviewed in the light of the latest scientific evidence, the European Commission has

requested at the European Food Safety Authority (EFSA) to give an opinion on the suitable age for the initiation of CF for infants in the EU. They concluded that **"***there is no convincing evidence that at any age investigated in the included studies (< 1 to < 6 months), the introduction of complementary foods is associated with adverse health effects or benefits (except for infants at risk of iron depletion). For nutritional reasons, most infants need complementary foods from around 6 months of age***.** *Infants at risk of iron depletion (exclusively breastfed infants born to mothers with low iron status, or with early umbilical cord clamping (< 1 min after birth), or born preterm***,** *or born SGW or with high growth velocity) may benefit from earlier introduction of complementary foods that are a source of iron"* [(67)].

All the national and international organisations and scientific societies are agreed to confirm that the timing for introduction of CFs associated with composition of the diet during this critical period and the type of milk feeding, should have health effects not just in the short term but also in the medium and long term [(47,62,66,67)]. For example, salt and sugars should not be added to CFs and the intake of sugars-containing drinks should be avoided [(60)]. Honey should not be introduced before 12 months of age unless the heat-resistant spores of Clostridium botulinum have been inactivated because the consumption of honey has been repeatedly associated with infant botulism [(47)]. Moreover avoidance or delayed introduction of other potentially allergenic foods, such as fish and eggs, has not been convincingly shown to reduce allergies, either in infants considered at risk for the development of allergy or in those not considered to be at risk [(47,67)].

However, recognizing that infants consume foods and diets rather than individual nutrients, some European countries have translated dietary intake recommendations for infants and young children into food-based dietary guidelines to help provide caregivers with an indication of suitable age-appropriate foods to meet dietary needs [(48)].

1.2. MOTHER-CHILD COHORT STUDIES

Mother-child cohort studies worldwide are many and various, although their primary objective in every case is to evaluate mothers' health status in relation to children's outcomes and to track children's development by monitoring their well-being and investigating their family, social and economic environment [68]. Unfortunately, there are very few studies determining energy and nutrients intake in detail and describing dietary habits during the first 1000 days. The most relevant are five large mother-child cohort studies: the Norwegian Mother and Child Cohort study (MoBa) [69], the German Dortmund Nutritional and Anthropometric Longitudinally Designed study (DONALD) [70], the Avon Longitudinal Study of Pregnancy and Children (ALSPAC) [71], the Infancia Y Medio Ambiente (Environment and Childhood) Project (INMA) [72] and the Public Health Impact of long-term, low-level Mixed Element Exposure in susceptible population strata study (PHIME) [73].

1.2.1. NORVEGIAN MOTHER AND CHILD COHORT STUDY (MoBa)

The Norvegian Mother and Child Cohort Study (MoBa) was planned in 1990s by researchers at the Medical Birth Registry of Norway (MBRN) and the Norwegian Institute of Public Health (NIPH) in order to estimate the association between exposures (including genetic, **nutritional**, environment factors) and diseases. The target population was all women who give birth in Norway (no exclusion criteria), that were recruitment during routine ultrasound examination at 17-18 weeks of pregnancy from July 1999 to December 2008. However, after the initial phase of recruitment, it was decided also to invite the fathers-to-be and so the cohort included 95368 mothers, 75618 fathers and 114622 children. The study design involved the administration of different questionnaires in order to collect data concern mother's and father's socio-economic status, educational and occupational information, **mother's dietetic habits during pregnancy**, mother's previous and present health problems and exposure, children's lifestyle exposure, children's learning, language and neurocognitive development, children's somatic diseases (asthma and allergies). Furthermore, there were also collected a blood samples from fathers and mothers and umbilical cord samples from children [69].

1.2.2. GERMAN DORTMUND NUTRITIONAL AND ANTHROPOMETRIC LONGITUDINALLY DESIGNED STUDY (DONALD)

The German Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD) is an ongoing open cohort study that, since 1985, has been collecting detailed

information concerning **diet**, growth, development, and metabolism in healthy subjects between infancy and early adulthood in order to evaluate their relationship. The study provides to recruit approximately 40 infants every year which are monitored at 3, 6, 9, 12, 18 and 24 months and then annually until young adulthood. The study design involves the administration of a **3-day weighed diet records**, which is compiled at each follow-up and provides **nutritional** data concern **infant's diet** and the collection of information about breastfeeding duration from birth until infant is fully weaned. Furthermore, at requirement, parents are interviewed by the study paediatrician about family characteristics, and are weighed and measured by the study nurses [70].

1.2.3. AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN (ALSPAC)

The Avon Longitudinal Study of Parents and Children (ALSPAC), known to its participants as 'Children of the 90s', is an ongoing transgenerational prospective observational study aims to understand how genotype and environmental characteristics influence health and development across the life course. It considers multiple genetic, epigenetic, biological, psychological, social, and other environmental exposures in relation to a similarly diverse range of health, social and developmental outcomes. The ALSPAC data are collected using a variety of different mechanisms, reported as follow: (a) self-completion questionnaires answer by mothers, fathers, children and child's school teachers; (b) health records; (c) biological sample from mothers, fathers and children; (d) education records; (e) environmental monitoring of subsamples of homes, including levels of air pollutants, magnetic radiation and noise; (f) hand-on assessment of a randomly selected 10% sample of study group from ages 4 months to 5 years – Children in Focus (CIF); (g) in-depth interviews and examination of subgroups and their controls; (h) annual hand-on assessments in the standardized environment from 7 years onwards [71]. Moreprecisley, mothers need to complete 20 questionnaires (4 antenatal and 16 postnatal) in order to collect data concern demographics and socioeconomics characteristics, health-related behaviours (smoking, alcohol, **diet** using a FFQ), life course characteristics, health (e.g. mental health, reproductive health, family health). The study design also involves 34 child-completed questionnaires of which 9 'focus' clinical assessments (accelerometer, **food diary**, neurocognitive and neurodevelopment test) and 25 questionnaires about child's health and behaviours completed by the mother or other caregiver. Moreover, the biological samples provided are blood, urine, hair, toenails, teeth, saliva and placenta which constitute a wide and detailed biobank with also genetic and epigenetic information [74].

1.2.4. INFANCIA Y MEDIO AMBIENTE PROJECT (Environment and Childhood – INMA)

The Infancia Y Medio Ambiente (Environment and Childhood) Project (INMA) is a network of a prospective population-based cohort studies that aim to investigate the associations between pre- and post-natal environmental exposures of pollutants in air, water and diet and growth, health and development from early foetal life until adolescence. The studies population includes pregnant women resident in each study area (Ribera d'Ebre, Menorca, Granada, Valencia, Sabadell, Asturias and Gipuzkoa) and their children, which have been recruitment in different periods. Moreover, each cohort varies in some degree in the follow-up time-points and methods, although follow-up survey is performed in all cohorts at the main time-points such as pregnancy, birth, and 4 years of child's age. Until now, it has been enrolled 3137 pregnant women and 2998 newborn, of which 2843 was followed at 1-1-5 years (the follow-up point that all participants have completed). The studies design involves that information could be gathered from a variety of sources: *ad hoc* questionnaires in face-to-face interviews by trained INMA personnel for collecting data concern social and environmental factors, lifestyles, maternal and childhood health and diseases; clinical data; physical examination (e.g. anthropometric measures, prick test, spirometry etc.); ultrasound scans; biological samples (blood, placenta, urine, saliva, hair, nails and mother's milk); biomarkers; **diet determinants** concern **mother and child** and environmental measures (air pollution, water pollution and persistent and semi-persistent pollutants). Data collected at each wave vary slightly among cohorts according to internal interests, but the main common variables are included in all cohorts [72].

1.2.5. PUBLIC HEALTH IMPACT OF LONG-TERM, LOW-LEVEL MIXED EXPOSURE IN SUSCETIBLE POPULATION STRATA PROJECT (PHIME)

The Public Health Impact of long-term, low-level Mixed Exposure in susceptible population strata (PHIME) was a 5-year integrated project (2006 - 2011), financed within EU´s Sixth Framework Programme for Research & Technological Development, that involved 35 Partners from 22 Countries worldwide. The aim was to improve the integrated health risk-assessment of long-term, low-level environmental exposure to toxic and essential metals (mercury - Hg, lead - Pb, cadmium - Cd, arsenic - As, manganese - Mn, uranium - U, platinum - Pt, palladium - Pd, radium - Ra) on health of susceptible population strata (foetus, infants, women, elderly). Within this larger framework, a prospective cohort study has been designed in order to assess the association between low-level mercury exposure from food consumption during pregnancy and child neurodevelopment among residents of the Mediterranean coastal area of 4 neighbouring countries (Mediterranean PHIME cohort) [73]. Moreprecisley, the pregnant women were resident, and enrolled, in four different coastal regions of Italy (coastal Province of Trieste), Slovenia (the city of Ljubljana and surroundings – up to 50 km), Croatia (the coastal city of Rijeka and its county) and the four Greek islands in the eastern Aegean. Recruitment took place at the Burlo Garofolo Children's Hospital in Trieste, Italy during the routine morphological ultrascan (20-22 gestational week); at the Maternity Hospital of the University Medical Centre of Ljubljana, Slovenia at delivery; at the University Hospital of Rijeka, Croatia during routine visits (34-38 gestational week) and at delivery; at the general regional hospitals of each islands in Greek at delivery. Times for enrolment were chosen according to logistic considerations in each country because there is no evidence that a particular gestational age is optimal for enrolment in this type of study, as reported in literature. Pregnant women were eligible if they were complied with a following inclusion criteria: were permanent residences of the study areas for at least 2 years, were at least 18 years of age, and had no absence from the study area for more than 6 weeks during pregnancy, no history of drug abuse, no serious health problems or complications of pregnancy, and no twin gestation. Moreover, researchers excluded from further follow-up any preterm births (<37 weeks of gestational age), babies with congenital malformations or severe perinatal problems, and those with severe health problems that presented in the following months and potentially compromised their neurological development, in order to avoid the potential confounders [73]. The Mediterranean PHIME cohort involved **2189** pregnant women of which 233 from Croatia, 466 from Greece, **900** from Italy, and 590 from Slovenia [75]. The study design involved the administration of three questionnaires, the collection of biological samples concerns mothers and children and the

evaluation of mother and child's neurodevelopment. Due to the logistics issues, the timing and the amount of data and samples collected were different among the four countries, as shown in **Table 5.**

Table 5. Protocol summary: study phases, samples, and data collected during each phase in each country of the Mediterranean PHIME cohort [73]

	ITALY	SLOVENIA	CROATIA	GREECE
Recruitment	20-22 weeks EGA	at delivery	34 weeks EGA - delivery	at delivery
Short questionnaire	20-22 weeks EGA	at delivery	34 weeks EGA - delivery	at delivery
Maternal hair (1st sample)	20-22 weeks EGA	at delivery	34 weeks EGA - delivery	at delivery
Maternal blood	20-22 weeks EGA	-	34 weeks EGA - delivery	-
Maternal urine	20-22 weeks EGA	-	34 weeks EGA - delivery	-
Maternal intelligence evaluation (Raven's Progressive Matrices - IQ)	20-22 weeks EGA	-	at delivery	-
Long questionnaire	28-week EGA – 1 month after delivery	1 month after delivery	1 month after delivery	3-6 months after delivery
Cord blood	at delivery	at delivery	at delivery	at delivery
Meconium	-	at delivery	at delivery	at delivery
Maternal hair (2nd sample)	1 month after delivery	-	-	-
Human milk	1 month after delivery	1 month after delivery	1 month after delivery	3-6 months after delivery
Child hair	18 months	-	-	-
Child's neurodevelopmental evaluation (BSID III test)	18 months	18 months	18 months	18 months
Child's neurodevelopmental evaluation (M-CHAT)	18 months	18 months	18 months	18 months
Supplementary questionnaire	18 months	18 months	18 months	18 months
7-day dietary record	18 months	-	-	-

Abbreviation: EGA, estimated gestational age; IQ, intelligent quotient; BSID III test, Bayley Scales of Infant and toddler Development; M-CHAT, Modified Checklist for Autism in Toddlers

The three questionnaires administered to mothers during the study were designed at the University of Udine, Italy and they were as follow: (a) a short questionnaire to identify any excluding conditions and to provide some brief information on family and lifestyles during pregnancy (dietary and physical activity habits, smoking attitude); (b) a long questionnaire to collect information on demographic, socioeconomic and health status, on pregnancy and delivery, on lifestyles and dietary habits (FFQ); (c) a supplementary questionnaire to update information on the family and child [75]. Mothers from Italy also compiled a 7-DD record to collect dietary habits on their child at 18 months of age.

Furthermore, biological samples was collected according to the protocol developed by the Josef Stefan Institute, Ljubljana and by the University Medical Centre, Clinical Institute of Clinical Chemistry and Biochemistry, Ljubljana, using standardized sample containers [73]. In **Table 6.** are reported a description of biological samples collected and the analysis that should be done.

Table 6. Biological samples collected and related analysis [73]

SAMPLE	QUANTITY	ANALYSIS
Maternal hair (1st sample)	~ 1 g (lock)	THg, MeHg
Maternal blood	5 mL of serum	Fe, Mg, Ca, PUFAs
	7 mL of plasma in NaH	THg, MeHg, Cd, Pb, Mn, Cu, Zn, Se, As
	3 mL of plasma in K_3EDTA	Genetic polymorphisms ABCA1, ABCB1, ABCC1, ABCC2
Maternal urine	50 mL	THg, Cd, U-Creatinine
Cord blood	5 mL of serum	Fe, Mg, Ca, PUFAs
	7 mL of plasma in NaH	THg, MeHg, Cd, Pb, Mn, Cu, As
Meconium (optional)	5 – 10 g	THg, MeHg, Pb, Se, PCBs
Cord tissues (optional)	3 – 5 cm	THg, MeHg, Pb, Se, PCBs
Human milk	50 mL	THg, MeHg, Cd, Pb, Cu, Zn, Se, PCBs
Maternal hair (2 nd sample)	~ 1 g (lock)	THg, MeHg
Child hair	~ 1 g (lock)	THg

Abbreviation: THg, total mercury; MeHg, metilmercury; Fe, iron; Mg, magnesium; Ca, calcium; PUFAs, polyunsaturated fatty acids; NaH, sodium hydrate; EDTA, ethylenediaminetetraacetic acid; Cd, cadmium; Pb, lead; Mn, manganese; Cu, copper; Zn, zinc; Se, selenium; As, arsenic; PCBs, polychlorinated biphenyl

Finally, mothers from Italy and Croatia took the Raven's Progressive Matrices Test, a test for general intelligence [76] while children at 18 (range 16 – 20) months of age underwent neurodevelopmental evaluation using the Bayley Scales of Infant and Toddler Development, Third Edition, Screening Test (BSID III), a test that considers 5 different scales (cognitive, language, motor, social emotional, and adaptive behaviour) [77]. Moreover, at the same age, children were also screened for autism by using the Modified Checklist for Autism in Toddlers (M-CHAT) [78]. However, follow-up continued in Italy after age 18 months (40 months and 7 years) as a continuation of the original PHIME project. Morepreciseley, at 40 months of child's age, mothers completed a supplementary questionnaire in order to ri-update information concern family and child, children underwent a BSID III test and it was conducted the AIRE test at child's home (a method for evaluating family environment, designed by Capotorti [79] and based on HOME model [80]). At 7 years of child, mothers compiled a Child Behaviour Checklist (CBCL) questionnaire and a 3-DD record concern child's diet while children underwent a neurodevelopmental evaluation by using different test (Wechsler Intelligence Scale for Children - WISC IV test; A Developmental NEuroPSYchological Assessment – NEPSY II test; MT Reading Test and *Batteria per la Valutazione della Scrittura e della Competenza Ortografica* - BVSCO 2). Moreover, it has been collected a biological samples concern child such as saliva, hair, and urine.

The research protocol of PHIME study was approved by the Ethics Committees of the University of Udine, of the Institute for Maternal and Child Health IRCCS Burlo Garofolo, of the Clinical Center of Rijeka, of the Institute of Child Health of Athens and by the National Ethics Committee of the Republic of Slovenia. The European Commission monitored all aspects of the study, including ethics, annually

1.3. DIETARY PATTERN

Dietary pattern is defined as the quantities, proportions, variety, or combination of different foods, drinks, and nutrients (when available) in the diets, and the frequency with which they are habitually consumed [81]. They are conceptualized and defined in many ways: as an exposure or a behaviour, by numbers or labels, as univariate or multivariate constructs, as a research-driven or data-driven, and as statistic or dynamic [82]. However, a nutritional analysis conducted using dietary pattern approach could be overcome some limitations related to single food/nutrient approach (traditional approach) because it takes into account the potential cumulative and interactive effects of individual dietary components (nutrients and foods) and so it has real-world application. In particular, it has been used to described associations between diet and diseases and to disseminated dietary recommendations and so contribute to both risk factor definition and diseases prevention [83].

Dietary patterns are typically derived using two main approaches. The first is defined as **hypothesises oriented** (*a priori*) approach, because it allows to measure, using *a priori* scores, the degree of adherence to specific dietary guidelines/recommendations or a healthy diet defined by scientific evidence on diet and disease [84]. Moreprecisley, *a priori* scores are composite numeric scores of foods, food components, and/or nutrients that are assessed as dichotomous variables (with predefined cut-points), ordinal variables such as quintiles, or as continuous variables. The individual components are summed to derive a total score that could be used to create a rank from maximum to minimum score [81]. This led to indices such as the World Cancer Research Fund Index, which measures adherence to the guidelines provided by the WCRF, or the famous DASH index designed to contain the risk of cardiovascular events, or the Mediterranean Diet Score (MDS) used to measure the compliance with the Mediterranean Diet [83]. The second commonly used method is **data driven** (*a posteriori*) approach in which dietary patterns are empirically derived from the underlying dietary data using statistical methods, such as principal component analysis (PCA), factor analysis, cluster analysis, k-means. Moreprecisley, the dietary variables are often assessed using an FFQ, 24-hour recalls, or diet records and then aggregated in order to reduce their number. Each dietary pattern is designated by a descriptive name based on predominant food groupings (largest or smallest amount relative to the other patterns) [81]. Finally, few studies used an alternative approach namely Reduced Rank Regression (RRR) method, which combines *a priori* knowledge with *a posteriori* analyses and therefore benefits from using information on potential diet–disease associations [81].

However, the identification of **food consumption patterns** represents the first step needed to study the dietary habits in a specific population and to perform a number of research and surveillance activities in the area of consumer science, nutrition and food safety [85]. Moreover, estimating energy

and nutrients intake is an important activity in order to monitor nutritional status. It allows to identify groups nutritionally at risk cause malnutrition; to target, plan and evaluate nutritional intervention programmes; and to establish dietary recommendations, food regulations and nutrition policies [86].

1.4. LIPIDOMIC ANALYSIS

1.4.1. GENERAL ASPECTS AND WORKFLOW

Lipidomics is a subfield of metabolomics that focus on the global study of molecular lipids (lipidome) within cell, tissue and biofluids, including the qualitative and quantitative analysis of lipids as well as the studies of their biological activities, subcellular localization and tissues distribution [87]. Lipids belong to the last step in the "omic" cascade starting from genome (genotype; deoxyribonucleic acid - DNA), through transcriptome (ribonucleic acid - RNA), proteome (proteins) and finally to metabolome (phenotype). In the latter was also included sugars, nucleotides and amino acids [88]. Lipidomics emerged in 2003 and has greatly advantaged in recent years, largely due to the development of mass spectrometry (MS) and computational methods, including data acquisition, bioinformatics and systems biology approaches [87,88]. Although, lipidome is a subgroup of metabolome, the analytical approaches typically adopted differ significantly from methods established in metabolomics in relation to the particular chemical structure of the majority of lipids molecules (polar/ionic head groups and nonpolar fatty acyl chain) that tend to form an amphiphilic molecules with a specific physicochemical proprieties which must be taken into account during the method development [88]. Moreover, two types of analytics are commonly applied in lipidomic studies such as hypothesis-driven target analysis and a comprehensive hypothesis generating, no targeted profiling approaches. The first allows to analysed only a defined group of lipids (same lipid class) using a targeted analytical protocol while the second aims to cover molecular lipids across a wide range of structural classes in a single analysis [89].

A typical workflow for lipidomic analysis of biological samples include the following steps: **sample preparation**, **MS-based analysis,** and **data processing**. However, lipids can be sometimes analysed directly from the biological materials (tissues slice or cell samples) using MS-imaging technic.

Sample preparation:

Sample preparation are very important phase and could be conducted appropriately to obtain the highest reproducibility and data value, even so the care should be considered covering sample collection, sample storage and lipid extraction. The best sample condition is using fresh samples; however, this is almost very difficult due to the practical circumstances and so the most common samples are often available in refrigerated state (fridge at T equal to -80°C). The sample stability could be prolonged by maintaining samples in environment free of oxygen, metal ions, and peroxides, and by addition of antioxidants (e.g. butylated hydroxytoluene) and anticoagulants (e.g.

ethylenediaminetetraacetic acid – EDTA). Although, adapting proper processes for biofluids are easier than tissues because the latter require more handling steps before freezing. The lipid extraction should be conducted using different non-polar organic solvents such as chloroform, methyl-*tert*-butyl eter (MTBE), and heptane alone or more frequently combined with other solvents. Moreprecisley, the chloroform based extraction systems described by Bligh and Dyer [90] and Folch [91] are the most commonly used for extracting lipids from biofluids and tissues, respectively. More recently, modified protocols using MTBE [92] and butanol-methanol (BUME) [93] have been developed in order to improve the extraction process as less hazardous alternatives. A correct lipidomic analysis includes the addition of internal standards prior to lipid extraction to facilitate monitoring recoveries and absolute quantification. After the lipid extraction, the samples are typically dried down and reconstituted in appropriate solvents optimal for MS analysis.

MS-based analysis:

There are two categories of MS-based analysis: shotgun and chromatography-based lipidomics. Shotgun lipidomics is a direct-infusion based lipidomic approach in which lipids are analysed without pre-chromatographic separation prior to MS even so the complexity of extract has been strategically reduced either during sample preparation or during MS analysis via intrasource separation/selective ionization or both [87]. Moreover, the pioneering method was based on low mass resolution MS/MS (tandem MS), typically triple quadrupole instruments [88]. The advantages of this MS-based approach are its simplicity and speed, while its major limitation is the ion suppression, which hampers the sensitivity and quantitative robustness of the determination [89]. Chromatography-based lipidomics are an approach that employ chromatography to separate the complex lipid classes and/or individual species prior to MS analysis. In the past, the most frequent technique used were thin-layer chromatography (TLC) and gas chromatography (GC) after the derivation of polar functionalities. The latter is still the most widely used method for the analysis of FAs (free FAs and esterified FAs) and steroids [89]. Nowadays, the gold standard is the use of atmospheric pressure chemical ionization MS (APCI) either without separation or coupled with liquid-phase separation techniques, such as (ultra)-high-performance liquid chromatography ((U)HPLC) or (ultrahigh-performance) supercritical fluid chromatography ((UHP)SFC), which provide superior performance. Furthermore, the latter provides the separation and quantification of polar and non-polar lipid classes in extremely short analysis times [88].

Data processing:

Data processing is usually automatic process and includes the lipid species identification, the deconvolution of isotopic overlap, and relative or absolute quantitation [88]. Moreprecisley, the data acquired in shotgun platforms are solely mass spectra including full MS and MS/MS spectra while data outputted from LC-MS platforms include both chromatograms (total ion chromatogram - TIC or extracted ion chromatogram – XIC) and mass spectra (full MS and MS/MS spectra including product ion and selected/multiple reaction monitoring – SRM/MRM data). Finally, for quantification, LC-MS platforms largely use the chromatogram data (i.e., peak areas) while shotgun platforms use ion peak intensities detected in either full MS or MS/MS spectral data or both [87].

1.4.2. LIPIDOMICS APPLICATIONS IN NUTRITION AND FOOD REASERCH

Given the many essential roles that lipids play in cellular functions including cellular barriers, membrane matrices, signalling and energy depots, lipidomic analysis applied in nutritional studies and food research have emerged recently [87]. Moreprecisley, this analytical approach allows to determine the interactions between genes, diet, nutrients, and human metabolism, and how they together contribute to health and disease [89]. Lipidomics can be used to understand changes in the structure, composition, and function of cellular lipids after a dietary intervention. For example, Szymanska and colleagues have studied the variation of lipid metabolism in healthy mildly hypercholesteraemic subjects after a 4-week consumption of two plant sterol-enriched yogurt drinks with different fat content (low and high) versus a placebo control. They found a similar reduction in total cholesterol and low-density lipoprotein cholesterol, but the low-fat drink had the most significant impact on the serum lipidome, reducing the level of several sphingomyelins. This could reflected in the reduction of inflammation and atherogenic mechanism [94]. Lipidomic analysis is used to study diet-related diseases in particular metabolic syndrome and its related risk factors (obesity, diabetes), neurological disorders, cancer, eye diseases. The integration of lipidomic data with genetic, proteomic, and metabolomic data is expected to provide a powerful analytical approach for elucidating the mechanisms behind lipid-based diseases [89]. For example, in an analysis of lipidome of 267 human breast tissues conducted by Hilvo and colleagues in 2011, they found that progression of breast cancer is strongly associated with increase in membrane phospholipids containing de novo synthesized of SFAs, typically of a diet rich in red and processed meat [95]. Finally, lipidomics could also be applied in food research, such as in development of food products, in the evaluation of food quality, functionality, bioactivity, and toxicity. In particular, the specificity and sensitivity of MS-based methods is officially recognized by international quality system control agencies and the

application of multistage ion analysis has become mandatory to adhere to worldwide regulations regarding the recognition of fraud and bad practices in food manipulation [96].

2. AIMS OF THE PhD PROJECT

The overall aim of my PhD project was to investigate the relationship of different typology of epidemiological data collected and already available, obtained from the Italian component of PHIME study (food frequency questionnaires, 7-day dietary records – food diaries, biological samples). Causing a reduced and sometimes dated available biological data together with some problems that I encouraged during my work, I had to modify some aspects of initial proposed PhD project. Moreprecisley, the evaluation of oxidative stress was not possible to perform due to the lack of suitable infants' biological sample, such as blood sample, in combination to datated women's blood samples.

The first specific aim was to provide a descriptive analysis of energy, macro and micronutrients intake in a pregnancy women and children at 18 months of age with a special focus on their different eating behaviours in term of foods and food group's choices. The second specific aim was to evaluate the adherence to the Italian dietary recommendations [12]. Finally, the third specific aim was to assess the lipids fraction on a human milk, that represents the first most important infants' source of this macronutrient, and how its content, in term of quantity and quality, is influenced by some mothers' characteristics such as dietary habits, age, BMI and GWG.

This research offers the opportunity to better understand how eating patterns change during this sensitive time (first 1000 days) and to identify critical aspects related to them, which should be monitored and addressed by national and international recommendations. The final objective is to protect the mother' and child's short and long-term health, by involving health care services and health professionals.

3. RESULTS

3.1. SUBJECT CHARACTERISTICS

In total, 900 mothers were enrolled for the Italian component of PHIME cohort, 767 (85%) remained in the study at delivery and filled in the long questionnaire with the food frequency section and 632 children underwent neurodevelopmental test at age 18 months. However, based on the inclusion criteria reported in Material&Methods section, **646** (72%) mothers and **389** (62%) children were included in my PhD project. The characteristics of these mother-child pairs are shown in **Table 1.**

The mean maternal age was 33.1 (STD 4.3) years. The majority were Italian (93.0%), married or living together with partner (89.0%), in employment (84.5%) and had a medium-high level of education (83.1%). Seventy – two percentage (N=461) of the mothers started pregnancy with normal BMI (mean 22.7, STD 3.7) and less thirty-seven percentage of the mothers achieved adequate GWG at the end of pregnancy, with an average increment about 13.5 kg. Concerning some mother's habits, it is observed a consumption of cigarettes during pregnancy, less that one per day (median = 0). Moreover, approximately seventy-eight percentage of the mothers used supplements (vitamins, minerals, and herbal products) during pregnancy but only 39.0% of them had started the supplementation with folic acid three months before conception, as recommended. Regarding children 185 were male (47.6%) and 204 were female (52.4%), with a mean birth weight and length equal to 3353.8 (STD 460.9) g and 50.0 (STD 2.2) cm, respectively.

A dropout analysis did not highlight a statistically significant difference ($p>0.05$) in the case of mothers' characteristics while there were statistically significant differences concern child's gender ($p=0.01$) and child's birth weight ($p=0.002$) (data not shown).

3.2. MATERNAL DIETARY ASSESSMENT

The distribution of macro and micronutrients intake and the percentage of adherence to recommendations [12], are presented in **Table 2.**

The mean energy intake was 2309.7 (STD 616.2) kcal/day. The intake recommendations for available carbohydrates was met by 77.7% of women with a mean energy contribution equal to 50.7%. However, almost 89% of women exceeded the DRVs for soluble carbohydrates (mean E percentage equal to 21.5%) while 46% of them did not met the fiber recommendation (≥ 25 g/day). Total proteins intake was equal to 87.1 (STD 24.8) g/day and its contribution to energy intake was

about 15%; even so, the compliance with DRV was kept by 97.5% of women. Total fat intake was above the DRV for about half percentage of mothers associated with an unbalanced FAs distribution. The contribution of SFAs to the total energy intake was above the DRV (< 10 E %) for 74.3% of women while that of PUFAs appeared to be in line with recommendation for the majority of women. However, less than half percentage of women (46.8%) reached the recommended intake for LCPUFAs ω3 series, such as DHA and EPA: median (IQR) intake of DHA plus EPA equal to 337.7 (231.9-489.4) mg/day vs. 350 – 450 mg/day as recommended. Unfortunately, there are not available DRVs for starch, oleic acid, and ARA.

Table 3 presents the women's estimated average daily intake of micronutrients compared to the DRVs [12]. A high percentage of women reported intake below the DRV for the following micronutrients: 96.6% for iron (median = 13.8 mg/day vs. 27 mg/day as recommended), 67.7% for vitamin B1 (median = 1.2 mg/day vs. 1.4 mg/day as recommended), 72.3% for niacin (median = 18.3 mg/day vs. 22 mg/day as recommended) and 86.2% for folate (median = 406.3 µg/day vs. 600 µg/day as recommended). All women (100%) reported vitamin D intake below the DRV, with a median (IQR) intake equal to 2.8 (2.1 – 3.8) µg/day vs. 15 µg/day, recommended. On the other hand, the assumption of calcium, sodium, phosphorus, vitamin B6, vitamin B12, vitamin C and vitamin E were higher than recommended values for 40.1%, 67.7%, 99.4%, 78.0%, 94.7%, 91.8% and 67.0% of women, respectively. The median (IQR) sodium intake was 1793.8 (1466.4 – 2383.0) mg/day vs. 1500 mg/day suggested by Italian DRVs, without the consideration of added salt.

The percentage distribution of the 17 food groups in the total intake of macronutrients are shown in **Figure 1** and **2**. In the figures are only highlighted the main food groups contributor concern each macronutrient.

The main source of total protein was meat (26.7%) follow by milk and dairy products (22.4%) and cereal-based products (16.9%). The principal source of available carbohydrates came from cereal-based products (36.6%) which also represented the first food group contributor of starch (64.1%) and the second food group contributor of fiber (21.2%). The soluble carbohydrates mainly derived from fruits (38.1%) and sugars, sweets, and desserts (35.0%). The main source of fiber was fruits (28.7%). Sugars, sweets and desserts, meat and cured meat and milk and dairy products represented the main sources of total fats, SFAs and cholesterol with a percentage contributions equal to 16.4%, 17.2% and 19.7% for total fats, 22.3%, 15.4% and 34.0% for SFAs, 27.7%, 29.5% and 19.2% for cholesterol, respectively. ARA content came from sugars, sweets and desserts (38.5%) and meat and cured meat (48.8%). However, also cooked and fresh vegetables have been contributed to total fats intake (7.0% and 13.1%) and the latter represented the second source of MUFAs (16.9%) and the first source of PUFAs (20.4%) in particular LA and ALA (22.4% and 18.2%). The reason

behind all this was represented by the fact that cooked and fresh vegetables in the nutritional database [97] were dressed by using a standard portion of condiment (1 spoon of vegetables oil). Finally, the main source of EPA and DHA was fish with a percentage contribution equal to 60.5% and 82.3% respectively, as expected.

The percentage distribution of the 17 food groups in the total intake of vitamins and minerals are shown in **Figure 3** and **4**. In the figures are only highlighted the main food groups contributor concern each micronutrient.

The main source of vitamin D was fish follow by meat and cured meat with a percentage contribution equal to 44.3% and 25.2% respectively. Moreover, the latter represented the principal contributor of vitamin B1 (18.1%), vitamin B6 (22.2%), vitamin B12 (37.4%) and niacin (30.2%). The vitamin B12 came also from milk and dairy products (25.9%) as vitamin B2 (29.8%). Finally, cooked and fresh vegetables represented the main sources of vitamin E (15.0% and 24.7%) and folate (19.7% and 17.6%) while the vitamin C intake came from fruits and vegetables, as expected 46.7% from fruits, 18.5% from cooked vegetables and 19.4% from fresh vegetables. The principal source of calcium and phosphorus was milk and dairy products with a percentage contribution equal to 48.9% for calcium and 30.1% for phosphorus. However, meat and cured meat (15.1%) and cereal-based products (14.5%) also provided the latter. Potassium came from fruits (18.7%) and fresh vegetables (13.5%) while cereal-based products (27.9%), milk and dairy products (21.1%) and cured meat (17.2%) provided sodium intake. Finally, the principal contributor of iron intake was cereal-based products (19.1%) while that of zinc was meat and cured meat (23.8%). However, milk and dairy products and cereal-based products with a percentage contribution equal to 21.2% and 17.9%, respectively also provided the zinc intake.

The consumption of alcoholic beverages was equal to 1.5 alcoholic units/day (2.7, STD = 5.2 g/day) corresponding to one and half can of beer (330 mL) or glass of wine (125 mL) or shot glass of liquor (40 mL). Moreover, the coffee consumption was equal to less than one cup per week (0.9, STD 0.9) (data not shown).

Finally, a weak statistical significant correlation was found between mother's age at delivery and maternal GWG ($r = -0.19449$; $p<0.0001$), fibre ($r = 0.15586$; $p<0.0001$), calcium ($r = 0.009006$; $p = 0.0221$), potassium ($r = 0.09696$; $p = 0.0137$), phosphorus ($r = 0.08421$; $p = 0.0324$), zinc ($r = 0.07473$; $p = 0.0577$), vitamin E ($r = 0.09426$; $p = 0.0166$), folate ($r = 0.08702$; $p = 0.0270$). Moreover a weak statistical significant correlation was found between maternal GWG and the intake of DHA ($r = - 0.10226$; $p = 0.0170$) and arachidonic intake ($r = 0.09203$; $p = 0.0319$).

3.3. CHILDREN DIETARY ASSESSMENT

The food diaries were available for 389 children (50.7%) of which 272 (69.9%) were compiled for 7 days, 8 (2.0%) for 6 days, 13 (3.3%) for 5 days, 11 (2.8%) for 4 days, 74 (19.0%) for 3 days, 5 (1.3%) for 2 days and 6 (1.5%) for one day. The total number of recipes prepared by mothers was 350. In addition, were loaded in the program Microdiet V.2.8.6, 183 *'standard recipes*' and 165 food labels (dietetics and non-dietetics foods).

The distribution of macro and micronutrients intake and the percentage of adherence to recommendations [12] are presented in **Table 4** and **5**. Same data divided by sex are shown in **Annex III**.

The mean energy intake was 916.0 (STD 196.1) kcal/day with a statistically significant difference between females and males (892.5, STD 190.1 kcal/day vs. 942.0, STD 199.9 kcal/day respectively). Overall, it is noted an unbalanced energy contribution from macronutrients. In particular, 95.4% of children had an excessive total proteins intake compared to DRVs (16.5 E % vs. 8-12 E %, respectively) while only 11.3% of children met the recommendation concern total fat (33.2 E % vs. 35-40 E %, respectively). Moreover, a more detailed comparison in a subgroup of children (N = 84) shown that “real” total proteins intake was greater than estimated proteins requirement for all children (100%): 37.4 (STD 9.6) g/day vs. 11.0 (STD 1.3) g/day respectively (data not shown). The estimated proteins intake was calculated using the DRV (PRI equal to 1 g/kg weight/day) and children’s weight reported by paediatricians during periodic health check. There was an unbalanced FAs distribution with an energy contribution of SFAs higher than DRV (< 10 E %) for 87.9% of children while that of PUFAs appear to be in line with recommendation only for 5% of children. In particular, the intake of LA and ALA was met by 4.9% and 29.6% of children, respectively (mean E % equal to 2.3% and 0.4%, respectively). Total carbohydrates intake was met by 77.7% of children with a contribution to energy intake equal to 53.5%. However, approximately 93% of children exceeded the DRVs for soluble carbohydrates (mean E % equal to 22) while 42% of them did not met the fiber recommendation (8.4 g/1000 kcal). Unfortunately, there are not available DRVs for cholesterol, starch, and oleic acid.

A high percentage of children reported intake below the DRV for the following micronutrients: 76.3% for potassium (median = 1382 mg/day vs. 1700 mg/day as recommended), 69.7% for zinc (median = 4.3 mg/day vs. 5 mg/day as recommended), 87.7% for vitamin E (median = 3.2 mg/day vs. 5 mg/day as recommended), 62.7% for niacin (median = 6.4 mg/day vs. 7 mg/day as recommended) and 72.8% for folate (median = 116.0 µg/day vs. 140 µg/day as recommended). Moreover, all children (100%) did not met the recommendation concern vitamin D with a median

(IQR) intake equal to 0.6 (0.4 – 1) µg/day vs. 15 µg/day recommended, while only 3.1% of children reached the AI concern iron (median = 4.4 mg/day vs. 8 mg/day as recommended). On the other hand, the assumption of calcium, sodium, vitamin group B and vitamin C were higher than recommended values for 51.7%, 68.1%, ~ 90% and 68.9% of children, respectively. Statistically significant differences between females and males were also observed in intake of available carbohydrates, soluble carbohydrates, starch, fibre, and vitamin E α-tocopherol equivalent (p-value < 0.05).

The percentage distribution of the four food types in the total intake of food's quantity, energy, and macronutrients are shown in **Figure 5**. I decided to report only these variables in relation to the low percentage of missing data instead of other nutrients (FAs, minerals, vitamins) for which data concern commercial products (food labels) were often missed.

Overall, it is noted a prevalence consumption of non-commercial products for all the variables considered with a percentage contribution approximately 80% (mean quantity = 757.0, STD 140.4 g/day) while that of commercial products was approximately 15% (mean quantity = 78.9, STD 37.1 g/day). The contribution of human milk and formula milk were minimal: ~1.5% (mean quantity = 17.3, STD 15.4 g/day) and ~0.4% (mean quantity = 4.7, STD 6.4 g/day). In fact, 65 (16.7%) and 21 (5.3%) children already consumed human milk and formula milk, respectively.

In **Figure 6.** and **7.** are reported the percentage distribution of the 19 food groups to the total intake of food's quantity and macronutrients. In the figures are only highlighted the main food groups contributor for each variable considered.

Overall, it is shown that, the main source of energy and quantity of foods was milk and dairy products with a percentage contribution equal to 33.6% and 45.0%, respectively. In the case of energy, the others food sources were cereal-based products (20.9%) and baby foods and snacks (13.0%) while concerning quantity of foods, the second and third food groups' contributor were soups (7.1%) and cereal-based products (6.7%). The consumption of fish (1.7%), eggs (0.4%), cured meat (1.0%) and pulses (1.6%) was negligible. Milk and dairy products also represented the principal source of total proteins (41.8%) and soluble carbohydrates (40.3%) and the second food source of available carbohydrates (20.0%). The total protein came also from meat (14.7%) and cereal-based products (13.9%) while the available carbohydrates intake derived from cereal-based products (34.6%), baby foods and snacks (16.2%) and fruits (10.4%). Fruits (27.7%) and cereal-based products (73.4%) represented the main contributor of fiber and starch intakes, as expected. Furthermore, the principal source of total fats and the different typology of FAs was milk and dairy products with a percentage contributions equal to 51.0% for total fats, 64.7% for SFAs, 43.1% for MUFAs, 39.8% for oleic acid, 19.0% for PUFAs, 44.2% for LA and 44.6% for cholesterol. The other two food groups that contributed to the fats intake were fats and oils and baby foods and snacks: 14.1% and 11.1% for

total fats, 8.2% and 11.2% for SFAs, 25.7% and 7.3% for MUFAs, 28.4% and 7.6% for oleic acid, 16.1% and 8.7% for PUFAs, 11.5% and 5.2% for LA. PUFAs came also from cereal-based products (10.2%), meat (8.7%) and cured meat (8.7%). Finally, the other sources of cholesterol were meat (14.2%), eggs (12.3%) and baby foods and snacks (9.9%).

The percentage distribution of the 19 food groups to the total intake of vitamins and minerals are shown in **Figure 8** and **9**. In the figures are only highlighted the main food groups contributor for each micronutrient.

The principal contributor of vitamin B group and folate intake was milk and dairy products with a percentage equal to 26.6% for vitamin B1, 62.6% for vitamin B2, 23.8% for vitamin B6, 55.5% for vitamin B12 and 24.9% for folate. Fish (23.9%) and meat (12.4%) also provided vitamin B12 intake and the latter food group represented the main contributor of niacin (19.4%). Moreover, the main source of vitamin D was fish follow by milk and dairy products with a percentage contribution equal to 35.2% and 21.6%, respectively. Concerning vitamin C, the principally food sources were fruits (41.5%) and vegetables (16.6%) while in the case of vitamin E, it came from fats and oils (27.7%), fruits (13.4%) and milk and dairy products (10.5%). The other two food groups that contributed to folate intake were vegetables (16.2%) and cereal-based products (15.9%). The principal source of all the minerals considered in this analysis, except iron, was milk and dairy products with a percentage contribution equal to 32.0% for sodium, 38.9% for potassium, 72.2% for calcium and 40.7% for zinc. However, this food group represented the second source of iron (13.9%) in combination with baby foods and snacks (13.8%) while the main contributor was cereal-based products (18.2%). Moreover, other sources of sodium were cereal-based products and soups with a percentage contribution equal to 21.0% and 10.3%, respectively.

I have explored the possibility that there may be an association between energy and nutrients intake of the mothers and their children. My results shown that such a weak positive significant statistical correlation exists for energy and some nutrients but it is of negligible entity ($r = 0.1\text{-}0.2$).

3.4. LIPIDOMIC ANALYSIS OF HUMAN MILK

The lipidomic analysis was carried out on 61 human milk samples from mothers belong to Italian component of PHIME cohort and considered in my PhD project for the nutritional analysis.

The most abundant lipid class was represented by triacylglycerol, followed by a low proportion of diacylglycerol while monoacylglycerol and phospholipids were presented in trace amounts.

As described in the Materials and Methods section, the human milk cell (HMC) membrane FA profile of mothers was based on a FA cluster of eighteen cis FAs and three trans FAs isomers expressed as relative percentage of the cluster, proposed in the approach of fatty acid-based functional lipidomics taking into account the most representative FA families and their roles in the membrane properties. From these values, the HMC membrane FA levels and indexes are shown in **Table 6**.

HMC membrane FA of these women was characterized by 41.1 (STD 5.1) % SFAs, 42.7 (STD 4.6) % MUFAs and 15.9 (STD 4.2) % PUFAs. The saturated palmitic acid provided approximately 28% of all human milk FAs and hence a major part of the total SFA content. A little contribution of total SFA content was also provided by myristic acid and stearic acid with a relative percentage equal to 6.0 (STD 1.9) % and 7.3 (STD 1.9) % of total FAs, respectively. MUFA were, along with SFAs, the most represented FA family in human milk of these mothers, and among them, oleic acid was the most abundant with a 38.5 (STD 4.4) % of total FAs. It also represented the principal FA detected in human milk samples. The longest SFA represented the circulating pool for oxidation. Stearic acid could rapidly be converted to oleic acid and this pathway indicated the strict metabolic relationship between the SFA and the MUFA pool. In fact, the SFA content of human milk samples negative correlated with MUFA content ($r = -0.60714$; $p < 0.0001$), oleic acid content ($r = -0.60846$; $p < 0.0001$). Moreover, it also negative correlated with PUFA content ($r = -0.55347$; $p < 0.0001$), LA content ($r = -0.56762$; $p < 0.0001$) and ALA content ($r = -0.37186$; $p < 0.0001$).

Among LCPUFAs, the precursor of ω-6 series, LA dominated, with 12 (STD 3.7) % of the pool of FAs in human milk and a strong positive correlation with total PUFA ($r = 0.96216$; $p < 0.0001$). It was followed by dihomo-gamma-linolenic acid and eicosadienoic acid, with a relative percentage around 1% while the content of ARA was low 0.4 (STD 0.1) %, as expected. In the ω-3 series, the precursor from this group, ALA also dominated. However, the amount was 10 times less than LA, at around 0.7%. EPA and DHA content were low: 0.06 (STD 0.03) % and 0.3 (STD 0.2) %, respectively. Moreover, the total amount of PUFA ω-6 series and PUFA ω-3 series were equal to 14.6 (STD 4.0) % and 1.3 (STD 0.4) % of total FAs resulting in a high value of inflammatory risk index (12.4, STD 4.3). Moreover, human milk samples were characterized by an unbalanced content of PUFAs with a low amount of PUFA ω-3 series, as reflected in a low value of PUFA balance index (8.0, STD 2.0).

The total trans FA content was equal to 0.1 (STD 0.06) % of total FAs and it was principally represented by the trans isomer of oleic acid such as elaidic acid (0.06, STD 0.04 %). Concerning BMC membrane characteristics, it was detected an unsaturation and peroxidation indexes equal to 74.0 (STD 7.8) and 32.0 (STD 14.0), respectively. Finally, there were not statistically significant difference for each FAs and indexes between the four mothers' group, that were created considering energy, available carbohydrates, total fats intakes from dietary assessment and inflammatory risk index, better specifying in Material and Methods section.

In **Table 7.** are shown the daily dietary FAs intake of the 61 women considered for lipidomic analysis. The mean total fat intake was equal to 97.3 (STD 31.9) g/day distributed as follow: 31.9 (STD 11.8) g/day from SFAs, 40.2 (STD 13.3) g/day from MUFAs and 17.3 (STD 6.1) g/day from PUFAs.

The most representative FA in maternal diet was oleic acid with an average intake equal to 37.3 (STD 12.4) g/day, those reflecting a commonly used of vegetable oils (e.g. olive oil) as dressing of many dishes as also detected in the maternal dietary assessment reported previously (Paragraph 3.2). The intake of total PUFA ω3 series was 2.3 (STD 0.8) g/day while that of total PUFA ω6 series was 14.8 (STD 5.3) g/day and so the inflammatory risk index was unbalanced in favour of the latter class of PUFAs (mean 6.3, STD 0.8). Moreprecisley, among the PUFA ω-6 series, LA was the most representative with an average intake equal to 14.3 (STD 5.2) g/day while the intake of ARA was 0.5 (STD 0.3) g/day. In the PUFA ω-3 series, the intake of ALA was 1.90 (STD 0.7) g/day while that of EPA and DHA were very low: 0.2 (STD 0.1) g/day and 0.3 (STD 0.2) g/day, respectively, those reflecting the very low consumption of fish.

A Spearman correlation analysis between FAs profile from maternal dietary intake and FAs profile derived from lipidomic analysis of human milk are shown in **Table 8.** It was detected a negative statistically significant correlation between ARA in human milk and the dietary intake of total MUFA ($r = -0.27329$; $p = 0.0331$), oleic acid ($r = -0.27247$; $p = 0.0336$), LA ($r = -0.28960$; $p = 0.0236$) and ALA ($r = -0.28007$; $p = 0.0288$). No other correlation was found, also considering other mothers' characteristics such as age and prepregnancy BMI (**Annex 4 and 5**).

4. DISCUSSION

4.1. MATERNAL DIETARY ASSESSMENT

The assessment of dietary intake of pregnant women belong to an Italian component of mother-child cohort and lived in a Mediterranean area, shows that most women did not adhere with a national recommendations [12], as reported in other studies conducted in a developmental countries [98,99].

Considering that the increase in energy requirement during the third trimester of pregnancy should be of 500 kcal/day, all the women reported a mean energy intake lower than recommended: 2309.7 kcal/day vs. 2544.3 kcal/day. This result is in line with those reported in a systematic review and meta-analysis published in 2012 by Blumfield and colleagues, that evaluated the energy and macronutrients intake during pregnancy in developed countries [98]. On the other hand, these data stand in contrast with the increase in prevalence of overweight and obesity, and of excessive GWG observed in developed countries [100,101], suggesting that, despite not achieving the target energy intake recommended for pregnancy, women are not in energy deficit during gestation. This trend is also evident in this cohort where about 1 out of 5 women started pregnancy in an overweight and/or obesity status probably due to inadequate eating habits and poor physical activity, as widely demonstrated [102] and the situation does not improve during pregnancy. Only about 40% of women in this cohort reached an adequate GWG at delivery, suggesting that they did not improve their nutrition status during this important period of life. Similarly, our results show that underweight before pregnancy and insufficient GWG affect 7.3% and 21% of women, respectively. These findings could, in part, explain the inadequate caloric intake of about ¼ of the women included in the present analysis.

The majority of women in this study had a total proteins and available carbohydrates intake within RI levels, as already observed in other studies [98,99,103]. For almost all women, the soluble carbohydrates intake exceeded the DRV and they came principally from fruits. The intake of the total fats and SFAs was above the recommended level and was consistent with data from a meta-analysis involving pregnant women in HICs [98]. As, expected, the most part of fats derived from animal-based products such as meat and cured meat and milk-dairy products, whereas, the contribution of fish was very low, in spite of its nutritional benefits and geographical location of this cohort. Furthermore, the majority of women reported an excessive intake of SFAs and cholesterol, mainly deriving form milk-dairy products and sugars, sweets, and desserts. A diet high in both total fats and SFAs during pregnancy, seems to be common in many women from developed countries [98] and has been linked to adverse health outcomes in both mothers and children [104,105]. A significant proportion of fats also derived from vegetable oils (mainly olive oil), commonly used in the Mediterranean area as dressing

in many dishes and representing, for these women, the main dietary contributor of PUFAs. However, the low consumption of fish and seafood (frequency equal to 1.0, STD 1.1 times/week) is reflected in a scarce intake of essential PUFAs, such as EPA and DHA, with a half percentage of women that did not reach the DRVs [12]. However, this results is in line with those reported in the third Italian National Consumption Survey 2005-2006 [85] and in the Sicilian study [106].

A low adherence to recommended intake of vitamin D, folate and iron, was observed in more than half of pregnant women from this cohort and seems to be in line with the results obtained by other authors [98,99,107–109] and could potentially have serious consequence for the developing foetus [110-112].

The intake of vitamin D deriving from diet alone was insufficient in all women and it came from fish source (the principal contributor of vitamin D intake). This is not surprising as vitamin D is contained in only few foods (fatty fish, egg yolk, butter, cod liver oil) and is mostly produced by endogenous synthesis following exposure to ultraviolet radiation. Furthermore, at the time of the study, there were no clear-shared clinical recommendations regarding vitamin D supplementation during pregnancy. It is only recently (since 2016) that the WHO has issued a recommendation for 15 µg/day vitamin D supplement during pregnancy, under individual medical advice [16]. Therefore, in order to accurately assess the adequacy of Vitamin D status during pregnancy, dietary assessment should be combined with direct measurement of Vitamin D levels and sun exposure [107,109]. Low folate intake in pregnancy is in line with inadequate periconceptional folic acid supplementation, as reported by the majority of women. Almost seventy-three percentage of mothers have reported to use vitamins/supplements during pregnancy that probably include also folic acid as other important micronutrients. Moreover, the principal source of folate was represented by cooked and fresh vegetables, which were also the main contributor of vitamin E. This is due to the fact that vegetables in the nutritional database [97] were dressed by using a standard portion of condiment (1 spoon of vegetables oil). The iron intake below to the DRV detected in more than half percentage of women derived probably to the fact that the main source of iron was represented by cereal-based products characterized by iron non-heme form (less biodisponibility) while the contribution of meat and cured meat was less than fifteen percentage, as reported for the Nordic mother-child population based cohort [99]. A number of other micronutrients were found to be barely adequate for approximately half of pregnant women population. This was the case niacin and vitamin B1, provided principally from meat and cured meat. However, the median intakes of all these nutrients were very close to DRVs. As also reported in other studies [98,99], the majority of the women had an excessive sodium intake, and, since the sodium contained in salt used in food preparation was not considered in the nutritional analysis, it is very likely that our data could underestimate the consumption of this micronutrient. In fact,

cereal-based products and milk and dairy-products represented the main source of this micronutrient for these women.

The findings of this dietary assessment indicate that, overall, the diet of these pregnant women could not adequately cover the requirements of several key micronutrients. Although it is important to underline that, these mothers should not be considered as deficient for specific micronutrients but they showed an increased risk of deficiency. In fact, using AR value as dietary reference, the percentage substantially decreased for some micronutrients such as iron (67.5%), vitamin B2 (18.1%) and niacin (41.5%) (data not shown).

Finally, all women respected the recommendations to not exceed caffeine intake above the 200 mg/day of caffeine, equivalent to just over 2 cups of filter coffee or 4 cups of tea [113], unlike what was detected in the Nordic mother-child cohort where about 20% of women exceeded the recommendations (median of caffeine equal to 90 mg/day). Although, the adverse effects of alcohol on foetus are well known and it is recommended to totally abstain from alcoholic in pregnancy, 68.4% of women in this cohort consumed alcoholic beverages with a mean intake equal to 1.5 alcoholic units/day (2.7, STD = 5.2 g/day of alcohol).

4.2. CHILDREN DIETARY ASSESSMENT

The results emerge from the nutritional analysis of the 7-DD records show that the diet of the children was characterized by a low variety with an excessive intake of proteins, mainly from animal sources and a scarce intake of PUFAs in particular essential FAs (EPA and DHA) and vitamin D. This could lead to negative short and long-term health consequences such as obesity, rickets, kidney diseases, delays in neurodevelopment [(49)].

Considering a DRVs concern energy equal to 790 – 1280 kcal/day for females and 870 – 1380 kcal/day for males referred to 1 – 3 years age range, 35.3% of female and 39.5% of males had a daily energy intake outside the Italian recommendation ranges reported above. However, the energy DRVs are calculated using the Schofield equation [(15)], that take into account a median weight for each age extrapolated from the Cacciari's growth charts [(114)], and a median PAF equal to 1.39 for children under 3 years of age [(12)]; even so probably the percentage values of children that did not meet the Italian recommendations could be under or overestimate.

Milk and dairy products represented the main component of children's diets at 18 months of age and were always the main sources of total proteins, available carbohydrates, SFAs, cholesterol and most of minerals and vitamins. This was reflected in an excessive intake of this macro and micronutrients compared to Italian recommendations [12].

Almost all children in this study had a total proteins intake above the RI levels (8-12 E%) and it was in line with other studies: 16.5 E% for children of this cohort, 16.8 E% for Italian children participated to the study conducted by Damianidi and colleagues [(115)] and 15 E% for children participated to Nutrintake 636 study conducted by Zuccotti and colleagues [(116)]. However, these proteins came mainly from dairy-products and so less contributed to iron intake that, in fact, appeared to be lower than AI value for almost all children. For the majority of children, the available carbohydrates intake was within the RI value while that of the soluble carbohydrates was excessive respect the DRV. Unfortunately, only 20% of soluble carbohydrates intake derived from fruits, which represents the better source instead of baby foods, snacks, sweets and desserts. This situation is also reported in a Nutrintake 636 study where about 6/66 (9%) children at 18 months of age met the AI concern soluble carbohydrates with a median intake equal to 49.3 g/day for females and 56.9 g/day for males [(116)], as happen for the children of this cohort (median intake of sugars equal to 51.5 g/day).

The majority of children had a total fats intake below the RI level with an unbalanced distribution between the different typology of FAs. Moreprecisley, their diet was characterized by a high intake of SFAs and a low intake of PUFAs, as compared to the Italian recommendations. This could reflect to an inadequate intake of essential FAs, EPA and DHA that are fundamental for

children's neurodevelopment. The most part of fats came from milk and dairy products while the contribution of fish and nuts was very low (5% and 1%, respectively). The reason was probably due to the parents or caregivers' fear to give these foods causing suffocation or allergic onset. A significant proportion of fats also derived from vegetable oils (mainly olive oil), commonly used in the Mediterranean area as dressing in many dishes and representing, for these children, the main dietary contributor of PUFAs. Despite national and international recommendations suggest limiting the assumption of snacks and desserts because rich in SFAs and soluble carbohydrates, their consumption are very common during infancy as reported in other studies [115,116] and also detected in this children cohort.

Concerning micronutrients, the results emerged that vitamin D, vitamin E, folate, iron were the nutrients most likely to be associated with insufficient intakes while sodium intake tended to be higher than recommended.

Moreprecisley, the totally of children had a very low intake of vitamin D and this deficiency seems to be a worldwide health problem with a similar data that have been described in infants in Netherlands [117], Spain [118], Finland [119], Belgium [120]. The high prevalence of inadequate intake could be justified by the fact that this micronutrient is found naturally only in few foods (oily fish, egg yolk, and liver) and even food fortification (e.g. milk, breakfast cereals, margarine) has a modest effect on vitamin D status [61]. Although, the consumption of these foods was negligent in the children belong to this cohort, fish represents the main source of this vitamin follow by milk and dairy products. Additionally, it is known that vitamin D could be also synthesized by sunlight, but sun exposure is not always sufficient to offset the low dietary intake during infancy when a higher skeletal growth rate occurs [12,61]. However, there is no homogenous consensus between national and international organization concern vitamin D supplementation in children over 12 months of age even if its use is strongly recommended in children who may be at higher risk of deficiency due to limited sun exposure (Nordic European population) or those with darker skin pigmentation [61]. The usual mean vitamin E intake was less than DRV in approximately 90% of children and this deficiency is more frequently found during infancy compared to adulthood, in relation to the limited stores and growing rapidly. A recent worldwide review concluded that vitamin E intake was insufficient in 61% of the reviewed studies and that 13% of the subjects, mainly newborns and children, were below the functional deficiency threshold for vitamin E and so its status is inadequate in a substantial part of the studied populations [121]. Moreover, almost all the children had an iron intake about a half of recommended value with a possible negative consequence on cognitive development. In fact, iron-deficiency represents the most common cause of anaemia in infants and toddlers [117] and an evaluation of iron intake performed with reference methods is especially important concern the first

1000 days. The main sources of this mineral were milk and dairy products, cereal-based products while the contribution of meat, and cured meat, characterized by high bioavailable iron form, did not reach the 10% of total iron intake. The intake of sodium exceeded the DRV in a considerable percentage of children and came principally from milk and dairy products and cereal-based products. Similar results have been shown for children in Netherland [117], Spain [118] and Italy [116]. Nevertheless, it is necessary to take account the difficulty to precisely measure added salt even, so it is likely that sodium intake is greater than that recorded. In fact, the best method for estimating sodium intake is the measurement of its excretion over 24 h while dietary survey and food composition database tend to underestimate its assumption.

Finally, the nutritional evaluation of children's diet highlight that its conduct was not completely adequate to cover the requirement of some important macro and micronutrients because characterized by a consumption of fruits and vegetables, fish and pulses less than recommended according to the Italian Food-based Dietary Guidelines [19] and typically of Mediterranean diet. However, the percentage of infants that did not reach the reference value changed for some micronutrients if AR was used instead of PRI. In particular, this percentage decreased for the intake of iron (37.5%), zinc (38.6%), niacin (24.7%) and folate (42.7%) (data not shown).

Finally, the consumption of non-commercial products (home-made foods) was prevalent in terms of all variable considered but after calculating the mean energy contribution per gram of each food type, it is interesting to note that it is higher in commercial products if compared to non-commercial products (1.6 kcal/g vs. 0.7 kcal/g, respectively) (data not shown).

4.3. LIPIDOMIC ANALYSIS OF HUMAN MILK

Human milk FAs profile of 61 mothers belong to an Italian component of PHIME cohort shows higher levels of total SFAs and total MUFAs reflecting a diet rich in carbohydrates and animal-based foods (Western diet), typically of HICs, as reported elsewhere [122]. On the other hand, the low content of LC-PUFAs, in particular EPA and DHA, confirms the scarce consumption of fish and fish-products, although these women lived in Mediterranean area. This aspect was already found in previous analysis of all mothers belong to the Italian component of PHIME cohort and it could be one of the possible reasons of a weak correlations between maternal fish intake and maternal serum concentration of DHA and with its percentage with of total serum FAs ($r = 0.08$, 0.0569 and $r = 0.09$, $p = 0.298$ respectively). Moreprecisley, the authors reported an average concentration of total PUFAs, EPA and DHA in maternal blood serum equal to 0.70±0.20 mg/mL, 0.04±0.03 mg/mL and 0.04±0.03 mg/mL respectively [123].

There were three dominating FAs in these human milk samples: palmitic acid (28%), oleic acid (40%) and LA (12%). Similar results have been shown in other studies worldwide [124]. Dominance of unsaturated FAs over saturated FAs helps to retain human milk fluidity (an average value of saturation index equal to 1.0, STD 0.2) and lipid digestion for breastfed infants. However, in the recent years, palmitic acid at the sn2 position of human milk triacylglycerol's are started to receive increased attention in relation to its capacity to enhance fat and mineral (e.g. calcium) absorption, intestinal comfort, bone density and to positive influence lipoprotein and non-esterified FAs metabolism [125]. Scientific literature suggests that a diet rich in carbohydrates also increase the concentration of medium-chain fatty acids (MFCAs) in human milk, whose are synthesized in the mammary gland from carbohydrates carbon skeletons. While in most tissues the synthesized is determinated after building a 16 carbon chain, the cytosol of mammary epithelial cells contains an acyl thioester-hydrolase (thioesterase II) that terminate FA synthesis already one a carbon chain length of 8-14 carbons is achieved [126]. However, in these human milk samples, the MFCAs content were not determinated.

Linoleic acid (12%) and alfa-linolenic acid (0.7%) detected in human milk samples of these mothers were similar to values concerning lactating women from Latvia [124] and women from other studies across Europe [127]: 11% and 1.1%, respectively. These essential FAs are either directly derived from the diet (short-term effect) or they had been incorporated into FA storage pools (e.g. adipose tissue or hepatic lipids) and so depended of dietary habits during the third trimester of pregnancy (long-term effect). Moreprecisley, data from scientific literature indicate that about 30% of the LA is directly derived from dietary intake with a human milk peak enrichment reached about

12 hours after assumption, whereas about 70% derives from fat deposits [126]. Instead, the proportion of ALA in the human milk derived directly from maternal diet during breastfeeding period is 65% [128]. Both these FAs originating in the diet are used for the synthesis of PUFA ω-6 series and PUFA ω-3 series and converted into CO_2. However, ALA coming from the body stores and contributing to its content in human milk seems to be much lower than the contribution of stored LA to human milk LA. The dietary intakes of PUFA ω-6 series and PUFA ω-3 series were within the DRVs but close to the lower limit: 5.3 E % and 0.9 E % vs. 4 – 8 E% and 0.5 – 2.0 E % recommended, respectively.

Arachidonic acid (0.4%) levels in human milk of these mothers were similar of the worldwide level of this FA (0.47%) [129] . Data from scientific literature reported that, the ARA content of human milk is quite stable and does not vary in relation to its maternal intake and the intake of its precursor (1.2% of ARA in human milk comes from maternal LA intake) but come from endogenous stores. This was reflected in a negative correlation between ARA level in these human milk samples and the dietary intake of LA. Moreover, the negative correlation between ARA level in these human milk samples and the dietary intake of ALA was the result of the competitor enzymatic cascade pathways for the synthesis of eicosanoids (prostaglandins, leukotrienes), and EPA and DHA, respectively. Mean DHA levels in human milk of these mothers (0.3%) was equal to mean worldwide level of this FA (0.32%), reported elsewhere [129]. It is well documented that, DHA levels in human milk are affected by maternal intake in particular during breastfeeding period, as remarkable by the strong or positive correlation reported elsewhere [126]. However, this correlation was not found probably due to less maternal dietary intake of this FA in association with EPA intake, resulting in a low consumption of fish and fish products. The lack of correlation between ALA in the diet and DHA in human milk was primarily the result of the human body's low efficiency in converting ALA into LCPUFAs, as suggest by several authors. In fact, a recent worldwide review published by Bobinski and Bobinska reports that about 2-10% of ALA are transformed into EPA and DHA while other authors suggest that conversion percentage is equal to 7% for EPA and only 0.013% for DHA. The total amount of trans FAs (0.1%) in human milk of these mothers were lower compared to data concern human milk lactating women from Latvia [124], in particular for elaidic acid (0.06% vs. 0.1%, respectively). Finally, the EFSA states that currently there are insufficient data to set an accurate value for the PUFA ω6: PUFA ω3 ratio, but it should be as low as possible (5:1) in order to reduce the risk of many chronic diseases (cardiovascular, inflammatory, autoimmune) [130]. This ratio in human milk samples of these mothers was approximately 13, which was higher than found in human milk samples among women from Latvia (~ 7) [124] and Croatia (~11) [131] but lower than values from Greece women (~26) [132] and Turkey women (~32) [133]. This high ratio is typically of contemporary diets (e.g. Western diet) characterized by an amount of PUFA ω6 series in particular ARA and a less amount of DHA.

Scientific societies and national and international recommendations agreed to affirm that differences in the amount of various FAs in human milk indicate that a women's body is probably adapted evolutionarily and genetically to the biosynthesis of milk with a composition adjusted to the need and state of the health of the child. In fact, an adequate supply from mothers concern essential FAs, such as LA and ALA, PUFA ω6 series and PUFA ω3 series, such as DHA and ARA, as well as total MUFAs and MCFAs is necessary in order to satisfy a child's growth and metabolic needs [128].

4.4. STRENGHT AND LIMITATIONS

4.4.1. MATERNAL DIETARY ASSESSMENT

A major strength of the maternal dietary assessment belongs to the Italian component of PHIME cohort and considered in my PhD project was the adoption of a rigorous design (cohort study), that allowed to accurate data collection and permit a comprehensive evaluation of variations in dietary habits during a vulnerable period of women life. In addition, the adoption of a method based on the prospective assessment of the diet of pregnant women eliminated the risk of recall bias. On the other hand, however, assessing the diet only once time in the third trimester of gestation, using a FFQ questionnaire, introduced the risk of missing dietary changes that may have taken place at different stages of the pregnancy. All FFQs are subject to large between-person errors because study participants tend to answer questions in a way that will be viewed favourably by others, which leads to over-reporting of good dietary habits and under-reporting of unhealthy habits, as also detected by Saunders and colleagues in the dietary assessment of Nordic pregnant women [(99)] . Nevertheless, the FFQ remains the most widely used instrument for the assessment of dietary habits, especially in large cohort.

Finally, this nutritional evaluation did not consider the supplements used by women during pregnancy, which can limit the completeness of nutritional data. . While this omission may limit the comprehensiveness of our data, it is in line with the indications provided by international recommendations for a varied and balanced diet with adequate intake of macro and micronutrients during pregnancy, which only consider nutrients derived from the diet.

4.4.2. CHILDREN DIETARY ASSESSMENT

The main strengths of the children dietary assessment belong to the Italian cohort and considered in my PhD project were the adoption of a rigorous design (prospective cohort study) that allowed for accurate data collection; the use of a 7-day dietary (7-DD) record which is the gold standard for the assessment of dietary intake during infancy [88]; the extraction of food composition and nutritional data using an opportunely integrated version of the Microdiet software. Nevertheless, Microdiet contains the Italian food composition database for epidemiological studies, that it was compiled using standard methods established by the EuroFIR project (www.eurofir.org) [93] and represents an important tool for epidemiological research, public health nutrition and education, clinical practice, and nutrition declaration of food labels.

The main limitation was the incomplete and not very detailed compilation of the 7-DD record for a good part of children, probably due to their length and complexity, which often required adopting standard recipes and measures. However, it is still comparable to those reported in other studies of this kind. Moreover, the use of commercial products in particular baby foods, which are generally characterized by poor nutritional labelling, may have limited the precision with regards to the intake of micronutrients such as vitamins and some minerals. Instead, the data on energy components (total proteins, available and soluble carbohydrates, and total lipids and SFAs) and sodium were completely covered [130]. The methodology adopted to estimate the consumption of human milk (frequency of feeds, perceived length of each feed by mothers) and the use of a literature-derived composition, constitute a weakness because it does not take into consideration inter-and intrasubject variability and may therefore contribute the inaccuracy of the assessment of nutrients intake [130] even if, the percentage of children that were still breastfed at 18 months were low (16.7%). Nevertheless, this methodology has been used before in other infants' cohort studies [72, 76]. Finally, the nutritional comparison between this cohort' nutrient intakes and the DRVs [12] may be hampered by the fact that the latter covers a wider age range (infants: 1-3 years).

4.4.3. LIPIDOMIC ANALYSIS OF HUMAN MILK

The main strength concern lipidomic analysis of human milk was that these sophisticated and detailed analytical methods provide the opportunity to obtain better insights into the physiological roles of fat and each FAs in early life, which may lead to further improvements in nutritional strategies. The use of a pooled human milk samples for 24-hour period (morning, afternoon, and night time) and two feeding points (hindmilk and foremilk) allowed considering the possible diurnal variations in human milk FAs composition.

The main limitation was the use of limited (N = 61) and undated human milk samples available. Nevertheless, a preliminary analysis of the human milk cell (HMC) membrane degradation state conducted in two samples revealed a membrane integrity associated with an adequate amount of each FAs, as also shown by a low peroxidation index value (mean = 32.0, STD 14.0).

5. MATERIAL and METHODS

5.1. STUDY POPULATION

The subjects involved in my PhD project are a mother-child pairs be part of the Italian component in the PHIME study [73]. Moreprecisley, in addition to the inclusion criteria previously reported [73], women with an energy intake during pregnancy lower than 1000 kcal/day or greater than 4000 kcal/day or with missing items (in the FFQ section) over 10% were excluded from the analysis. The energy intake cut-offs were defined using the range proposed by Willet for general population (500 – 3500 kcal/day) [134], increased by 500 kcal/day, which represents the energy requirement increase by Italian DRVs [12] in the third trimester of gestation. Concerning the percentage of missing data, it was based on the methodology adopted for the dietary assessment in the ALSPAC cohort study [135]. Finally, only children who completed the 7-DD record at 18 months were considered for nutritional analysis.

5.2. MATERNAL DIETARY ASSESSMENT

Dietary data were collected using the long questionnaire (**Annex I**) that was admistered to mothers at 28 week of gestation – 1 months after delivery. It included a detailed food frequency section concern the consumption of 138 food items during pregnancy adapted from a validated food frequency questionnaire [136–138] and a qualitative part investigating the consumption of over 22 fish species commonly fished or marketed in the study areas, in order to better identify and quantify the main source of metilmercury (MeHg). It was also collected sociodemographic and health status information on the mother and her family, information on the pregnancy and delivery and the health status of the newborn child, a detailed residential and occupational history of the mother, a record of maternal smoking, drinking and general dietary habits [75].

Moreprecisley, the food frequency section included data on the consumption of: hot beverages, milk and sugar (16 item); bread, cereals and first course (17 item); main course (28 item); vegetables (21 item); fruits (22 item); sweets and various food (e.g. popcorn, chips etc.) (21 item); non-alcoholic beverages (7 item) and alcoholic beverages (6 item). The portion size of each item was indicated in the questionnaire (grams, number of pieces, cup, plate, glasses, can). The response category used to indicate the usual frequency of food consumption over the time course were: never, less than once per month, 1-3 times per month, once per week, 2-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day and more than 3 times per day. Only, for non-alcoholic (7 item) and alcoholic

beverages (6 item) the response category was: never, less than once per month, 1-3 times per month, once per week, 2-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day and more than 5 times per day. The response categories for each item were converted into continuous values of intake by assigning to each category a consumption level equal to the median value for that category (for example, 2-4 times/week became 3 times/week). Finally, each response category were converted in weekly consumption for each item.

The nutritional analysis was performed using the Italian Food Composition database for Epidemiological Studies in Italy (version 1998) [97] and considering 30 food components reported as follow: total proteins, carbohydrates (available and soluble, starch, fiber), lipids (total, SFA, MUFA and PUFA; oleic, LA, ALA, ARA, EPA and DHA; cholesterol), minerals (calcium, phosphorus, potassium, sodium, iron, zinc) and vitamins (vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E expressed as α-tocopherol equivalent, niacin, folate). Some nutrients such as iodine and magnesium, which are particularly important during pregnancy, were not considered for this study due to the high percentage of missing data in the food composition database used for the analysis. Energy was calculated using the mean quantity intake of each macronutrient and applying the corresponding energy conversion factors.

Foods and beverages were regrouped into 17 food groups based on the methodology proposed by Talamini et al. (2006) [139] and modified as follows: cereal-based products; meat and cured meat; milk and dairy products; fruits; dried fruits; cooked vegetables; fresh vegetables; sugars, sweets and desserts; fish; eggs; coffee, tea and chocolate; soy-based products; soups; tubers; pulses; alcoholic beverages and water.

5.3. CHILDREN DIETARY ASSESSMENT

Dietary data were collected using a 7-day dietary (7-DD) record (food diary) (**Annex II**) provided to mothers at 18 months of children's age, together with the instructions on how to complete its. Specifically, the information required in the food diary were as follows: time and place of consuming food and/or recipe; type of food specifying where possible any brand; type of recipe with the list of ingredients, composition and quantities; portion of food eaten raw and/or cooked. Mothers of breastfed infants were asked to record an estimate of feeding duration expressed as "long" "medium" and "short" feed that was converted in volume of milk consume (30, 50, 70 mL), as suggested by Cameron & Hofvander [140]. Was also required to report the state of child health and any medicine taken during period of compiling the food diary. Moreover, mothers were asked to report weight and length of their children as measured by their community paediatrician during periodic

health checks. The instructions attached to the food diary also included a table with a list of common kitchen utensils that could be used, as an alternative to weighting, to measure solids and fluids (teaspoon, tablespoon, glass and cup) and an indication of the estimated equivalent in grams. When the 7-DD record was given to mothers, the table was explained, and the mothers were asked to test the utensils they had at home and to validate our estimate. The measures recorded by mothers in their food diaries (e.g. half a teaspoon) were then translated into grams in our database. If the amount of salt, herbs and other condiments were reported in the form of common domestic measure (e.g. salt pinch), they were translated using weight standards reported in the validation study of an Italian food frequency questionnaire [138]. Serving sizes were indicated using food weight (e.g. 50 g of baked potatoes), "natural" serving size (e.g. one apple, one jar of baby food or one baby's bottle of milk) or common portion size. (e.g. "one plate of pasta"). However, in the absence of information about quantities of foods consumed, the following criteria were used: recommended portions for Italian infants indicated by Italian DRVs [12]; recommended portions for the nurseries in Friuli Venezia Giulia [141]; portion sizes obtained from the manufacturer's indication.

Coding and conversion of foods intakes in nutrients were carried out using the Microdiet V2.8.6 software (Microdiet software – Downlee Systems Ltd., High, Peak, UK), which contains the Italian Food Composition database for Epidemiological Studies in Italy – BDA (*Banca Dati di composizione degli alimenti per studi epidemiologici in Italia*) [97], the Food Standard Agency database [142] and the USDA National Nutrition database [143]. Food composition data were mostly drawn from BDA, with the exception of nine food items, which came from the USDA database (tomato ketchup, curry powder, poppy seed, cereals ready-to-eat Kellogg, hummus, game meat, and couscous dry and cooked millet raw). Since BDA only contains simple foods, it was necessary to create a specific food composition database (User Database) including data from food labels and, in the case of human milk, from literature [144]. The food labels are recovered by consulting the websites of the major infant formulae and infant food manufacturers and of other food industries, from September 2017 to July 2018. Whenever detailed information about the recipes prepared from mothers were missing, were taken from Italian traditional cookbooks and from the Photographic Food Atlas [145], both of which had been previously used in a large case-control study conducted in Italy [137,138]. Composite foods and recipes that did not have an equivalent in the food tables were broken down into their components and allocated codes and weights appropriately. The 7-DD records were subsequently extracted in text format, converted into excel format and decomposed as shown in **Examples 1** and **2**.

The nutritional analysis was performed on 27 food components: total proteins, carbohydrates (available and soluble, starch, fiber), lipids (total, SFA, MUFA and PUFA; oleic, LA and ALA; cholesterol), minerals (sodium, potassium, calcium, iron, zinc) and vitamins (vitamin B_1, vitamin B_2,

vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E expressed as a-tocopherol equivalent, niacin, folate).

Foods were classified into 19 food groups based on the methodology proposed by Talamini et al. (2006) [139] and modified as follows: cereals-based products, milk and dairy products, eggs, vegetables, fruits, seeds and nuts, herbs and spices (also salt), fish, meat, fats and oils, beverages (water and beverage without sugar), sugars, sweets and desserts, sauces, tubers, baby foods and snacks, soups, beverages (fruit juices, camomillae), pulses and cured meat.

Based on the methodology used by Noble and colleagues in the ALSPAC study [146], foods were also classified into 4 food types: commercial products (data from food labels), non-commercial products (data already present in the BDA), human milk and follow-on formula.

Example 1. A one day of 7-DD record loading in Microdiet V2.8.6

(It is highlighting the recipe to be decomposed)

Code	Item	FoodCode	FoodName	Grams	MealCode	DayCode	Protn	Fat	CHO	Sugars
IB0XX	35	ALI128	human milk 18 months (HM)	80	S	3	0.88	2.56	5.6	5.6
IB0XX	36	ALI128	human milk 18 months (HM)	40	S	3	0.44	1.28	2.8	2.8
IB0XX	37	C406	Banana [musa sapientium]	80	B	3	0.96	0.24	12.4	10.24
IB0XX	38	C1630	Yoghurt, whole milk	100	B	3	3.5	3.9	3.6	3.6
IB0XX	39	ALI26	Bread	30	M	3	2.36	0.25	17.94	0.66
IB0XX	40	IB0XX_5	**vegetables soup 5 IB0XX**	140	L	3	1.21	0.28	7.65	2.96
IB0XX	41	ALI6	Homogenized turkey baby foods (E)	40	L	3	3.12	1.08	2.39	ns
IB0XX	42	C1714	Grana cheese	10	L	3	3.55	2.5	0.37	0.37
IB0XX	43	C702901	Vegetable oil, olive, extra virgin	3	L	3	0	3	0	0

Abbreviation: Protn, protein; CHO, available carbohydrates; S, snack; B, breakfast; M, mid-morning snack; L, lunch

Example 2. A one day of 7-DD record loading in Microdiet V2.8.6

(It is highlighting the recipe decomposed)

Code	Item	FoodCode	FoodName	Grams	MealCode	DayCode	Protn	Fat	CHO	Sugars
IB0XX	35	ALI128	human milk 18 months (HM)	80	S	3	0.88	2.56	5.6	5.6
IB0XX	36	ALI128	human milk 18 months (HM)	40	S	3	0.44	1.28	2.8	2.8
IB0XX	37	C406	Banana [musa sapientium]	80	B	3	0.96	0.24	12.4	10.24
IB0XX	38	C1630	Yoghurt, whole milk	100	B	3	3.5	3.9	3.6	3.6
IB0XX	39	ALI26	Bread	30	M	3	2.36	0.25	17.94	0.66
IB0XX		C312	**Carrots [daucus carota]**	33.33	L	3	0.37	0.00	2.53	33.33
IB0XX		C381	**Potatoes [solanum tuberosum]**	26.67	L	3	0.56	0.27	4.80	26.67
IB0XX		C101	**Green beans [phaseolus vulgaris]**	13.33	L	3	0.28	0.01	0.32	13.33
IB0XX		C999999	**Water**	66.67	L	3	0.00	0.00	0.00	66.67
IB0XX	41	ALI6	Homogenized turkey baby foods (E)	DA	L	3	3.12	1.08	2.39	ns
IB0XX	42	C1714	Grana cheese	10	L	3	3.55	2.5	0.37	0.37
IB0XX	43	C702901	Vegetable oil, olive, extra virgin	3	L	3	0	3	0	0

Abbreviation: Protn, protein; CHO, available carbohydrates; S, snack; B, breakfast; M, mid-morning snack; L, lunch

5.4. LIPIDOMIC ANALYSIS

The lipidomic analysis was carried out on human milk samples collected one month after birth by trained research personnel at the participant's homes (majority of samples) or by mothers us and delivered to research staff at the study hospital. Moreprecisley, human milk was collected from three feedings (approximatively 50 mL) over a 24-hour period (morning, afternoon, and night-time) and at two different points in each feeding occasion (i.e. at the initiation and the end of the feeding episode). It was squeezed directly from the breast or was extracted using a breast pump; however, a plastic container previously delivered to lactating mother was used for the collection and storage of sample in fridge at a temperature equal to -4°C between each feeding. Each human milk sample was then transferred and storage in a fridge in the University of Udine at a temperature equal to -80°C, prior to the analysis.

The lipidomic analysis was conducted at The Institute of Organic Synthesis and Photoreactivity (ISOF) of the National Research Council of Italy (CNR) in Bologna using an automated protocol that provides the following steps: a sampling of 200 µL of human milk from each tubes and weighted; a phospholipids extraction from cells using the Bligh and Dyer's method [(90)] (water 0.5 mL and chloroform: methanol 2:1); a transesterification of fatty acids metil ester (FAMEs) by treatment with a potassium hydroxide (KOH)/ methyl alcohol (MeOH) solution for 30 minutes at room temperature and extraction with *n*-hexane (2 mL). FAMEs were analysed using capillary column gas chromatography. GC analysis was run on the Agilent 6850 Network GC System, equipped with a fused silica capillary column Agilent DB23 (60 m _ 0.25 mm _ 0.25 _m) and a flame ionisation detector (FID), using specific conditions (T injector: 240°C; Volume injector: 1 µL; Split ratio: 1/50; Carrier gas: helium; Carrier gas flow rate: 1 mL/minute; Vapour pressure: 29 psi; Oven temperature: from 165 °C to 240°C). Optimal separation of all FAs and their geometrical and positional isomers was achieved. Identification was made by comparing them to commercially available standards (Sigma Aldrich and Larodan). The amount of each FAs was calculated as a percentage of the total FAs content (relative %), being > 97% of the GC peaks recognized with appropriate standards.

The fatty acid (FA) panel considered 21 fatty acids and three FA families: SFAs, myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0); MUFAs, C16:1(6+7)c, palmitoleic acid (C16:1; 9c), oleic acid (C18:1; 9c), vaccenic acid (C18:1; 11c), gadoleic acid (C20:1; 9c); PUFAs, ALA (C18:3), EPA (C20:5), DPA (C22:5), DHA (C22:6); LA (C18:2), gamma-linolenic acid (18:3), eicosadienoic (20:2), dihomo-gamma-linolenic acid - DGLA (C20:3); ARA (C20:4); trans isomers, considering palmitelaidic acid (C16:1 total trans), elaidic acid (C18:1; 9t); C18:2 monotrans.

Considering these FAs, the following indexes were calculated: total PUFAs ω-3 series, total PUFAs ω-6 series, total trans, inflammatory risk index (%PUFA ω6/%PUFA ω3), membrane fluidity index or saturation index (SI) (%SFA/%MUFA), Unsaturation index (UI) ((%MUFA*1)+(%LA*2)+(%DGLA*3)+(%AA*4)+(%EPA*5)+(%DHA*6)), Peroxidation index (PI) ((%MUFA*0.025)+(%LA*1)+(%DGLA*2)+(%AA*4)+(%EPA*6)+(%DHA*8)), PUFA balance ((%EPA + %DHA)/total PUFA*100).

5.5. STATISTICAL ANALYSIS

The general characteristics of mother-child pairs are presented as frequency and percentage distribution or mean, standard deviation (STD), median, respectively for categorical and continuous variables. Mothers' pre-pregnancy weight (kilograms) and height (centimetres) were self-reported. BMI (kg/m^2) was calculated (weight (kg)/ height (m^2)) and categorised, according to WHO definitions as: underweight (BMI<18.5 kg/m^2), normal weight (18.5 $kg/m^2 \leq$ BMI<25.0 kg/m^2), overweight (25.0 $kg/m^2 \leq$ BMI<30 kg/m^2) and obese (BMI$\geq$30.0 kg/m^2). The GWG was calculated by subtracting the mother's pre-pregnancy weight from the weight at delivery, and was categorised as insufficient, appropriate and excessive, based on current IOM recommendations [147].

Concerning children's dietary assessment, the average daily intake of energy, macro-, and micronutrients was calculated on a 7-day observation basis, excluding the use of supplements. The supplements' use were also not considered in mothers' dietary assessment.

Mean, standard deviation (STD), median and interquartile range (IQR) were computed for each macro and micronutrient intake for all mothers and for those selected for lipidomic analysis, for all children, and for each FA levels and indexes detected in human milk samples. The percentage contribution of macronutrients to energy intake was estimated for all mothers and children. Nutrients intake was compared with the Italian DRVs [12] and was expressed using different indexes (AI, RI, PRI). The percentage of mothers and children with intake below, within or above the DRV was estimated for each macro and micronutrient. Differences between children's gender for energy and nutrients intake were assessed with U Mann-Whitney test.

Based on dietary intake of energy, available carbohydrates, total lipids and PUFA ω6: PUFA ω3 ratio, mothers' selected for lipidomic analysis were classified in 4 groups: high intake of all variables considered; high intake of E, CHO, lipids but low PUFA ω6: PUFA ω3 ratio; low intake of all variables considered; low intake of E, CHO, lipids but high PUFA ω6: PUFA ω3 ratio. Differences between mothers' group for human milk FA levels and indexes were determined using Kruskal-Wallis test.

The percentage distribution of the food groups (N food groups mothers = 17, N food groups children = 19) in the total intake of each nutrient was estimated with the aim of establishing the principal food sources. Moreover, concerning children dietary assessment, the percentage distribution of each food type (N food type = 4) in the total intake of foods (quantity), energy and each macronutrients were determinated. The correlation between maternal prepregnancy BMI, maternal age at delivery and maternal GWG and dietary intake of mothers and children was assess using Spearman rank test. The same statistical test was performed in order to detected the correlation

between FAs profile and indexes from dietary intake, FAs profile and indexes from lipidomic analysis of human milk, mother's age, maternal prepregnancy BMI and maternal GWG.

The normality of the mothers' and children variables was tested using Kolmogorov-Smirnov test. Statistical significance for all tests was set at a p-value of 0.05. For all analyses was used SAS 9.3 for Windows (SAS Institute Inc., Cary, NC, USA).

6. TABLES

7. FIGURES

Table 1. General characteristics of mothers and children ($N_{mothers}$ = 646; $N_{children}$ = 389)

CHARACTERISTICS	
Mother's age at delivery, mean±STD (median)	33.1±4.3 (33)
Maternal BMI before pregnancy, n (%):	
Underweight	47 (7.3)
Normalweight	466 (72.1)
Overweight	97 (15.0)
Obese	36 (5.6)
Weight gain during pregnancy, n (%):	
Insufficient	136 (21.0)
Adequate	240 (37.2)
Excessive	168 (26.0)
Not reported	102 (15.8)
Maternal nationality, n (%)	
Italian	596 (93.0)
Foreigner	45 (7.0)
Mother's occupation, n (%):	
Employed on maternity	493 (76.3)
Employed worker	53 (8.2)
Housewife	51 (7.9)
Other condition	42 (6.5)
Not reported	7 (1.1)
Mother's marital status, n (%):	
Married/Living together with partner	575 (89.0)
Widow/single/never married/Separated/divorcing	65 (10.1)
Not reported	6 (0.9)
Mother's educational level, n (%):	
Elementary and middle school	107 (16.6)
High school	312 (48.3)
University degree	225 (34.8)
Not reported	2 (0.3)
Cigarettes smoked during pregnancy, mean±STD (median)	163.3±598.1 (0)
Use of supplements[a] during pregnancy, n (%):	
Yes	471 (72.9)
No	167 (25.8)
Not reported	8 (1.3)
Use of folic acid before pregnancy, n (%):	
Yes	252 (39.0)
No	394 (61.0)
Child's gender, n (%):	
Male	185 (47.6)
Female	204 (52.4)
Birth weight (g), mean±STD	3353.8±460.9
Birth length (cm), mean±STD	50.0±2.2

Abbreviation: STD, standard deviation; n, number of subjects
[a]including vitamins, minerals, and herbal products

Table 2. Daily macronutrients intake of mothers compared with dietary recommendations [12] (N = 646)

MACRONUTRIENTS	MEAN±STD	MEDIAN (IQR)	Italian DRVs[12]	% below DRV	% within DRV	% above DRV
Total proteins (g)	87.1±24.8	85.0 (72.8-109.8)				
Total proteins (E %)	*15.1*		10 – 20 E%[a]	0.3	97.5	2.2
Total fats (g)	89.5±27.1	86.0 (73.7-114.7)				
Total fats (E %)	*34.9*		20 – 35 E%[a]	-	52.2	47.8
Satured fatty acids (g)	29.5±10.0	27.9 (24.3-37.4)				
Satured fatty acids (E %)	*11.5*		< 10 E%[b]	-	25.7	74.3
Monounsatured fatty acids (g)	36.8±11.3	35.5 (30.5-47.5)				
Monounsatured fatty acids (E %)	*14.3*		10 – 15 E%[a]	1.1	63.5	35.5
Oleic acid* (g)	34.0±10.6	32.9 (28.4-44.2)	-			
Polyunsatured fatty acids (g)	15.7±5.3	15.1 (12.7-20.3)				
Polyunsatured fatty acids (E %)	*6.1*		5 – 10 E%[a]	17.0	82.0	0.8
Linoleic acid (g)	13.0±4.5	12.4 (10.5-16.8)				
Linoleic acid (E %)	*5.1*		4 E%[b]	15.0	-	85.0
Alfa - linolenic acid (g)	1.7±0.6	1.6 (1.4-2.2)				
Alfa - linolenic acid (E %)	*0.7*		0.5 – 2.0 E%[b]	7.9	92.1	
Arachidonic acid* (g)	0.5±0.3	0.4(0.3-0.6)				
Eicosapentaenoic acid (mg)	152.0±91.0	130.0 (90-190)				
Docosahexaenoic acid (mg)	252.0±179.0	209.0 (140-300)				
EPA + DHA (mg)	404.6±264.1	337.7 (231.9-489.4)	350 – 450 mg/day[a]	52.8	18.4	28.8
Cholesterol (mg)	308.6±117.1	284.4 (234.5-389.2)	< 300 mg/day[C]	54.8		45.2
Available carbohydrates (g)	292.8±85.5	284.3 (244.5-372.4)				
Available carbohydrates (E %)	*50.7*		45 – 60 E%[a]	16.7	77.7	5.6
Soluble carbohydrates (g)	132.6±52.0	125.1 (99.2-165.2)				
Soluble carbohydrates (E %)	*21.5*		< 15 E%[b]	-	10.2	89.8
Starch* (g)	159.9±55.3	153.3 (131.6-214.2)	-			
Fiber (g)	27.7±10.9	26.2 (21.5-36.6)	≥ 25 g/day[c]	46.0	-	54.0

Abbreviation: STD, standard deviation; IQR, InterQuartile Range; DRV, Dietary Reference Value; E%, Energy percentage; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

[a]Below DRV: extreme of the range not included (<); within DRV: extremes included above DRV: extreme not included (>). [b]Below DRV: extreme included (≤); above DRV: extreme not included (>). [c]Below DRV: extreme not included (<); above DRV: extreme included (≥).

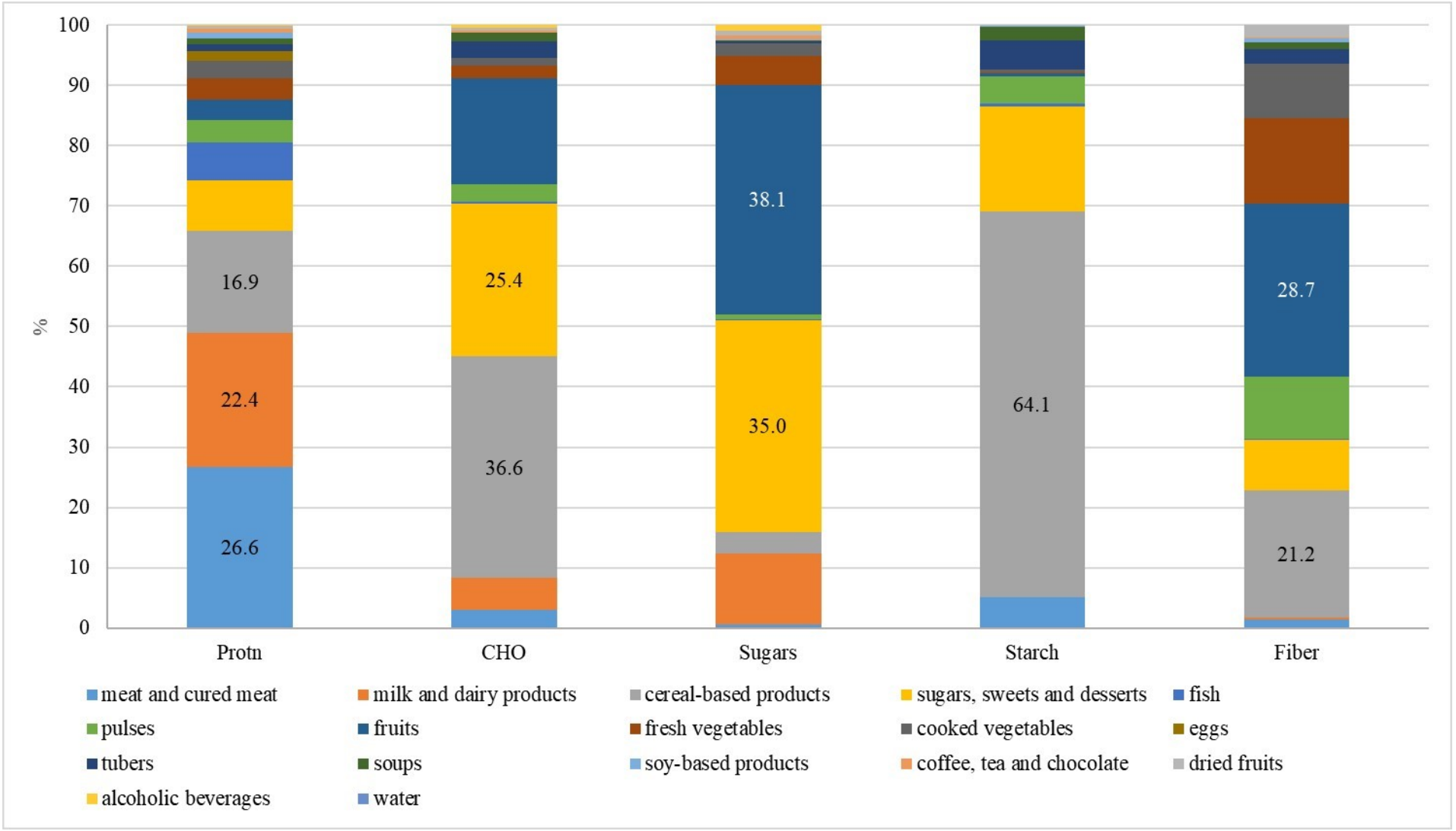
*not DRVs available

Table 3. Daily micronutrients intake of mothers compared with dietary recommendations [12] (N = 646)

MICRONUTRIENTS	MEAN±STD	MEDIAN (IQR)	Italian DRVs [12]	% below DRV[a]	% above DRV[b]
Calcium (mg)	1142.5±390.9	1100.9 (930.5-1432.0)	1200 mg/day	39.3	60.7
Phosphorus (mg)	1534.1±440.3	1513.2 (1281.6-1932.4)	700 mg/day	0.2	99.8
Potassium (mg)	4204.3±1454.8	4047.2 (3397.8-5330.1)	3900 mg/day	35.5	64.6
Sodium (mg)	1880.3±646.7	1793.8 (1466.4-2383.0)	1500 mg/day	32.4	67.7
Iron (mg)	14.4±4.6	13.8 (11.7-18.2)	27 mg/day	67.5	32.5
Zinc (mg)	11.5±3.3	11.3 (9.7-14.7)	11 mg/day	47.4	52.6
Vitamin B1 (mg)	1.3±0.4	1.2 (1.0-1.5)	1.4 mg/day	67.7	32.4
Vitamin B2 (mg)	2.0±0.6	1.9 (1.5-2.4)	1.7 mg/day	51.4	48.6
Vitamin B6 (mg)	2.5±0.8	2.4 (1.9-2.9)	1.9 mg/day	22.0	78.0
Vitamin B12 (µg)	6.1±3.5	5.3 (4.2-7.5)	2.6 µg/day	5.3	94.7
Vitamin C (mg)	249.7±131.6	220.2 (161.6-319.6)	100 mg/day	8.2	91.8
Vitamin D (µg)	3.1±1.4	2.8 (2.1-3.8)	15 µg/day	100.0	-
Vitamin E α-tocopherol equivalent (mg)	13.5±4.9	12.9 (10.0-16.2)	12 mg/day	33.0	67.0
Niacin (mg)	19.0±5.9	18.3 (15.2-23.8)	22 mg/day	72.3	27.7
Folate (µg)	423.4±161.5	406.3 (323.0-542.8)	600 µg/day	86.2	13.8

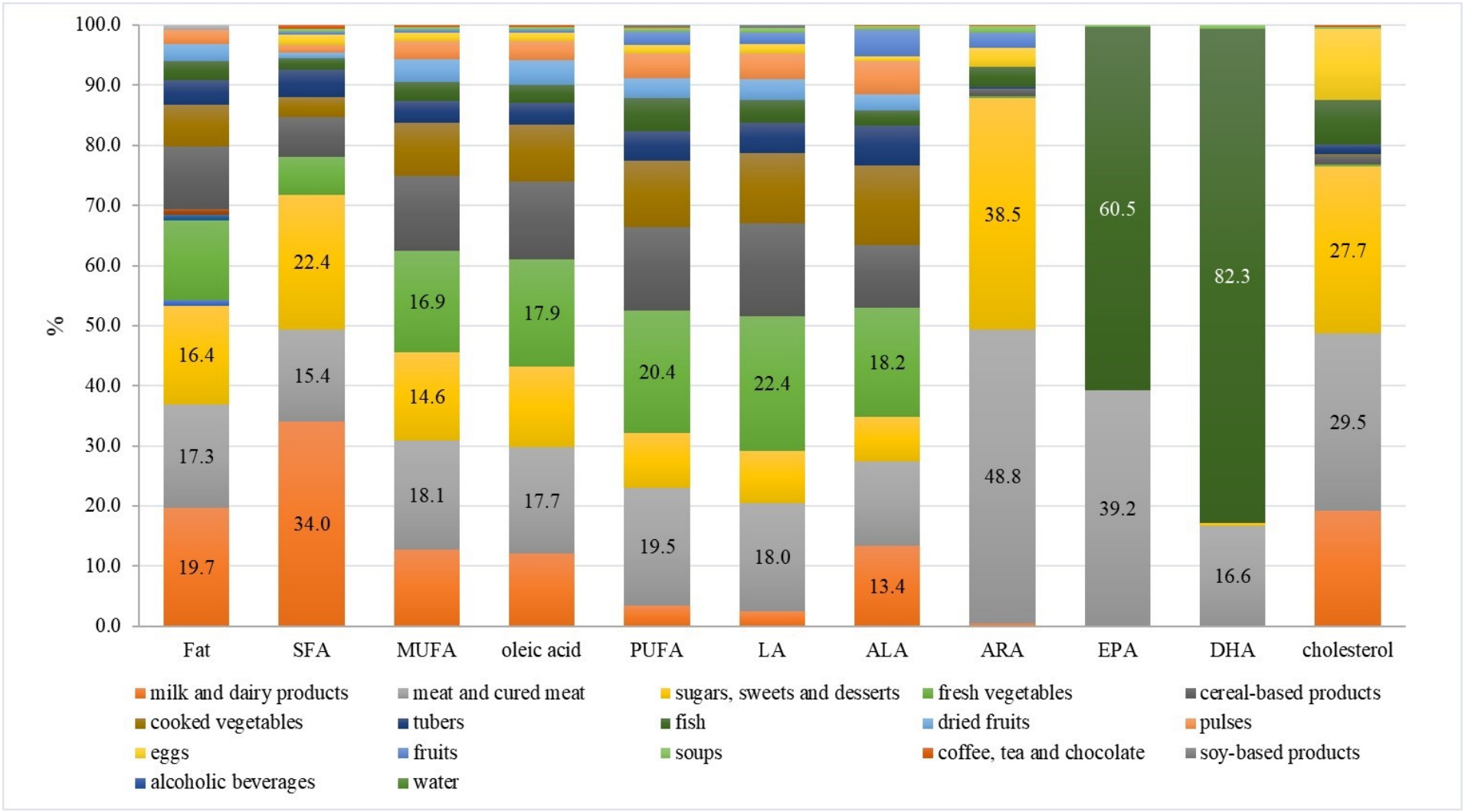
Abbreviation: STD, standard deviation; IQR, InterQuartile Range; DRV, Dietary Reference Value
[a]Below DRV: not included reference value (<). [b]Above DRV: reference value included (≥)

Figure 1. Percentage distribution of the different food groups of total proteins, available carboidrates, sugars, starch and fiber based on mothers' intake (N = 646)



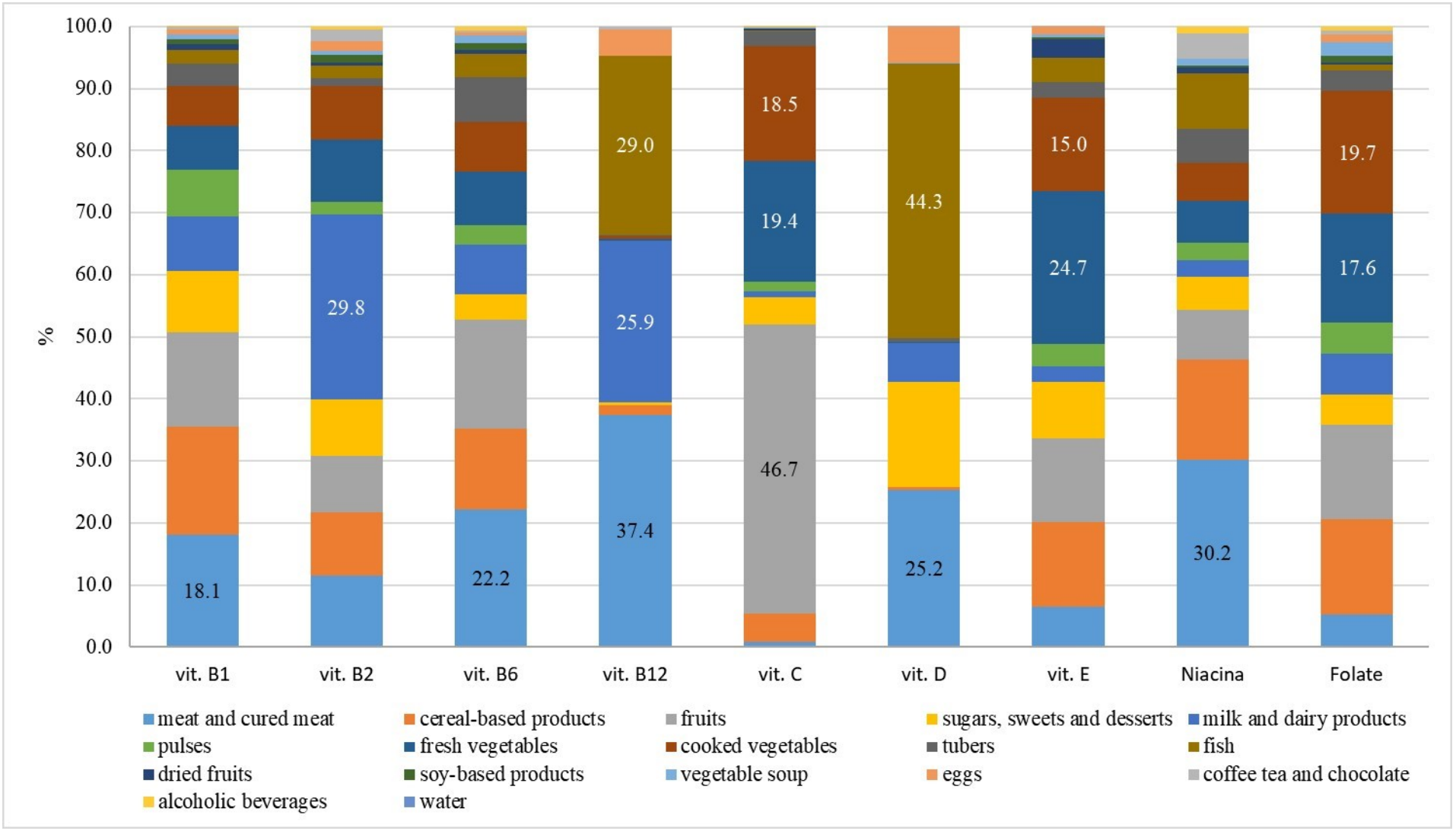
Abbreviation: Protn, total proteins; CHO, available carbohydrates

Figure 2. Percentage distribution of the different food groups of total fats based on mothers' intake (N = 646)



Abbreviation: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

Figure 3. Percentage distribution of the different food groups of vitamins based on mothers' intake (N = 646)



Abbreviation: vit., vitamin

Figure 4. Percentage distribution of the different food groups of minerals based on mothers' intake (N = 646)

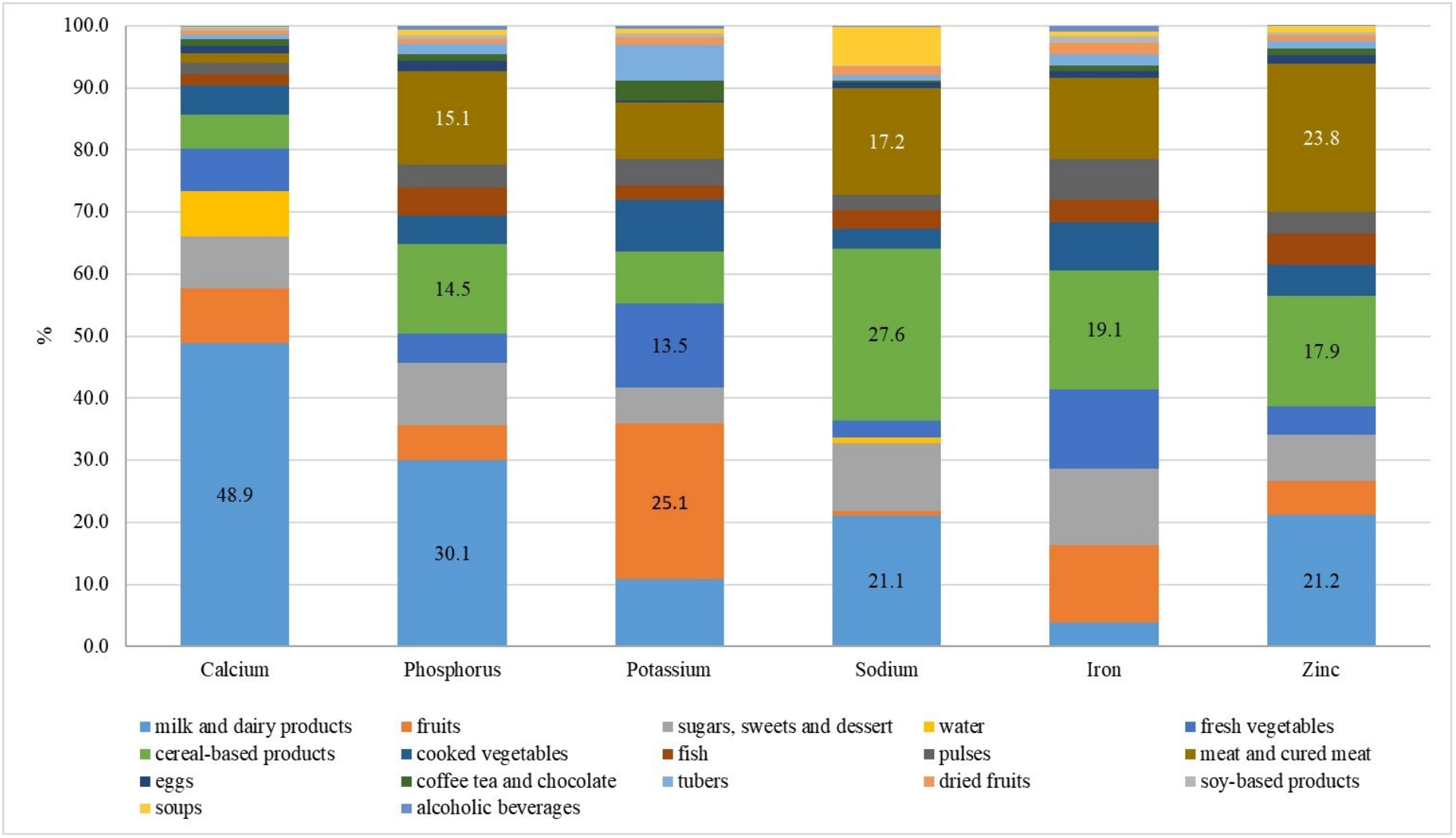


Table 4. Daily macronutrients intake of children compared with dietary recommendations [12] (N = 389)

MACRONUTRIENTS	MEAN±STD	MEDIAN (IQR)	Italian DRVs[12]	% below DRV	% within DRV	% above DRV
Total proteins (g)	37.8±10.0	36.9 (31.3-44.3)				
Total proteins (E %)	*16.5*		8 – 12 E%[a]	-	4.6	95.4
Total fats (g)	33.9±9.4	32.7 (27.2-39.8)				
Total fats (E %)	*33.2*		35 – 40 E%[a]	64.0	11.3	24.7
Satured fatty acids (g)	14.1±4.6	13.5 (10.7-17.2)				
Satured fatty acids (E %)	*13.8*		< 10 E%[b]	12.1	-	87.9
Monounsatured fatty acids (g)	11.6±4.0	11.1 (8.8-13.9)				
Monounsatured fatty acids (E %)	*11.4*		10 – 15 E%[a]	33.9	56.1	10.0
Oleic acid* (g)	10.2±3.9	9.8 (7.4-12.4)				
Polyunsatured fatty acids (g)	3.2±1.3	3.0 (2.3-3.8)				
Polyunsatured fatty acids (E %)	*3.1*		5 – 10 E%[a]	94.9	5.1	-
Linoleic acid (g)	2.4±1.1	2.2 (1.7-2.8)				
Linoleic acid (E %)	*2.3*		4 – 8 E%[b]	95.1	4.9	-
Alfa - linolenic acid (g)	0.4±0.1	0.4 (0.4-0.5)				
Alfa - linolenic acid (E %)	*0.4*		0.5 – 2.0 E%[b]	70.4	29.6	-
Cholesterol* (mg)	119.7±42.6	118.9 (90.8-143.7)				
Available carbohydrates (g)	122.6±30.1	120.9 (101.7-139.1)				
Available carbohydrates (E %)	*53.5*		45 – 60 E%[a]	11.1	73.0	15.9
Soluble carbohydrates (g)	53.8±16.8	51.5 (42.2-62.6)				
Soluble carbohydrates (E %)	*22.0*		< 15 E%[b]	6.7	-	93.3
Starch* (g)	56.0±22.7	54.5 (40.1-70.4)				
Fiber (g)	7.3±2.9	7.0 (5.2-9.2)	8.4 g/1000 kcal[c]	42.4	-	57.6

Abbreviation: STD, standard deviation; IQR, InterQuartile Range; DRV, Dietary Reference Value; E%, Energy percentage

[a]Below DRV: extreme of the range not included (<); within DRV: extremes included above DRV: extreme not included (>). [b]Below DRV: extreme included (≤); above DRV: extreme not included (>). [c]Below DRV: extreme not included (<); above DRV: extreme included (≥).

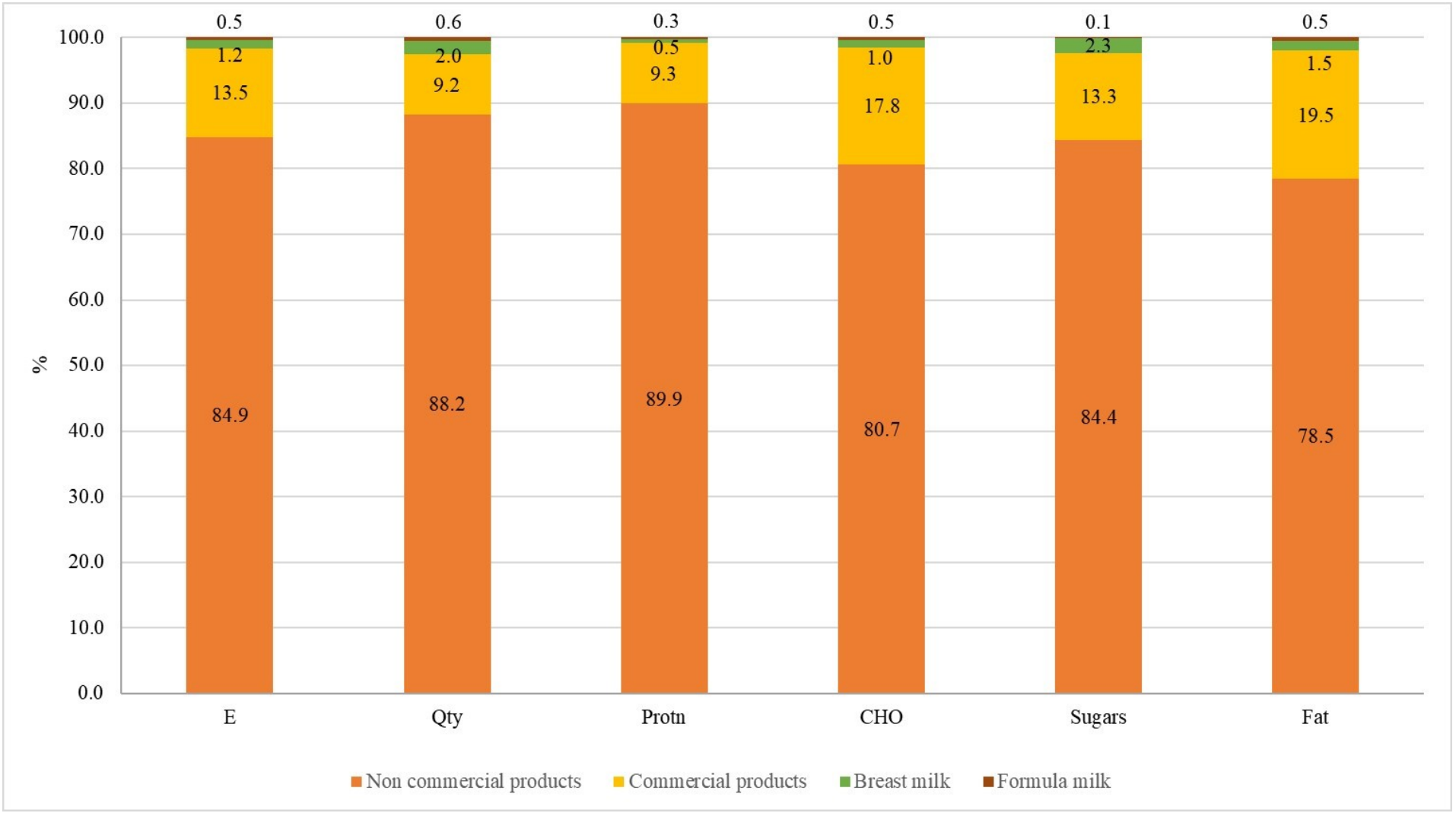
*not DRVs available

Table 5. Daily micronutrients intake of children compared with dietary recommendations [12] (N = 389)

MICRONUTRIENTS	MEAN±STD	MEDIAN (IQR)	Italian DRVs[12]	% below DRV[a]	% above DRV[b]
Calcium (mg)	643.2±234.8	622.1 (479.5-797.7)	600 mg/day	48.3	51.7
Potassium (mg)	1417.8±412.6	1382.5 (1147.6-1683.8)	1700 mg/day	76.3	26.7
Sodium (mg)	880.0±310.9	846.1 (661.2-1053.8)	700 mg/day	31.9	68.1
Iron (mg)	4.5±1.6	4.4 (3.5-5.3)	8 mg/day	96.9	3.1
Zinc (mg)	4.4±1.3	4.3 (3.5-5.2)	5 mg/day	69.7	30.3
Vitamin B1 (mg)	0.6±0.2	0.6 (0.5-0.7)	0.4 mg/day	12.3	87.7
Vitamin B2 (mg)	1.0±0.3	1.0 (0.8-1.3)	0.5 mg/day	4.9	95.1
Vitamin B6 (mg)	1.0±0.3	1.0 (0.8-1.2)	0.5 mg/day	4.4	95.6
Vitamin B12 (µg)	2.3±1.1	2.2 (1.5-2.9)	0.9 mg/day	7.2	92.8
Vitamin C (mg)	55.5±34.8	46.1 (30.6-69.0)	35 mg/day	31.1	68.9
Vitamin D (µg)	1.1±2.1	0.6 (0.4-1.0)	15 µg/day	100	-
Vitamin E α-tocopherol equivalent (mg)	3.5±1.6	3.2 (2.4-4.4)	5 mg/day	87.7	12.3
Niacin (mg)	6.5±2.4	6.4 (5.0-7.7)	7 mg/day	62.7	37.3
Folate (µg)	121.7±44.1	116.0 (31.6-145.0)	140 µg/day	72.8	27.2

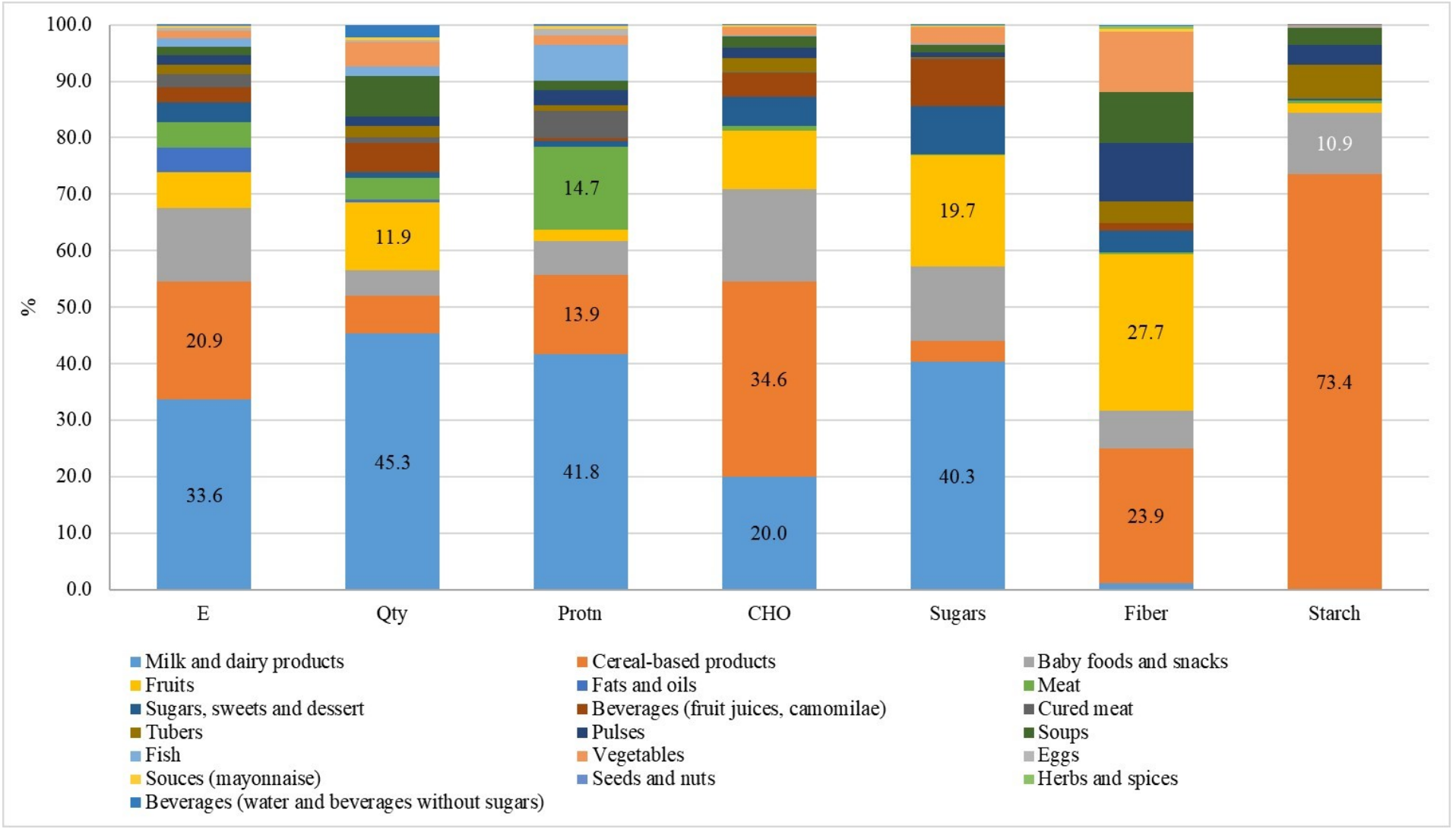
Abbreviation: STD, standard deviation; IQR, InterQuartile Range; DRV, Dietary Reference Value
[a]Below DRV: not included reference value (<). [b]Above DRV: reference value included (≥)

Figure 5. Percentage distribution of the different food type of quantity, energy, and macronutrients based on children's intake (N = 389)



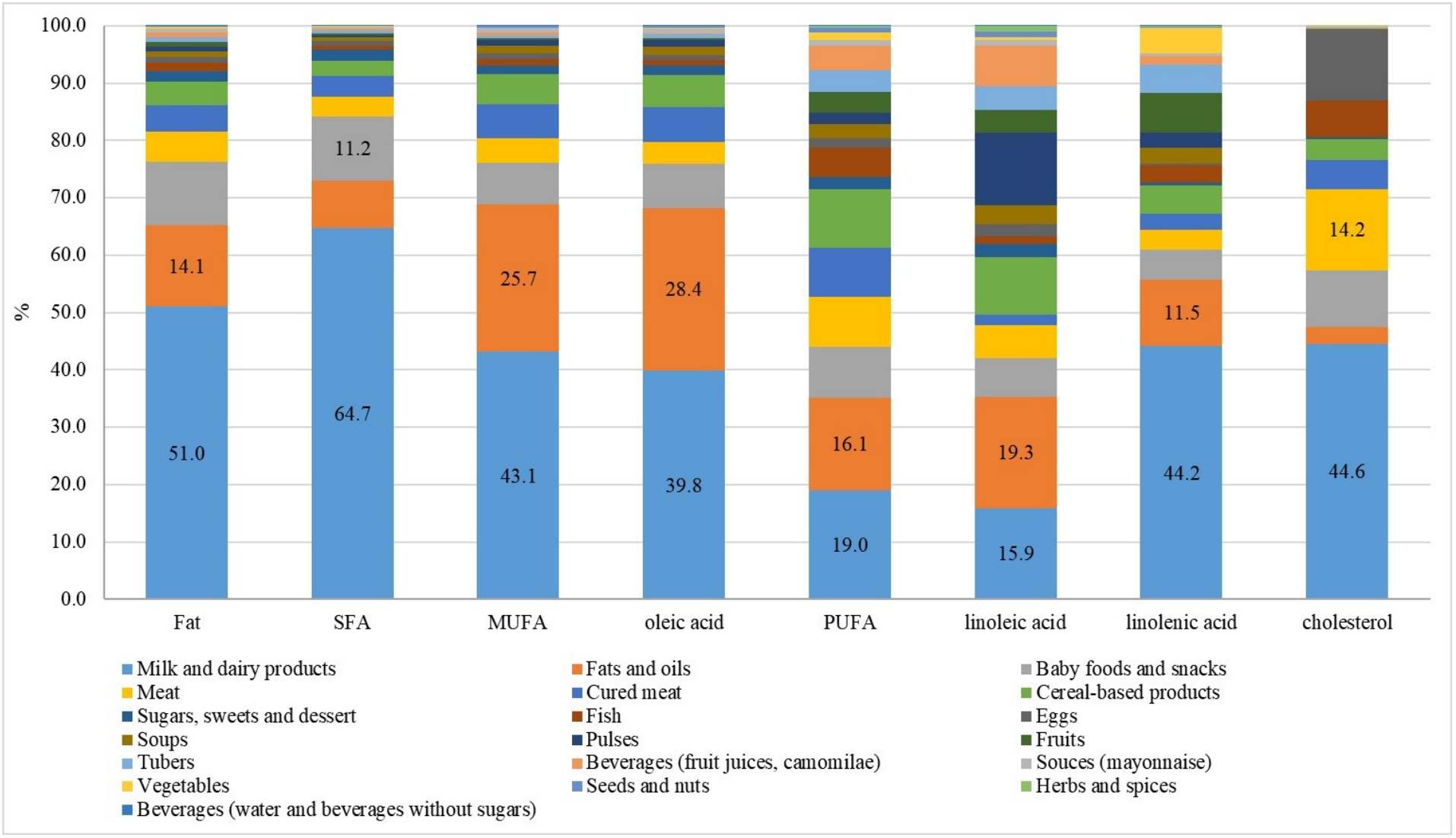
Abbreviation: E, energy; Qty, quantity; Protn, total protein; CHO, available carbohydrates

Figure 6. Percentage distribution of the different food groups of quantity, energy, total proteins, available carboidrates, sugars, fiber and starch based on children's intake (N = 389)



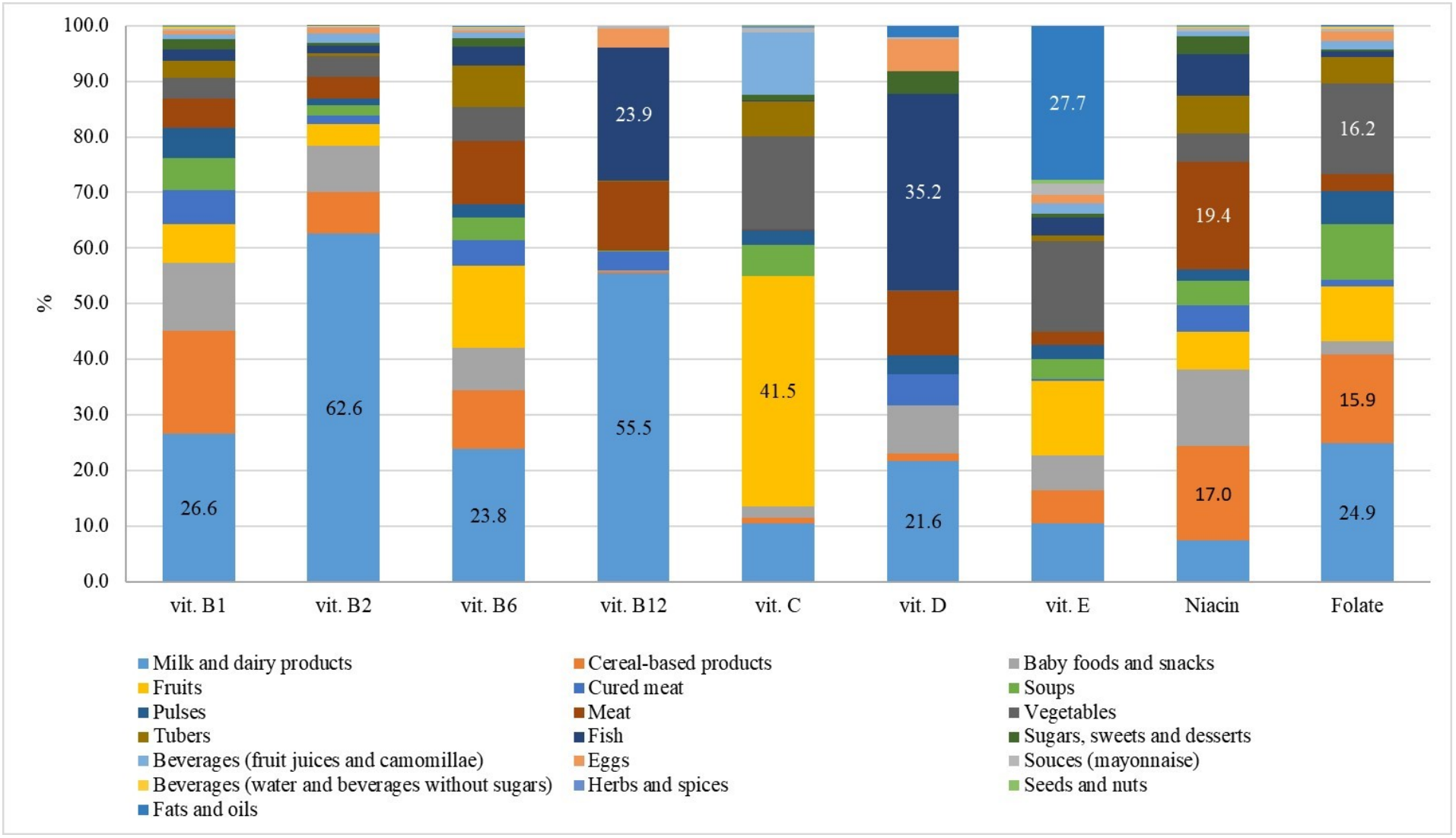
Abbreviation: E, energy; Qty, quantity; Protn, total proteins; CHO, available carbohydrates

Figure 7. Percentage distribution of the different food groups of fats based on children's intake (N = 389)



Abbreviation: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Figure 8. Percentage distribution of the different food groups of vitamins based on children's intake (N = 389)



Abbreviation: vit, vitamin

Figure 9. Percentage distribution of the different food groups of minerals based on children's intake (N = 389)

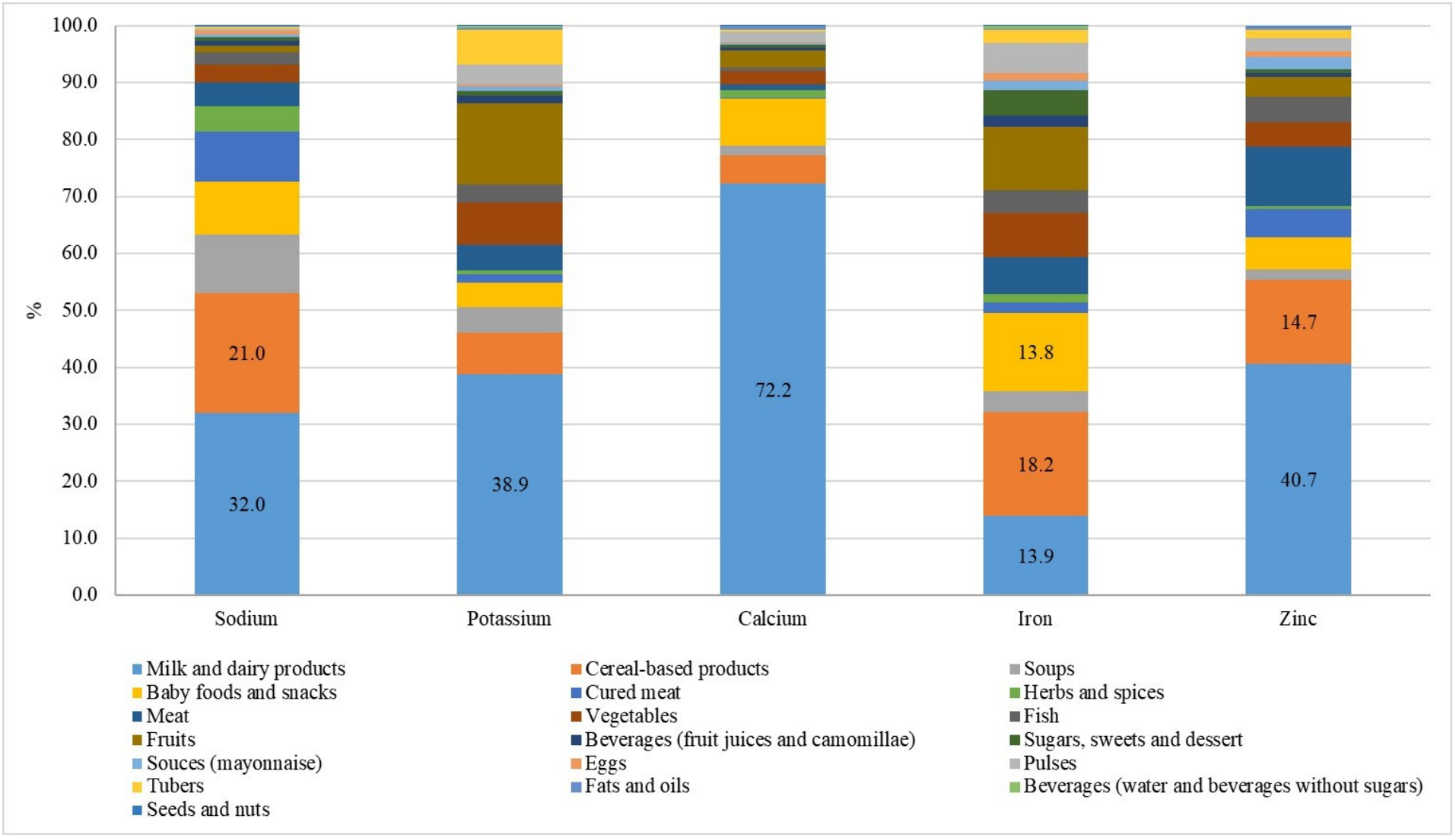


Table 6. Human milk cell (HMC) membrane fatty acid (FA) levels of mothers (N = 61)

HMC membrane FA (% rel)	MEAN±STD	MEDIAN (IQR)
Saturated fatty acids		
Myristic acid (C14:0)	6.0±1 9	6.0(4.7-6.8)
Palmitic acid (C16:0)	27.7±3.2	28.3(25.4-29.9)
Stearic acid (C18:0)	7.3±1.9	7.1(6.1-8.5)
Arachidic acid (C20:0)	0.2±0.1	0.2(0.2-0.3)
Monounsaturated fatty acids		
C16:1(6+7) cis	0.4±0.06	0.4(0.3-0.4)
Palmitoleic acid (C16:1 9c)	1.9±0.6	1.8(1.4-2.1)
Oleic acid (C18:1 9c)	38.5±4.4	37.7(34.1-41.7)
Vaccenic acid (C18:1 11c)	1.6±0.3	1.6(1.4-1.8)
Gadoleic acid (C20:1 9c)	0.4±0.1	0.4 (0.4-0.5)
Polyunsaturated fatty acids ω6		
Linoleic acid (C18:2)	12.0±3.7	11.0(9.6-13.3)
Gamma-linolenic acid (C18:3)	0.2±0.07	0.2(0.1-0.2)
Eicosadienoic acid (C20:2)	1.0±0.3	0.9(0.8-1.2)
Dihomo-gamma-linolenic acid (20:3)	1.1±0.3	1.1(0.9-1.3)
Arachidonic acid (20:4)	0.4±0.1	0.4(0.4-0.5)
Polyunsaturated fatty acids ω3		
Alfa-linolenic acid (C18:3)	0.7±0.3	0.6(0.5-0.8)
Eicosapentaenoic acid - EPA (C20:5)	0.06±0.03	0.05(0.03-0.07)
Docosapantaenoic acid - DPA (C22:5)	0.2±0.06	0.1(0.1-0.2)
Docosahexaenoic acid – DHA (C22:6)	0.3±0.2	0.3(0.2-0.4)
Trans fatty acids		
Trans C16:1	0.03±0.02	0.02(0.01-0.03)
Elaidic acid (C18:1 9t)	0.06±0.04	0.04(0.03-0.07)
Monotrans C18:2	0.04±0.03	0.04(0.03-0.06)
Total fatty acids		
Total SFA	41.1±5.1	41.3(36.9-45.5)
Total MUFA	42.7±4.6	41.8(39.5-46.4)
Total PUFA	15.9±4.2	14.5(13.5-17.7)
Total PUFA ω3	1.3±0.4	1.17(0.94-1.36)
Total PUFA ω6	14.6±4.0	13.4(12.1-16.3)
Total trans	0.1±0.06	0.1(0.08-0.2)
Fatty acids index		
Inflammatory risk index	12.4±4.3	11.9(9.7-13.2)
Saturation index	1.0±0.2	1.0(0.8-1.1)
PUFA balance	8.0±2.0	8.0(7.0-9.0)
Unsaturation index	74.0±7.8	72.7(68.4-79.6)
Peroxidation index	32.0±14.0	29.5(24.0-36.5)

Abbreviation: STD, standard deviation; IQR, InterQuartile Range; rel, relative quantity; c, cis; t, trans; SFA, saturated fatty acids, MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Table 7. Daily dietary FAs intake of mothers participating at the lipidomic analysis (N = 61)

FATTY ACIDS	MEAN±STD	MEDIAN (IQR)
Total lipids	97.3±31.9	91.5(73.0-120.3)
Monounsaturated fatty acids		
Oleic acid (C18:1 9 cis) (g)	37.3±12.4	35.5(26.8-46.7)
Polyunsaturated fatty acids		
Linoleic acid (C18:2) (g)	14.3±5.2	13.3(10.2-17.4)
Arachidonic acid (20:4) (g)	0.5±0.3	0.4(0.3-0.6)
Alfa-linolenic acid (C18:3) (g)	1.9±0.7	1.8(1.4-2.43)
EPA (C20:5) (mg)	0.2±0.08	0.2(0.1-0.2)
DHA (C22:6) (mg)	0.3±0.2	0.3(0.2-0.3)
Total fatty acids		
Total SFA (g)	31.9±11.8	29.7(25.2-38.2)
Total MUFA (g)	40.2±13.3	37.6(29.5-50.3)
Total PUFA (g)	17.3±6.1	15.9(12.3-21.4)
Total PUFA ω3 (g)	2.3±0.8	2.2(1.8-2.9)
Total PUFA ω6 (g)	14.8±5.3	13.7(10.5-18.0)
Fatty acids index		
Inflammatory risk index	6.3±0.8	6.3(6.0-6.8)
PUFA balance	0.1±0.02	0.13(0.13-0.14)

Abbreviation: STD, standard deviation; IQR, InterQuartile Range; rel, relative quantity; SFA, saturated Fatty Acids, MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

Table 8. Spearman Correlation between FAs profile from dietary intake and that derived from lipidomic analysis of human milk

FFQ (g) / Human milk (% rel)	SFA	MUFA	Oleic acid (C18:1 9c)	Linoleic acid (C18:2)	Alfa-linolenic acid (C18:3)	Arachidonic acid (C20:4)	EPA (C20:5)	DHA (C22:5)	Inflammatory risk index
SFA	0.16779 0.1962	0.08704 0.5047	0.07647 0.5581	0.08250 0.5273	0.05542 0.6714	0.04389 0.7370	-0.00931 0.9432	0.01169 0.9288	-0.11237 0.3885
MUFA	-0.15140 0.2441	-0.07208 0.5809	-0.06610 0.6128	-0.04860 0.7099	-0.03733 0.7751	-0.03099 0.8126	0.02105 0.8721	-0.07197 0.5815	0.03707 0.7767
Oleic acid (C18:1 9c)	-0.14035 0.2806	-0.06441 0.6219	-0.05971 0.6476	-0.04741 0.7167	-0.03448 0.7919	-0.00598 0.9635	0.07026 0.5906	-0.03800 0.7713	0.01116 0.9320
PUFA	-0.03096 0.8127	-0.03739 0.7748	-0.03051 0.8154	-0.03332 0.7988	-0.00783 0.9523	0.05428 0.6778	0.02811 0.8297	0.07324 0.5748	0.04350 0.7393
Linoleic acid (C18:2)	0.04532 0.7287	0.04918 0.7066	0.05582 0.6692	0.04804 0.7131	0.07454 0.5681	0.06499 0.6187	0.05254 0.6876	0.10965 0.4002	0.07647 0.5580
Alfa-linolenic acid (C18:3)	-0.00259 0.9842	0.04463 0.7327	0.04397 0.7365	0.07976 0.5412	0.06521 0.6176	0.04976 0.7033	0.05574 0.6696	0.08140 0.5328	-0.00590 0.9640
Arachidonic acid (C20:4)	-0.22038 0.0879	-0.27329 **0.0331***	-0.27247 **0.0336***	-0.28960 **0.0236***	-0.28007 **0.0288***	-0.00180 0.9890	-0.07549 0.5631	-0.07287 0.5768	-0.15058 0.2467
EPA (C20:5)	0.21154 0.1017	0.14373 0.2691	0.13292 0.3072	0.11425 0.3806	0.07756 0.5524	0.01994 0.8788	-0.07458 0.5678	0.18641 0.1503	-0.00900 0.9451
DHA (C22:5)	-0.03490 0.7895	0.02426 0.8528	0.01592 0.9031	0.00961 0.9414	0.03243 0.8040	0.17872 0.1682	0.16099 0.2152	0.07986 0.5407	0.03148 0.8097
Saturation Index	0.17737 0.1715	0.08456 0.5170	0.07578 0.5616	0.04109 0.7532	0.07081 0.5876	0.04754 0.7160	0.05299 0.6851	0.00984 0.9400	-0.08694 0.5053
Inflammatory risk index	-0.09448 0.4689	0.02813 0.8296	-0.01912 0.8837	-0.02240 0.8640	-0.04958 0.7044	0.07924 0.5438	-0.00690 0.9579	0.00412 0.9748	0.02782 0.8315

Abbreviation: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid
*the statistically significant correlation ($p<0.05$) are reported in bold type

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NORMATIVE REFERENCE

REGULATION (EU) No 609/2013 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control

COMMISSION DELEGATED REGULATION (EU) No 127/2016 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding

COMMISSION DIRECTIVE (EC) No 141/2006 on infant formulae and follow-on formulae and amending Directive (EC) No 21/1999

COMMISSION DIRECTIVE (EC) No 125/2006 on processed cereal-based foods and baby foods for infants and young children

Conferenza Stato Regioni Del. 20.12.2007 – Gazzetta Ufficiale n. 32 del 7-2-2008- Suppl. Ordinario n.32 Linee di indirizzo nazionali sulla protezione, la promozione ed il sostegno dell'allattamento al seno

9. ANNEX

Annex 1	Prototype of long questionnaire: Food frequency questionnaire section
Annex 2	Prototype of 7-day dietary record (food diary) (Italian version)
Annex 3	Daily macro and micronutrients intake of children divided by sex
Annex 4	Spearman Correlation between mother's age, pre-pregnancy BMI and FAs profile and indexes from dietary intake
Annex 5	Spearman Correlation between mother's age, pre-pregnancy BMI and FAs profile and indexes from lipidomic analysis of human milk

Annex 1. Prototype of long questionnaire: Food frequency questionnaire section (Italian version)

ABITUDINI ALIMENTARI

BEVANDE CALDE – LATTE - DOLCIFICANTI	Mai	Meno di 1 volta al mese	1-3 volte al mese	1 volta a sett	2-4 volte a sett	5-6 volte a sett	1 volta al giorno	2-3 volte al giorno	Più di 3 volte al giorno	Anche prodotto in casa?
Tè (1 tazza)	1	2	3	4	5	6	7	8	9	
Tisane, camomilla, tè deteinato, etc. (1 tazza)	1	2	3	4	5	6	7	8	9	
Caffè (1 tazzina)	1	2	3	4	5	6	7	8	9	
Caffè decaffeinato (1 tazzina)	1	2	3	4	5	6	7	8	9	
Latte aggiunto nel caffè o nel tè (1 cucchiaino)	1	2	3	4	5	6	7	8	9	Si
Cappuccino o Caffelatte (1 tazza)	1	2	3	4	5	6	7	8	9	
Cioccolata calda (1 tazza)	1	2	3	4	5	6	7	8	9	
Latte di vacca, intero (1 tazza|1 bicchiere)	1	2	3	4	5	6	7	8	9	Si
Latte di vacca parz. scremato o scremato (1 tazza|1 bicchiere)	1	2	3	4	5	6	7	8	9	Si
Latte di capra (1 tazza|1 bicchiere)	1	2	3	4	5	6	7	8	9	Si
Latte di soia (1 tazza|1 bicchiere)	1	2	3	4	5	6	7	8	9	
Yogurt intero bianco o alla frutta (1 vasetto)	1	2	3	4	5	6	7	8	9	Si
Yogurt parz. scremato o scremato bianco o alla frutta (1 vasetto)	1	2	3	4	5	6	7	8	9	Si
Zucchero, aggiunto a bevande a altri cibi (1 cucchiaino)	1	2	3	4	5	6	7	8	9	
Dolcificanti artificiali (saccarina, aspartame, fruttosio, ecc.) (1 bustina, 1 compressa)	1	2	3	4	5	6	7	8	9	
Cioccolato in polvere (tipo Nesquik) (1 cucchiaio)	1	2	3	4	5	6	7	8	9	

PANE – CEREALI - PRIMI PIATTI	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**Più di 3 volte al giorno**	**Anche prodotto in casa?**
Pane bianco (1 panino, 50g)	1	2	3	4	5	6	7	8	9	Si
Pane, di tipo integrale (1 panino, 50g)	1	2	3	4	5	6	7	8	9	Si
Crackers (1 pacchetto), grissini (n. 5), fette biscottate (n. 3)	1	2	3	4	5	6	7	8	9	
Crackers (1 pacchetto), grissini (n. 5), fette biscottate (n. 3) di tipo integrali	1	2	3	4	5	6	7	8	9	
Cereali per la prima colazione (corn flakes, etc) (1 porz, circa 30 g)	1	2	3	4	5	6	7	8	9	
Pizza (1 al piatto o 1 trancio)	1	2	3	4	5	6	7	8	9	Si
Polenta (1 fetta)	1	2	3	4	5	6	7	8	9	Si
Pasta o riso in bianco, risotto (1 piatto)	1	2	3	4	5	6	7	8	9	
Pasta o riso al pomodoro (1 piatto)	1	2	3	4	5	6	7	8	9	
Pasta o riso con ragù di carne (1 piatto)	1	2	3	4	5	6	7	8	9	
Pasta o riso con verdure (broccoli, zucchini, etc.) (1 piatto)	1	2	3	4	5	6	7	8	9	
Lasagna, cannelloni, tortellini, moussakà etc. di carne (1 piatto)	1	2	3	4	5	6	7	8	9	
Lasagna, cannelloni, tortellini, moussakà etc. di magro (1 piatto)	1	2	3	4	5	6	7	8	9	
Minestrina in brodo – anche con pasta e riso (1 piatto)	1	2	3	4	5	6	7	8	9	
Minestrone di verdura, pasta e fagioli (1 piatto)	1	2	3	4	5	6	7	8	9	
Minestre o altri primi piatti con cereali tipo orzo, faro, miglio (1 piatto)	1	2	3	4	5	6	7	8	9	
Formaggio grattugiato, su primi asciutti e minestre (1 cucchiaino)	1	2	3	4	5	6	7	8	9	

SECONDI PIATTI	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**Più di 3 volte al giorno**	**Anche prodotto in casa?**
Uovo sodo, in camicia, alla coque o crudo (n. 1)	1	2	3	4	5	6	7	8	9	Sì
Uova fritte, frittata, omelette (n. 1)	1	2	3	4	5	6	7	8	9	Sì
Pollo o tacchino, poco condito o lesso (200 g)	1	2	3	4	5	6	7	8	9	Sì
Pollo o tacchino arrosto, fritto o in umido (200 g)	1	2	3	4	5	6	7	8	9	Sì
Coniglio, preparato in qualsiasi modo (200 g)	1	2	3	4	5	6	7	8	9	Sì
Carne magra di manzo, vitello, cavallo *(roast-beef, bistecca, lesso, filetto, carne tritata magra, etc)* (120g)	1	2	3	4	5	6	7	8	9	
Spezzatino (gulasch), brasato, polpette, cotolette, arrosto di manzo, vitello o cavallo (150 g)	1	2	3	4	5	6	7	8	9	
Carne di capra, preparata in qualsiasi modo (150 g)	1	2	3	4	5	6	7	8	9	
Carne di maiale, preparata in qualsiasi modo (150 g)	1	2	3	4	5	6	7	8	9	
Carne di agnello, preparata in qualsiasi modo (150 g)	1	2	3	4	5	6	7	8	9	
Fegato, di qualsiasi animale (150 g)	1	2	3	4	5	6	7	8	9	Sì
Altre frattaglie: cuore, cervello, rognone, trippa, ecc. (150 g)	1	2	3	4	5	6	7	8	9	Sì
Prosciutto crudo, bresaola, speck (4 fette)	1	2	3	4	5	6	7	8	9	
Prosciutto cotto (4 fette)	1	2	3	4	5	6	7	8	9	
Salame, mortadella, pancetta, etc. (50 g)	1	2	3	4	5	6	7	8	9	
Salsicce, würstel, cotechino, (*Cevapcici, musetto, etc)* (3 fette, 120 g)	1	2	3	4	5	6	7	8	9	
Selvaggina *(cervo, fagiano, capriolo, etc)* (150 g)	1	2	3	4	5	6	7	8	9	
Pesce: bollito, alla griglia, al forno (150 g)	1	2	3	4	5	6	7	8	9	
Crostacei (aragosta, astice, gamberi, scampi, etc) bolliti, alla griglia, al forno (150 g)	1	2	3	4	5	6	7	8	9	
Molluschi (polpo, calamari, vongole, cozze, etc) bolliti, alla griglia, al forno (150 g)	1	2	3	4	5	6	7	8	9	
Pesce fritto (150 g)	1	2	3	4	5	6	7	8	9	

SECONDI PIATTI	Mai	Meno di 1 volta al mese	1-3 volte al mese	1 volta a sett	2-4 volte a sett	5-6 volte a sett	1 volta al giorno	2-3 volte al giorno	Più di 3 volte al giorno	Anche prodotto in casa?
Crostacei (aragosta, astice, gamberi, scampi, etc), fritti (150 g)	1	2	3	4	5	6	7	8	9	
Molluschi (polpo, calamari, vongole, cozze, etc), fritti (150 g)	1	2	3	4	5	6	7	8	9	
Tonno, sgombro, sardine sott'olio (1 scatoletta, 80g)	1	2	3	4	5	6	7	8	9	
Formaggi freschi (ricotta, mozzarella, caciotta, etc) come secondo piatto (100 g)	1	2	3	4	5	6	7	8	9	
Feta, come secondo piatto (80 g)	1	2	3	4	5	6	7	8	9	
Altri formaggi (grana, latteria, fontina, emmenthal, groviera, etc) come secondo piatto (80 g)	1	2	3	4	5	6	7	8	9	
Formaggi (tutti i tipi) come snack (25 g)	1	2	3	4	5	6	7	8	9	

VERDURE - CONTORNI	Mai	Meno di 1 volta al mese	1-3 volte al mese	1 volta a sett	2-4 volte a sett	5-6 volte a sett	1 volta al giorno	2-3 volte al giorno	Più di 3 volte al giorno	Anche prodotto in casa?
Spinaci, bieta, cicoria, tarassaco (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Carote (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Carciofi (n. 1), cardi (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Cavolfiore (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Cavoli, verze, broccoli, broccoletti di Bruxelles, etc (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Cipolle, crude o cotte, porri (n. 1) (escludendo quelle usate per i soffritti o in altre preparazioni)	1	2	3	4	5	6	7	8	9	Sì
Finocchi, crudi o cotti (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Funghi (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Insalata verde o rossa (indivia, scarola, radicchio, etc) (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Insalata mista con carote, pomodori, cetrioli, etc. (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Fagiolini verdi (tegoline, piattoni) (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Fagioli, freschi e secchi (1 porzione)	1	2	3	4	5	6	7	8	9	Sì

VERDURE - CONTORNI	Mai	Meno di 1 volta al mese	1-3 volte al mese	1 volta a sett	2-4 volte a sett	5-6 volte a sett	1 volta al giorno	2-3 volte al giorno	Più di 3 volte al giorno	Anche prodotto in casa?
Piselli, freschi e secchi (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Altri legumi (ceci, lenticchie, fave, etc) (freschi e secchi) (1 porzione	1	2	3	4	5	6	7	8	9	Sì
Melanzane (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Patate: lesse o in purea (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Patate: fritte o arrosto (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Peperoni (rossi, gialli e verdi) (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Pomodori (1, medio)	1	2	3	4	5	6	7	8	9	Sì
Soia e prodotti derivati (fagioli, germogli, tofu, etc) (1 porzione)	1	2	3	4	5	6	7	8	9	Sì
Zucchine (1 porzione)	1	2	3	4	5	6	7	8	9	Sì

FRUTTA	Mai	Meno di 1 volta al mese	1-3 volte al mese	1 volta a sett	2-4 volte a sett	5-6 volte a sett	1 volta al giorno	2-3 volte al giorno	Più di 3 volte al giorno	Anche prodotto in casa?
Mele (n. 1)	1	2	3	4	5	6	7	8	9	Sì
Pere (n. 1)	1	2	3	4	5	6	7	8	9	Sì
Arance (n. 1)	1	2	3	4	5	6	7	8	9	
Mandarini, (n. 2), clementine (n. 3), altri agrumi (mapo, etc) (n. 1)	1	2	3	4	5	6	7	8	9	
Pompelmo (mezzo)	1	2	3	4	5	6	7	8	9	
Limone: ad es. sulla verdure, su te o altro (1 spruzzata)	1	2	3	4	5	6	7	8	9	
Spremute di agrumi (1 bicchiere)	1	2	3	4	5	6	7	8	9	
Anguria (1 fetta)	1	2	3	4	5	6	7	8	9	Sì
Banana (n. 1)	1	2	3	4	5	6	7	8	9	
Fragole (1 tazza, 150 gr)	1	2	3	4	5	6	7	8	9	Sì
Ciliegie (n.15, 150 gr)	1	2	3	4	5	6	7	8	9	Sì
Kiwi (n. 1)	1	2	3	4	5	6	7	8	9	Sì
Melone (2 fette)	1	2	3	4	5	6	7	8	9	Sì

FRUTTA	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**Più di 3 volte al giorno**	**Anche prodotto in casa?**
Pesche (n. 1), pesche noci (n. 1), albicocche (n. 2)	1	2	3	4	5	6	7	8	9	Sì
Fichi (n. 2, medi)	1	2	3	4	5	6	7	8	9	Sì
Uva (1 grappolo)	1	2	3	4	5	6	7	8	9	Sì
Ananas (2 fette)	1	2	3	4	5	6	7	8	9	
Altra frutta esotica_____ (1 pezzo, 1 fetta)	1	2	3	4	5	6	7	8	9	
Frutta cotta zuccherata (1 coppetta)	1	2	3	4	5	6	7	8	9	
Frutta secca in guscio: mandorle, noci, nocciole, etc. (1 manciata)	1	2	3	4	5	6	7	8	9	Sì
Frutta essiccata: prugne secche, fichi secchi, albicocche secche, uvetta, etc. (1 manciata)	1	2	3	4	5	6	7	8	9	Sì

DOLCI – ALIMENTI VARI	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**Più di 3 volte al giorno**
Biscotti (tutti i tipi eccetto gli integrali) (n. 7)	1	2	3	4	5	6	7	8	9
Biscotti integrali (n. 7)	1	2	3	4	5	6	7	8	9
Brioche, croissant o bomboloni krapfen senza crema, merendine tipo Pan di Spagna, torta Margherita, dolci lievitati, panettone gubana, etc (n. 1 o 1 fetta)	1	2	3	4	5	6	7	8	9
Pasticcini alla crema (1 grande o 3 mignon) Bomboloni krapfen alla crema (n. 1)	1	2	3	4	5	6	7	8	9
Crostata alla frutta o alla marmellata (1 fetta) Pasticcini alla frutta (1 grande o 3 mignon)	1	2	3	4	5	6	7	8	9
Budino, crème caramel, dolci al cucchiaio (1 coppetta)	1	2	3	4	5	6	7	8	9
Cioccolata (1 quadretto, 10 g), cioccolatino (n. 1)	1	2	3	4	5	6	7	8	9
Snack al cioccolato tipo Mars, Kit-Kat, Bounty, etc. (n. 1)	1	2	3	4	5	6	7	8	9
Caramelle normali (n. 1)	1	2	3	4	5	6	7	8	9
Caramelle light (senza zucchero) (n. 1)	1	2	3	4	5	6	7	8	9
Gomme da masticare -normali (n. 1)	1	2	3	4	5	6	7	8	9

DOLCI – ALIMENTI VARI	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**Più di 3 volte al giorno**
Gomme da masticare -light (senza zucchero) (n. 1)	1	2	3	4	5	6	7	8	9
Gelato (n. 1)	1	2	3	4	5	6	7	8	9
Marmellata o miele (1 cucchiaino)	1	2	3	4	5	6	7	8	9
Burro sul pane (5 g)	1	2	3	4	5	6	7	8	9
Olive verdi o nere (al naturale o in salamoia) (n. 3)	1	2	3	4	5	6	7	8	9
Maionese, salsa rosa, salsa tonnata, etc. (1 cucchiaio)	1	2	3	4	5	6	7	8	9
Sottaceti (cetrioli, cipolline, peperoni, giardiniera, etc) (50 g)	1	2	3	4	5	6	7	8	9
Patatine fritte in sacchetto, salatini, etc. (1 pacchetto, 25 g)	1	2	3	4	5	6	7	8	9
Pop corn (1 tazza, 8 g)	1	2	3	4	5	6	7	8	9

BEVANDE ANALCOLICHE	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**4-5 volte al giorno**	**Più di 5 volte al giorno**
Coca-Cola, Pepsi-Cola, Chinotto (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Altre bevande gassate (aranciata, acqua brillante, Sprite etc), compresi gli aperitivi analcolici (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Bevande gassate "light" (senza zucchero) (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Succhi di frutta commerciali (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Acqua minerale (con o senza anidride carbonica) (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Acqua potabile – di acquedotto (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Acqua potabile – di pozzo (1 bicchiere)	1	2	3	4	5	6	7	8	9	10

BEVANDE ALCOLICHE	**Mai**	**Meno di 1 volta al mese**	**1-3 volte al mese**	**1 volta a sett**	**2-4 volte a sett**	**5-6 volte a sett**	**1 volta al giorno**	**2-3 volte al giorno**	**4-5 volte al giorno**	**Più di 5 volte al giorno**
Vino bianco, spumante, champagne (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Vino rosso o rosato (1 bicchiere)	1	2	3	4	5	6	7	8	9	10
Birra (1 lattina)	1	2	3	4	5	6	7	8	9	10
Aperitivi alcolici: es. vermouth, Campari, marsala, etc. (1 bicchierino)	1	2	3	4	5	6	7	8	9	10
Amari, digestivi, liquori dolci (1 bicchierino)	1	2	3	4	5	6	7	8	9	10
Whisky, cognac, brandy, grappa, vodka, acquavite, tequila etc. (1 bicchierino)	1	2	3	4	5	6	7	8	9	10

Annex 2. Prototype of 7-day dietary record (food diary) (Italian version)

Codice fiscale della mamma |_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|
Codice ricerca /_/_/_/_/_/_/
Data di nascita del bambino: /_/_/_/_/_/_/ D18 (mesi)

Per cortesia:

- Scriva su questo diario tutto quello che il suo bambino consuma: latte materno, latte artificiale, cibi, bevande, spuntini (ad es. spicchio di mela, caramelle, bevande fuori pasto, dolci) seguendo le istruzioni riportate nelle pagine seguenti
- Il diario, cioè le schede vuote che troverà alla fine del documento, dev'essere compilato per **7 giorni della medesima settimana** L'intero documento sarà ritirato al prossimo incontro
- **Per ogni problema o dubbio contatti XXXX al numero XXXX (Istituto per l'Infanzia Burlo Garofolo, Unità per la Ricerca sui Servizi Sanitari e la Salute Internazionale, Via dell'Istria 118, 34137 Trieste; Istituto di Igiene ed Epidemiologia Clinica, Università degli Studi di Udine, Via Colugna 50, 34070 Udine)**

ISTRUZIONI PER LA COMPILAZIONE

- Scrivere sempre in stampatello
- Compilare con precisione, all'inizio della settimana di diario, la scheda con le indicazioni per le unità di misura casalinghe (vedere pag. 2)
- Ogni giorno iniziare una nuova pagina di diario
- Ora: indicare l'ora di consumo e riferire il giorno alle 24 ore (dalle ore 0 alle ore 24)
- Luogo: indicare il luogo di consumo (casa propria, ristorante, da conoscenti)
- Descrizione del cibo e delle bevande e modo di preparazione: indicare tutte le informazioni il più precisamente possibile, specificando la marca e la quantità consumata nel caso si tratti di un prodotto confezionato (baby foods, latte artificiale, biscotti). Chiedere aiuto a chi ha confezionato il piatto nel caso di dubbio (ad es. all'asilo nido)
- Porzione: indicare il più precisamente possibile le diverse pezzature e unità di misura nonché il peso preciso. Inoltre:
- per le ricette a casa indicare:
  - il peso degli ingredienti a crudo, per quante persone è stata preparata la ricetta, il peso della porzione a cotto del bambino, facendo attenzione a togliere il peso del piatto (tara)
- per i pasti fuori casa:
  - riferire se si tratta delle stesse unità di misura utilizzate a casa (ad es. biberon, omogeneizzato in busta o in vasetto) oppure, se non note, le dimensioni e la pezzatura dei cibi, anche approssimativa

ALCUNE NOTE

LATTE MATERNO: specificare se la poppata è normale oppure più breve o lunga del solito

LATTE ARTIFICIALE: specificare il tipo di latte e la marca (in polvere o liquido, il numero per i latti di transizione, se di soia, ipoallergenico o altro) e specificare quanto il bambino ha realmente consumato (differenza tra la quantità preparata e quella rimasta nel biberon/tazza/bicchiere)

BABY FOODS: specificare la marca e la confezione

LATTE: specificare il tipo (intero, parzialmente scremato o scremato, fresco, UHT; delattosato) e la provenienza (di mucca, di asina, di capra, di soia, di riso)

GRASSO VISIBILE di CARNE ed AFFETTATI, compreso la pelle di pollo, tacchino o del pesce: specificare se il bimbo le consuma oppure no

CARNE e PESCE: specificare la parte mangiata (coscia, petto, costoletta, filetto, pesce intero)

SUGO e CONDIMENTI: specificare se il bimbo l'ha lasciato oppure no in fondo al piatto

VERDURA e FRUTTA FRESCA: pesare il frutto e la verdura prima di darlo al bimbo e riportare la quantità mangiata

ZUCCHERO ed OLIO: indicare il numero di cucchiaini da caffè o cucchiai da minestra

TÈ, INFUSI e BEVANDE: indicare il numero di tazze, bicchieri, porzioni commerciali intere o loro frazioni (1 lattina da xx ml intera o metà, 1 bottiglia da xx litri o ml o metà)

UNITÀ DI MISURA CASALINGHE
Prima di iniziare a compilare il diario, esegua per favore a casa sua le seguenti misurazioni: tutti i recipienti da misurare devono contenere acqua fino all'orlo. Per i 7 giorni di diario usi questi utensili come riferimento nel caso di consumi/preparazioni casalinghe.

	Grammi
20 cucchiaini da caffè	
20 cucchiai da minestra	
1 tazzina di caffè	
1 tazza da caffelatte	
1 bicchiere da liquore	
1 bicchiere da vino	
1 bicchiere d'acqua	
1 mestolo	

ESEMPIO 1. Attenzione: questo diario non rappresenta un modello nutrizionale, è solo un esempio.

Data: 19/06/2007 Giorno: MARTEDÍ

Ora	Luogo	Descrizione del cibo e delle bevande e modo di preparazione	Porzione (più precisa possibile)
6.00	A casa	Latte materno	Poppata breve
9.30	A casa	Latte materno	Poppata normale
12.45	A casa	Latte artificiale ″XYZ″ in polvere Orzo	125 ml 1 cucchiaio
13.30	Dalla nonna	Passato di verdura: olio di oliva extra vergine 1 carota 1 zucchina	 1 cucchiaio 50 gr a crudo 60 gr a crudo
16.20	A casa	Latte artificiale ″XYZ″ in polvere	125 ml
		Mela 1 spicchio	10 gr
		Prosciutto cotto	15 gr
20.00	A casa	Latte artificiale ″XYZ″ in polvere Orzo	125 ml 1 cucchiaio
		Omogeneizzato ″XYZ″ di vitello	3 cucchiai
21.40	A casa	Latte materno	Poppata breve
23.30	A casa	Latte materno	Poppata lunga

ESEMPIO 2. Attenzione: questo diario non rappresenta un modello nutrizionale, è solo un esempio.

Data: 21/06/2007 Giorno: GIOVEDÍ

Ora	Luogo	Descrizione del cibo e delle bevande e modo di preparazione	Porzione (più precisa possibile)
8.15	A casa	Latte di mucca Zucchero Orzo Amaretti	125 ml ½ cucchiaino 1 cucchiaio 2
10.25	Al parco	½ panino (pane) Clementina	30 grammi 1 (50 gr)
12.30	A casa	¾ filetto di pesce 1/4 fetta polenta	75gr 40 gr
17.20	Da amici	Latte vaccino Zucchero Orzo	125 ml ½ cucchiaino 1 cucchiaio
19.00	A casa	Pasta al sugo preparata per 4 persone *-Pasta a crudo 400g* *-Cipolla 70 g* *-Olio extra vergine d'oliva 2 cucchiai* *-Salsa "XY" 350 grammi* - porzione per il bambino Formaggio grana Formaggio montasio Clementina	 50g a cotto 2 cucchiaini 20 gr 1 (50gr)

1° GIORNO DEL DIARIO DELLA SETTIMANA: Data /_/_/_/_/_/_/_/ Giorno della settimana: ___________

Ora	Luogo	Descrizione del cibo e delle bevande e modo di preparazione	Porzione (più precisa possibile)

Durante questa giornata di diario suo/a figlio/a è: Sano/a ☐ Malato/a ☐
Se malato/a, di cosa soffre? ____
Ha preso dei farmaci? Sì ☐ No ☐
Se si, quali (nome commerciale dei farmaci e dosaggio

Annex 3. Daily macro and micronutrients intake of children divided by sex (N=389)

	FEMALES (n=204)		**MALES (n=184)**	
	Mean±STD	Median (IQR)	Mean±STD	Median (IQR)
Energy (kJ)	3743.9±812.0	3591.0(1137.0)	3947.6±837.2	3880.6(1193.1)
Energy (kcal)	892.5±190.1	855.9(274.8)	942.0±199.9	927.1(283.4)
Total proteins (g)	38.1±10.0	37.0(12.6)	37.5±10.1	36.9(12.9)
Total lipids (g)	33.1±8.4	32.0(11.4)	34.8±10.4	33.2(14.3)
Saturated fatty acids (g)	13.8±4.0	13.4(5.9)	14.4±5.2	13.6(6.9)
Monounsaturated fatty acids (g)	11.3±3.6	11.1(5.0)	11.9±4.5	11.3(5.3)
Oleic acid (g)	10.0±3.4	9.6(4.8)	10.5±4.3	10.1(4.8)
Polyunsaturated fatty acids (g)	3.1±1.2	3.0(1.3)	3.3±1.4	3.0(1.7)
Linoleic acid (g)	2.3±1.0	2.1(1.0)	2.5±1.2	2.2(1.4)
Alfa-Linolenic acid (g)	0.4±0.1	0.4(0.2)	0.5±0.2	0.4(0.2)
Available carbohydrates (g)	117.9±30.8	114.3(37.6)	127.8±28.4	124.6(36.6)
Soluble carbohydrate (g)	51.5±17.3	48.4(19.1)	56.3±16.0	55.2(19.7)
Starch (g)	53.0±22.6	50.6(32.5)	59.4±22.5	57.8(28.8)
Fibre (g)	6.9±2.9	6.5(3.9)	7.8±2.9	7.4(3.8)
Cholesterol (mg)	118.5±41.1	119.0(53.3)	121.0±44.2	117.7(49.4)
Sodium (mg)	858.2±290.3	819.1(397.7)	904.1±331.4	867.1(433.3)
Potassium (mg)	1393.2±396.4	1379.6(479.9)	1444.8±429.2	1392.5(587.4)
Calcium (mg)	649.4±220.5	644.1(290.4)	636.3±250.0	592.3(3)
Iron (mg)	4.5±1.6	4.3(1.9)	4.6±1.5	4.6(1.7)
Zinc (mg)	4.4±1.3	4.2(1.6)	4.4±1.3	4.4(1.9)
Vitamin B1 (mg)	0.6±0.2	0.6(0.2)	0.6±0.2	0.6(0.2)
Vitamin B2 (mg)	1.1±0.3	1.0(0.4)	1.0±0.3	1.0(0.5)
Vitamin B6 (mg)	1.0±0.3	1.0(0.3)	1.0±0.3	1.0(0.4)
Vitamin B12 (µg)	2.4±1.1	2.3(1.3)	2.2±1.1	2.0(1.5)
Vitamin C (mg)	55.0±37.9	43.6(38.9)	56.1±31.2	49.0(35.3)
Vitamin D (µg)	1.1±1.5	0.6(0.5)	1.1±2.6	0.6(0.6)
Vitamin E α-TE (mg)	3.3±1.4	3.1(1.8)	3.7±1.9	3.3(2.0)
Retinol (µg)	193.1±78.5	191.7(103.6)	187.0±93.9	175.9(119.5)
Retinol Eq. (µg)	509.2±227.9	469.6(287.6)	515.2±231.0	490.6(301.7)
Niacin (mg)	6.7±2.6	6.4(2.7)	6.4±2.2	6.1(2.7)
Folate (µg)	118.7±43.8	114.1(52.4)	125.0±44.3	119.0(53.6)

Abbreviation: STD, standard Deviation; IQR, InterQuartile Range

Annex 4. Spearman Correlation between mother's age, pre-pregnancy BMI and FAs profile and indexes from dietary intake

	Age	BMI	SFA (D)	MUFA (D)	c18:1 (D)	c18:2 (D)	c18:3 (D)	PUFA (D)	C20:5 (D)	C22:6 (D)	C20:4 (D)	Saturation Index (D)	Inflammatory risk index (D)
Age	1.00000	0.16288 0.2137	0.06582 0.6173	0.07110 0.5893	0.06655 0.6134	0.07230 0.5830	0.08129 0.5369	0.07434 0.5724	0.18775 0.1509	0.19683 0.1317	0.02670 0.8395	0.02265 0.8636	-0.12469 0.3425
BMI	0.16288 0.2137	1.00000	0.09385 0.4719	0.05302 0.6849	0.05601 0.6681	0.04747 0.7164	0.03509 0.7883	0.03935 0.7634	0.02089 0.8730	-0.01624 0.9012	-0.03337 0.7985	0.08433 0.5182	-0.03927 0.7638
SFA (D)	0.06582 0.6173	0.09385 0.4719	1.00000	0.88667 <.0001	0.86785 <.0001	0.74490 <.0001	0.80799 <.0001	0.76277 <.0001	0.25717 0.0454	0.16282 0.2099	0.67488 <.0001	0.31935 0.0121	0.11914 0.3604
MUFA (D)	0.07110 0.5893	0.05302 0.6849	0.88667 <.0001	1.00000	0.99810 <.0001	0.95537 <.0001	0.97144 <.0001	0.96240 <.0001	0.32792 0.0099	0.25981 0.0432	0.59651 <.0001	-0.08318 0.5239	0.27129 0.0344
c18:1 (D)	0.06655 0.6134	0.05601 0.6681	0.86785 <.0001	0.99810 <.0001	1.00000	0.96467 <.0001	0.97610 <.0001	0.97028 <.0001	0.32417 0.0108	0.26192 0.0414	0.56748 <.0001	-0.11777 0.3660	0.27948 0.0292
c18:2 (D)	0.07230 0.5830	0.04747 0.7164	0.74490 <.0001	0.95537 <.0001	0.96467 <.0001	1.00000	0.97641 <.0001	0.99625 <.0001	0.32850 0.0097	0.29989 0.0189	0.48154 <.0001	-0.31364 0.0138	0.33792 0.0077
c18:3 (D)	0.08129 0.5369	0.03509 0.7883	0.80799 <.0001	0.97144 <.0001	0.97610 <.0001	0.97641 <.0001	1.00000	0.98038 <.0001	0.31174 0.0145	0.26314 0.0405	0.48091 <.0001	-0.19016 0.1421	0.23469 0.0687
PUFA (D)	0.07434 0.5724	0.03935 0.7634	0.76277 <.0001	0.96240 <.0001	0.97028 <.0001	0.99625 <.0001	0.98038 <.0001	1.00000	0.37414 0.0030	0.34283 0.0068	0.51523 <.0001	-0.28858 0.0241	0.29482 0.0211
c20:5 (D)	0.18775 0.1509	0.02089 0.8730	0.25717 0.0454	0.32792 0.0099	0.32417 0.0108	0.32850 0.0097	0.31174 0.0145	0.37414 0.0030	1.00000	0.90888 <.0001	0.51327 <.0001	-0.14622 0.2608	-0.47705 0.0001
c22:6 (D)	0.19683 0.1317	-0.01624 0.9012	0.16282 0.2099	0.25981 0.0432	0.26192 0.0414	0.29989 0.0189	0.26314 0.0405	0.34283 0.0068	0.90888 <.0001	1.00000	0.40090 0.0014	-0.26240 0.0411	-0.43316 0.0005
c20:4 (D)	0.02670 0.8395	-0.03337 0.7985	0.67488 <.0001	0.59651 <.0001	0.56748 <.0001	0.48154 <.0001	0.48091 <.0001	0.51523 <.0001	0.51327 <.0001	0.40090 0.0014	1.00000	0.23485 0.0685	0.05907 0.6511
Saturation Index (D)	0.02265 0.8636	0.08433 0.5182	0.31935 0.0121	-0.08318 0.5239	-0.11777 0.3660	-0.31364 0.0138	-0.19016 0.1421	-0.28858 0.0241	-0.14622 0.2608	-0.26240 0.0411	0.23485 0.0685	1.00000	-0.33342 0.0086
Inflammatory risk index (D)	-0.12469 0.3425	-0.03927 0.7638	0.11914 0.3604	0.27129 0.0344	0.27948 0.0292	0.33792 0.0077	0.23469 0.0687	0.29482 0.0211	-0.47705 0.0001	-0.43316 0.0005	0.05907 0.6511	-0.33342 0.0086	1.00000

Abbreviation: BMI, Body mass index; SFA, saturated fatty acid; D, dietary; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Annex 5. Spearman Correlation between mother's age, pre-pregnancy BMI and FAs profile and indexes from lipidomic analysis of human milk

	SFA (HM)	MUFA (HM)	C 18:1_9 cis (HM)	C 18:2 (HM)	C 18:3 w3 (HM)	PUFA (HM)	C 20:5 (HM)	C 22:5 (HM)	C 20:4 (HM)	Saturation index (HM)	Inflammatory risk index (HM)
Age	0.10095 0.4428	0.03538 0.7884	0.07976 0.5446	-0.05471 0.6780	0.07954 0.5458	-0.10207 0.4377	0.22569 0.0829	0.24521 0.0590	-0.17225 0.1882	0.04703 0.7212	-0.17757 0.1747
BMI	0.18690 0.1492	-0.09337 0.4742	-0.11996 0.3571	-0.12005 0.3567	-0.00712 0.9566	-0.07006 0.5916	0.16444 0.2054	0.02572 0.8440	0.10481 0.4215	0.17315 0.1820	-0.09448 0.4689
SFA (HM)	1.00000	-0.60714 <.0001	-0.60846 <.0001	-0.56762 <.0001	-0.37186 0.0032	-0.55347 <.0001	-0.02143 0.8698	0.03530 0.7871	-0.13401 0.3032	0.91523 <.0001	-0.00703 0.9571
MUFA (HM)	-0.60714 <.0001	1.00000	0.96559 <.0001	-0.16835 0.1946	0.13056 0.3159	-0.21148 0.1018	-0.11843 0.3633	-0.16667 0.1992	-0.12552 0.3351	-0.85896 <.0001	0.15328 0.2382
C18:1_9cis (HM)	-0.60846 <.0001	0.96559 <.0001	1.00000	-0.12431 0.3398	0.09179 0.4817	-0.19520 0.1317	-0.11842 0.3634	-0.16791 0.1958	-0.22854 0.0765	-0.85001 <.0001	0.17259 0.1835
C18:2 (HM)	-0.56762 <.0001	-0.16835 0.1946	-0.12431 0.3398	1.00000	0.29281 0.0220	0.96216 <.0001	0.01185 0.9278	0.02111 0.8717	0.08206 0.5296	-0.26925 0.0359	-0.20532 0.1124
C18:3 ω3 (HM)	-0.37186 0.0032	0.13056 0.3159	0.09179 0.4817	0.29281 0.0220	1.00000	0.36862 0.0035	0.00129 0.9921	0.05171 0.6922	0.19044 0.1415	-0.29873 0.0194	-0.35416 0.0051
PUFA (HM)	-0.55347 <.0001	-0.21148 0.1018	-0.19520 0.1317	0.96216 <.0001	0.36862 0.0035	1.00000	0.07801 0.5501	0.11076 0.3954	0.26835 0.0365	-0.24104 0.0613	-0.20767 0.1083
C20:5 (HM)	-0.02143 0.8698	-0.11843 0.3633	-0.11842 0.3634	0.01185 0.9278	0.00129 0.9921	0.07801 0.5501	1.00000	0.41190 0.0010	0.29723 0.0200	0.01313 0.9200	0.03906 0.7650
C 22:5 (HM)	0.03530 0.7871	-0.16667 0.1992	-0.16791 0.1958	0.02111 0.8717	0.05171 0.6922	0.11076 0.3954	0.41190 0.0010	1.00000	0.34364 0.0067	0.10388 0.4256	0.06945 0.5948
C:20:4 (HM)	-0.13401 0.3032	-0.12552 0.3351	-0.22854 0.0765	0.08206 0.5296	0.19044 0.1415	0.26835 0.0365	0.29723 0.0200	0.34364 0.0067	1.00000	-0.02642 0.8399	-0.02736 0.8342
Saturation Index (HM)	0.91523 <.0001	-0.85896 <.0001	-0.85001 <.0001	-0.26925 0.0359	-0.29873 0.0194	-0.24104 0.0613	0.01313 0.9200	0.10388 0.4256	-0.02642 0.8399	1.00000	-0.10148 0.4364
Inflammatory risk index (HM)	-0.00703 0.9571	0.15328 0.2382	0.17259 0.1835	-0.20532 0.1124	-0.35416 0.0051	-0.20767 0.1083	0.03906 0.7650	0.06945 0.5948	-0.02736 0.8342	-0.10148 0.4364	1.00000

Abbreviation: HM, human milk; BMI, body mass index; SFA, saturated fatty acid; D, dietary; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

10. PUBLICATIONS

1. **Concina F,** Pani P, Bravo G, Barbone F, Carletti C, Knowles A, Ronfani L, Parpinel M. Nutrient intakes in an Italian population of infants during the complementary feeding period. *Public Health Nutrition*, 2018, 21(16): 3018-3026 doi: 10.1017/S136898001800201X

2. Carletti C, **Concina F***, Pani P, Monasta L, Knowles A, Parpinel M, Barbone F, Ronfani L. Age-Related Trends in the Diet of An Infant's Cohort in the Northeast of Italy from Six to Twelve Months of Age. *Nutrients*, 2019, 11(2): 230-241 doi: 10.3390/nu1102023

3. F. Fiori, **F. Concina**, P. Gnagnarella, G. Carioni, M. Parpinel. Update of "babyfoods" and "snacks" categories from the Food Composition Database used for the analysis of infants' diet at 18 months of age in PHIME study. *Nutrition, Metabolism & Cardiovascular Diseases*, 2020, 30(3):539 doi: https://doi.org/10.1016/j.numecd.2019.12.032

CO-SUPERVISOR OF STUDENT THESIS RELATED TO MY PhD PROJECT

1. Gabriele Arduini. ASSUNZIONE DI ENERGIA E NUTRIENTI IN UNA COORTE DI BAMBINI A 18 MESI NEL NORD-EST D'ITALIA: ANALISI PRELIMINARE. Laurea Magistrale in Scienze e Tecnologie Alimentari, Università degli Studi di Udine. Relatrice: Prof.ssa Maria Parpinel

2. Stefania Gagno. LE ABITUDINI ALIMENTARI NEL SECONDO ANNO DI VITA: RISULTATI DI UNO STUDIO DI COORTE NEL NORD-EST D'ITALIA. Laurea Magistrale in Scienze e Tecnologie Alimentari, Università degli Studi di Udine. Relatrice: Prof.ssa Maria Parpinel

3. Dora Del Savio. L' ALIMENTAZIONE NEI PRIMI DUE ANNI DI VITA: RISULTATI DI UNO STUDIO OSSERVAZIONALE IN FRIULI VENEZIA GIULIA. Laurea a Ciclo Unico in Medicina e Chirurgia. Università degli Studi di Udine. Relatrice: Prof.ssa Maria Parpinel

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Nutrient intakes in an Italian population of infants during the complementary feeding period

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Abstract

Objective: To describe the nutrient intakes of an Italian cohort of infants at 6, 9 and 12 months of age.

Design: Dietary data were collected using a food diary at three follow-ups (6, 9 and 12 months of age of infants). The infants' dietary data were used to estimate nutrient intakes using the Italian food composition database integrated with data from nutritional labels and the literature. The mean and standard deviation, median and interquartile range, minimum and maximum, and 5th, 25th, 75th and 95th percentiles were calculated for the daily intake of twenty-eight nutrients, with sex differences evaluated using parametric/non-parametric statistical methods.

Setting: A prospective population-based birth cohort.

Subject: Infants (*n* 400) living in the urban area of Trieste (Italy).

Results: The sex distribution was fairly balanced at each follow-up. The mean daily intakes of energy and the other twenty-seven nutrients considered were greater in males at all follow-ups. In particular, a significant statistical difference was observed in higher male consumption of cholesterol at 9 months and in energy and carbohydrate intakes at 12 months ($P < 0{\cdot}05$). The mean daily intake of proteins was greater than that recommended by the Italian Dietary Reference Values at all follow-ups.

Conclusions: These preliminary results provide a useful basis for understanding the nutrient intake patterns of infants in this area of Italy during the first year of life.

Keywords
Prospective cohort study
Infants
Complementary feeding
Energy and nutrient intakes
Italian Dietary Reference Values

Understanding the dietary habits and the nutritional intake of infants during the first 2 years of life is important because this period, which covers breast-feeding and complementary feeding, is characterized by high nutrient requirements and critical dietary changes that have an impact on the development of food preferences and on short- and long-term health[1,2].

Inadequate complementary feeding, as well as maternal malnutrition and inappropriate breast-feeding, can have direct and indirect negative consequences on child health, such as inadequate growth velocity, infections, obesity, CVD, autoimmune diseases (coeliac disease and type 1 diabetes) and atopic disorders[3].

In 2003, the WHO and UNICEF published a 'Global Strategy for Infant and Young Child Feeding' emphasizing that inadequate feeding practices are a major risk factor for morbidity and mortality in the first part of infancy[4]. Subsequently, in 2017, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition published a position paper on complementary feeding (latest edition), reviewing differences in knowledge and practices among countries and summarizing the limited available scientific evidence on the short- and long-term health effects of timing and composition of complementary foods[5].

Unfortunately, there are very few studies describing nutritional practices at different ages during the first 2 years of life and determining nutrient intakes in detail. Among international studies, the most relevant are four large cohort studies: the Norwegian Mother and Child Cohort Study (MoBa)[6], the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD)[7], the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC)[8] and the 'Public health impact of long-term, low-level mixed element exposure in susceptible population strata' study (PHIME)[9]. Although these studies all involve children and parents, their aims are substantially different. The MoBa study examines the causes of serious diseases by estimation of specific exposure–outcome associations among children

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and parents[6]. The DONALD study focuses on the relationship between diet, nutrition and development of children and adolescents during their growth period[7]. The ALSPAC study is designed to determine how the genotype combines with environmental pressures to influence health and development of children[8]. Finally, the PHIME study aims at improving the integrated health-risk assessment of long-term, low-level environmental exposure to toxic and essential metals via food[9]. In Italy, PHIME was designed to investigate the effects of prenatal low-level Hg exposure from fish and seafood consumption on the neuro- and physiological development of infants in the first 18 months of life[9]. During the same period, a Trieste Infants Food cohort (TIF cohort), partially overlapping with the Italian component of the PHIME study, was set up to study nutrition in the first 3 years of life in the north-east of Italy[10].

The main objective of the present paper is to provide a descriptive analysis of the intakes of nutrients by infants aged 6, 9 and 12 months, as part of the TIF cohort, according to sex[10]. The secondary objective is to compare the results with national and international recommendations[11,12].

To our knowledge, the present study is the first to provide a detailed description of the dietary intake of an Italian infant population at different ages during the second semester of life, when complementary feeding is established.

Methods

A total of 400 infants born at the Institute of Maternal and Child Health of Trieste, Italy, between 2006 and 2007 were enrolled at birth. The Ethics Committee of the Institute of Maternal and Child Health – IRCCS 'Burlo Garofolo' of Trieste approved the protocol of the study and all participating subjects were informed and consented to participate. The study design, protocol and sampling procedures are described elsewhere[10].

Dietary data were collected using a 3 d dietary record (food diary) at 6, 9 and 12 months of age. The food diary was given to mothers on the occasion of the first contact, together with instructions on how to record type, quantity and method of feeding over a 24 h period on three separate non-consecutive days, including one at the weekend. The instructions included a table with a list of household implements (e.g. teaspoons) that could be used at home to weigh foods and fluids, with an estimate of the equivalent in grams. A telephone contact number was provided to mothers in case they had questions regarding the completion of the food diary.

Data extracted from the food diaries were analysed using the Microdiet software version 2.8.6 (Downlee Systems Ltd, High Peak, UK) containing the Italian food composition database for epidemiological studies[13], integrated with information from nutritional labels (e.g. baby food) and, in the case of human milk, from the literature[14,15]. For human milk, the duration of each feed in combination with the frequency of breast-feeding was used to estimate the volume of milk, as suggested by Cameron and Hofvander[16]. The complete methodology for coding and conversion of food intakes into nutrient intakes is described in Concina *et al.*[17]. Additional demographic, education, social and anthropometric data on the mother, the father and the infant were obtained from a questionnaire[9]. All procedures were conducted by trained food technologists and nutritionists, who were fully familiar with brand names, composition of commercial products, food preparation methods and in the management of food composition data.

Twenty-eight food components, defined by the Italian food composition database research group[13], were considered for nutritional analysis: total proteins, carbohydrates (available, soluble, starch, fibre), lipids (total, saturated, monounsaturated and polyunsaturated fatty acids; oleic, linoleic and linolenic acids; cholesterol), minerals (Na, K, Ca, Fe, Zn) and vitamins (thiamin, riboflavin, vitamin B_6, vitamin C, vitamin D, vitamin E expressed as α-tocopherol equivalents, retinol, retinol equivalents, niacin, folic acid).

Statistical analysis

Categorical data are presented as number and percentage; continuous data as mean and standard deviation. For each infant, the mean daily intakes of energy, macronutrients and micronutrients were calculated on a 3 d observation basis, excluding the use of supplements.

The mean and standard deviation, median and interquartile range (IQR), minimum and maximum, and 5th, 25th, 75th and 95th percentiles were calculated for daily intake of each nutrient in all infants and by sex. The percentage contribution of each macronutrient to energy intake was estimated for all infants. The nutrient intakes were then compared with the Italian Dietary Reference Values (DRV)[11] and the WHO feeding and nutrition recommendations for infants and young children[12], when available. More precisely, the DRV were expressed using different indices such as the Suggested Dietary Target, Adequate Intake, Reference Intake range for macronutrients, Average Requirement and Population Reference Intake, while WHO recommendations were expressed using the Recommended Daily Intake.

To detect significant sex differences for each nutrient and in terms of energy intake, a comparative analysis was conducted at each follow-up using *t* tests or the corresponding non-parametric test (Wilcoxon, Mann–Whitney) if a non-normal distribution of data was seen by the Kolmogorov–Smirnof test. Statistical significance for all tests was set at a P value of 0·05.

Analyses were conducted using the statistical software package SAS version 9.4 for Windows.

Results

The characteristics of mothers and infants at enrolment and delivery, respectively, are reported in detail in Table 1. The differences in sample size reported in Table 1 for some variables are due to missing data in the questionnaires.

The mean maternal age was 33·0 (SD 4·7) years. Most women were Italian (87·8 %), married (80·0 %), in employment (77·0 %) and had a medium–high level of education (72·8 %).

Infants were equally distributed by sex: 173 males and 172 females; for fifty-five infants, gender information was not reported. The mean birth weight and length of infants at delivery was 3394·6 (SD 456·0) g and 50·4 (SD 2·1) cm, respectively. The number food diaries available for analysis were 268 at 6 months, 179 at 9 months and 176 at 12 months of age of the infants (Fig. 1).

A dropout analysis of data at 6, 9 and 12 months showed that the sample was statistically representative in terms of maternal age at enrolment ($P = 0{\cdot}03$ at 6 months and $P = 0{\cdot}02$ at 12 months), maternal age at delivery ($P = 0{\cdot}03$ at 6 and 9 months and $P = 0{\cdot}01$ at 12 months), education ($P = 0{\cdot}00$ at 6 months, $P = 0{\cdot}04$ at 9 months and $P = 0{\cdot}02$ at 12 months) and occupation ($P = 0{\cdot}03$ at 6 months; data not shown).

The rates of breast-feeding, alone or in combination with infant formula or cow's milk, or both, were 70·0 % at 6 months, 54·8 % at 9 months and 39·7 % at 12 months. Moreover, at 6 months only 6·0 % of infants were breast-fed exclusively while at the other follow-ups, none were breast-fed or formula-fed exclusively (data not shown).

Table 1 Characteristics of mothers and infants at enrolment and delivery in the Trieste Infants Food cohort (*n* 400)

Characteristic	*n*	%
Nationality of mother		
Italian	351	87·8
Foreign	49	12·3
Not reported	0	0·0
Marital status		
Married/living with partner	318	80·0
Separated/divorced	13	3·3
Single/not living with partner	18	5·0
Not reported	51	12·3
Maternal education level		
None	1	0·3
Completed primary school	6	2·0
Completed secondary school	52	13·0
Completed high school or equivalent	154	39·0
Bachelor degree or higher	135	33·8
Not reported	52	13·0
Maternal occupational status		
Yes	306	77·0
No	17	4·3
Not reported	77	19·3
Sex of child		
Male	173	43·3
Female	172	42·0
Not reported	55	13·8
	Mean	SD
Maternal age (years; *n* 348)	33·0	4·7
Birth weight of child (g; *n* 344)	3394·6	456·0
Birth length of child (cm; *n* 342)	50·4	2·1

Daily energy, macronutrient and micronutrient intakes for all infants, divided by sex, are shown in Tables 2, 3 and 4 (for infants at 6, 9 and 12 months of age, respectively).

The distribution of nutrient and energy intakes by percentile (5th, 25th, 75th and 95th), and minimum and maximum values, for all infants are reported in the online supplementary material (Tables S1, S2 and S3 for infants at 6, 9 and 12 months of age, respectively).

A wide range of variability in the intake values of some vitamins and minerals was observed in all infants at each follow-up. In particular, the mean daily intakes of Na and Ca were 373·1 (SD 321·5) mg and 383·5 (SD 184·0) mg at 6 months; 671·2 (SD 492·1) mg and 536·1 (SD 221·6) mg at 9 months; and 721·6 (SD 397·7) mg and 595·3 (SD 251·5) mg at 12 months, respectively.

The mean energy daily intake was greater in males than in females at all follow-ups, with a statistically significant difference at 12 months (3464·4 (SD 898·5) kJ *v.* 3242·8 (SD 907·6) kJ; $P = 0{\cdot}04$). Generally, the intakes of all twenty-eight nutrients considered were greater in males (Tables 2, 3 and 4), with the exception of Ca, that was always greater in females, without statistically significant differences, and thiamin, whose intake was very similar between sexes. A statistically significant difference between females and males was also observed in intakes of cholesterol ($P = 0{\cdot}04$) at 9 months and available carbohydrates ($P = 0{\cdot}001$), soluble carbohydrates ($P = 0{\cdot}01$) and starch ($P = 0{\cdot}03$) at 12 months. No statistically significant difference was observed at 6 months.

The percentage contributions of the main macronutrients to total energy intake are reported in Table 5. In particular, available carbohydrates contributed ~50 % of the total energy intake at each follow-up, while the contribution from total lipids ranged from ~40 % at 6 months to ~34 % at 12 months. The contribution of SFA to total energy intake ranged from 15·5 % at 6 months to 13·7 % at 12 months; the contribution of MUFA varied from 15·4 % at

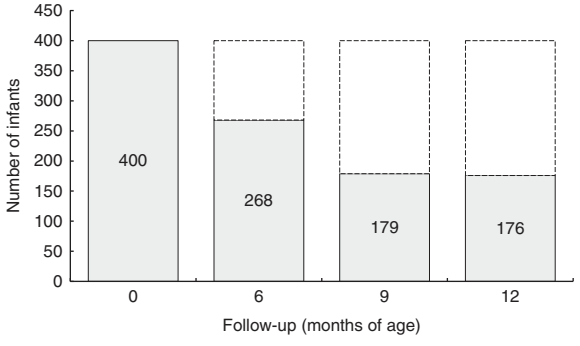

Fig. 1 Distribution of infants at each follow-up: the Trieste Infants Food cohort

Table 2 Daily energy, macronutrient and micronutrient intakes of Italian infants at 6 months of age in the Trieste Infants Food cohort

	All infants (*n* 268)				Females (*n* 132)				Males (*n* 136)			
Nutrient	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR
Energy (kJ)	2437·7	803·3	2312·7	887·3	2360·0	706·8	2250·7	773·9	2513·1	883·1	2344·2	1180·5
Total proteins (g)	15·6	9·6	14·1	9·2	15·3	7·8	14·2	7·8	15·9	11·1	14·1	10·5
Total lipids (g)	25·5	8·2	23·8	9·5	25·0	7·9	23·3	9·1	26·0	8·4	24·3	9·5
SFA (g)	9·5	2·9	8·9	3·4	9·3	2·8	8·8	3·1	9·6	2·9	9·0	3·8
MUFA (g)	9·9	4·0	9·1	4·5	9·6	3·7	9·0	4·7	10·1	4·2	9·2	4·1
Oleic acid (g)	9·5	3·9	8·7	4·0	9·2	3·7	8·5	4·3	9·8	4·0	9·0	3·8
PUFA (g)	3·3	1·6	2·9	1·7	3·3	1·6	2·8	2·1	3·3	1·5	3·0	1·6
Linoleic acid (g)	2·8	1·3	2·4	1·4	2·8	1·4	2·4	1·7	2·7	1·3	2·4	1·3
Linolenic acid (g)	0·9	0·5	0·8	0·5	0·9	0·5	0·8	0·6	0·9	0·4	0·8	0·4
Available carbohydrates (g)	77·6	28·9	71·4	33·7	74·1	25·8	70·1	27·4	80·9	31·3	74·0	42·3
Soluble carbohydrates (g)	47·9	13·2	46·7	16·9	46·0	12·0	44·8	15·4	49·7	14·2	47·5	17·9
Starch (g)	12·7	13·4	9·3	18·1	12·5	13·0	9·0	18·2	13·0	13·8	9·9	16·9
Fibre (g)	4·2	3·9	3·3	4·8	3·8	3·4	3·1	4·4	4·6	4·4	3·6	5·0
Cholesterol (mg)	62·8	43·7	66·1	72·4	59·5	43·5	65·1	75·0	66·1	43·7	67·5	63·2
Na (mg)	373·1	321·5	253·4	317·5	350·1	271·9	251·3	272·5	395·3	362·9	257·1	374·5
K (mg)	747·4	408·3	635·7	399·1	716·7	379·5	617·4	356·7	777·2	433·8	650·7	444·4
Ca (mg)	383·5	184·0	346·4	245·0	391·6	189·5	349·2	234·8	375·6	178·8	316·3	244·6
Fe (mg)	3·6	4·2	2·5	5·0	3·8	5·3	2·7	4·8	3·4	3·0	2·3	5·1
Zn (mg)	3·5	1·3	3·3	1·5	3·5	1·3	3·3	1·6	3·5	1·3	3·3	1·6
Thiamin (mg)	0·5	0·4	0·4	0·5	0·5	0·5	0·4	0·5	0·5	0·3	0·4	0·5
Riboflavin (mg)	0·7	0·5	0·5	0·6	0·7	0·6	0·6	0·6	0·6	0·4	0·5	0·6
Vitamin B_6 (mg)	0·5	0·5	0·5	0·5	0·5	0·6	0·4	0·4	0·5	0·4	0·5	0·5
Vitamin C (mg)	62·9	32·4	56·1	44·3	61·8	32·0	53·5	45·5	63·9	32·9	56·7	43·5
Vitamin D (μg)	2·7	3·3	0·3	5·1	2·9	3·4	0·6	5·4	2·5	3·2	0·3	5·0
Vitamin E, α-TE (mg)	1·4	1·4	1·0	1·6	1·2	1·3	0·8	1·3	1·5	1·5	1·2	2·1
Retinol (μg)	94·8	142·2	16·6	150·8	99·9	144·5	19·3	179·9	89·8	140·2	10·9	120·4
RE (μg)	279·8	366·5	144·0	393·1	253·1	347·1	133·4	315·7	305·8	383·9	165·7	469·7
Niacin (mg)	4·4	5·2	3·4	3·9	4·7	6·7	3·4	3·7	4·2	3·1	3·4	4·1
Folate (μg)	97·7	48·0	87·7	51·0	95·3	45·1	88·6	49·9	99·9	50·8	86·2	56·5

IQR, interquartile range; α-TE, α-tocopherol equivalents; RE, retinol equivalents.
No significant difference by sex was detected.

Table 3 Daily energy, macronutrient and micronutrient intakes of Italian infants at 9 months of age in the Trieste Infants Food cohort

	All infants (*n* 179)				Females (*n* 89)				Males (*n* 90)			
Nutrient	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR
Energy (kJ)	3213·2	890·2	3220·9	1000·5	3208·2	962·1	3167·6	1179·4	3218·2	818·4	3242·0	900·6
Total proteins (g)	27·9	11·4	26·7	13·1	27·3	10·3	26·7	13·4	28·5	12·5	26·4	13·1
Total lipids (g)	30·6	9·8	29·8	13·2	31·1	10·5	30·4	14·1	30·0	9·0	28·3	11·6
SFA (g)	10·6	3·7	10·3	4·2	10·6	3·8	10·5	4·6	10·6	3·6	10·0	3·7
MUFA (g)	11·5	4·5	10·6	5·6	11·5	4·6	10·6	5·6	11·4	4·4	10·6	5·2
Oleic acid (g)	10·6	4·6	10·1	5·6	10·7	4·7	10·1	6·0	10·6	4·4	10·0	4·3
PUFA (g)	3·5	1·6	3·2	1·9	3·6	1·8	3·2	2·0	3·4	1·4	3·1	1·9
Linoleic acid (g)	2·9	1·4	2·5	1·6	3·0	1·6	2·7	1·6	2·7	1·2	2·5	1·6
Linolenic acid (g)	0·5	0·2	0·4	0·3	0·5	0·2	0·4	0·3	0·5	0·2	0·4	0·3
Available carbohydrates (g)	101·7	30·5	98·6	36·2	100·6	33·3	98·6	40·6	102·7	27·6	98·8	31·0
Soluble carbohydrates (g)	43·4	15·4	43·4	19·0	41·2	15·9	40·4	19·8	45·5	14·6	45·3	16·1
Starch (g)	29·5	19·2	25·0	24·6	29·2	18·7	27·2	25·7	29·8	19·7	24·9	24·7
Fibre (g)	7·0	3·6	6·6	4·6	6·8	3·6	6·2	3·9	7·3	3·5	6·8	4·8
Cholesterol (mg)	69·3	42·0	64·1	57·2	62·6*	39·1	60·9	50·3	75·8*	43·9	70·0	47·5
Na (mg)	671·2	492·1	521·9	499·5	633·5	499·1	498·4	419·9	708·6	484·8	542·1	619·7
K (mg)	1080·5	505·5	1031·6	618·3	1039·4	448·0	1008·2	596·8	1121·1	556·1	1044·4	657·3
Ca (mg)	536·1	221·6	523·9	313·3	543·6	220·8	532·7	327·8	528·7	223·4	508·4	301·5
Fe (mg)	6·3	14·7	5·0	4·4	7·5	20·7	4·9	4·3	5·2	2·7	5·0	4·4
Zn (mg)	3·6	1·8	3·5	2·5	3·7	1·7	3·7	2·7	3·4	1·8	3·4	2·3
Thiamin (mg)	0·6	0·3	0·6	0·4	0·6	0·3	0·5	0·5	0·6	0·3	0·6	0·3
Riboflavin (mg)	0·8	0·4	0·8	0·5	0·8	0·4	0·8	0·5	0·8	0·4	0·7	0·5
Vitamin B_6 (mg)	0·8	0·4	0·8	0·4	0·8	0·4	0·8	0·4	0·8	0·4	0·8	0·4
Vitamin C (mg)	70·5	34·9	62·8	48·8	69·2	33·2	61·8	38·0	71·7	36·7	64·4	53·3
Vitamin D (μg)	2·3	2·5	0·7	4·1	2·6	2·7	1·9	4·4	1·9	2·4	0·5	3·2
Vitamin E, α-TE (mg)	2·8	1·6	2·4	2·2	2·6	1·6	2·3	2·3	2·9	1·7	2·5	1·8
Retinol (μg)	72·5	68·8	50·7	69·4	69·3	62·3	45·2	64·8	75·6	74·8	59·9	72·1
RE (μg)	515·0	394·4	423·7	503·6	506·5	398·3	384·6	513·4	523·5	392·6	453·1	461·9
Niacin (mg)	6·4	3·4	6·0	4·3	6·5	3·5	6·0	4·8	6·4	3·3	6·3	4·0
Folate (μg)	122·7	61·3	107·2	65·3	122·3	56·5	109·5	60·3	123·2	66·0	102·6	65·7

IQR, interquartile range; α-TE, α-tocopherol equivalents; RE, retinol equivalents.
Mean value was significantly different between sexes: *$P < 0·05$.

Table 4 Daily energy, macronutrient and micronutrient intakes of Italian infants at 12 months of age in the Trieste Infants Food cohort

	All infants (*n* 176)				Females (*n* 85)				Males (*n* 91)			
Nutrient	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR
Energy (kJ)	3357·3	907·1	3274·3	1027·3	3242·8*	907·6	3138·3	1147·6	3464·4*	898·5	3509·1	948·2
Total proteins (g)	32·5	11·2	32·1	13·4	32·6	11·4	32·3	12·7	32·5	11·1	32·0	14·3
Total lipids (g)	30·2	10·0	29·2	11·6	29·6	9·1	28·7	11·2	30·7	10·7	30·1	12·1
SFA (g)	12·1	4·6	11·1	6·3	12·0	4·3	11·1	5·8	12·1	4·9	11·1	6·7
MUFA (g)	11·2	4·7	10·6	5·3	10·8	4·0	10·0	4·9	11·6	5·3	11·4	6·6
Oleic acid (g)	10·2	4·5	9·8	4·7	9·9	3·9	9·3	4·7	10·6	5·0	10·3	4·7
PUFA (g)	3·0	1·4	2·6	1·7	2·9	1·3	2·5	1·5	3·1	1·5	2·8	1·7
Linoleic acid (g)	2·4	1·3	2·0	1·5	2·3	1·2	1·9	1·5	2·5	1·3	2·2	1·5
Linolenic acid (g)	0·5	0·2	0·4	0·3	0·5	0·2	0·4	0·3	0·5	0·2	0·4	0·3
Available carbohydrates (g)	106·8	30·8	104·0	44·8	100·8*	31·2	93·8	38·4	112·4*	29·4	113·0	45·8
Soluble carbohydrates (g)	46·1	15·7	45·0	17·0	43·1*	13·4	42·3	13·2	48·9*	17·2	48·3	22·8
Starch (g)	36·7	21·7	33·8	30·8	33·6*	21·5	29·6	25·5	39·7*	21·6	39·7	33·9
Fibre (g)	7·0	3·2	7·0	4·3	6·5	2·9	6·0	4·0	7·5	3·5	7·2	4·1
Cholesterol (mg)	100·6	59·0	88·0	56·5	100·8	6·0	87·9	57·9	100·5	60·3	88·1	56·1
Na (mg)	721·6	397·7	654·1	467·5	655·8	334·8	575·9	404·1	783·1	441·7	710·6	679·5
K (mg)	1290·1	510·2	1227·3	703·4	1291·9	492·0	1265·2	640·8	1288·3	529·3	1190·5	754·5
Ca (mg)	595·3	251·2	577·8	385·3	614·2	256·2	588·8	335·5	577·6	247·1	539·6	420·2
Fe (mg)	4·9	2·4	4·7	2·7	4·8	2·3	4·7	2·9	5·1	2·4	4·7	2·6
Zn (mg)	4·2	1·5	4·0	1·8	4·2	1·6	3·9	1·6	4·3	1·5	4·1	1·8
Thiamin (mg)	0·6	0·2	0·6	0·3	0·6	0·2	0·5	0·3	0·6	0·2	0·6	0·3
Riboflavin (mg)	0·9	0·4	0·9	0·6	0·9	0·4	0·9	0·5	0·9	0·4	0·9	0·6
Vitamin B_6 (mg)	1·0	0·4	0·9	0·5	1·0	0·4	0·9	0·5	0·9	0·4	0·9	0·5
Vitamin C (mg)	58·8	34·4	51·2	35·8	56·9	29·0	49·9	28·7	60·7	38·9	51·7	38·2
Vitamin D (μg)	1·3	1·7	0·5	1·2	1·3	1·7	0·5	0·9	1·3	1·7	0·5	1·6
Vitamin E, α-TE (mg)	2·9	1·6	2·7	2·2	2·8	1·3	2·6	2·1	3·1	1·9	2·9	2·4
Retinol (μg)	128·2	91·3	105·0	129·5	135·2	100·7	108·8	128·8	121·6	81·5	102·1	125·5
RE (μg)	506·1	302·5	460·7	387·9	504·3	294·6	454·5	331·5	507·9	311·4	469·6	412·0
Niacin (mg)	6·9	3·2	6·2	4·1	6·7	3·3	5·9	3·7	7·2	3·2	6·2	4·3
Folate (μg)	116·6	48·4	106·4	60·3	115·9	50·4	102·6	63·9	117·3	46·7	109·2	55·0

IQR, interquartile range; α-TE, α-tocopherol equivalents; RE, retinol equivalents.
Mean value was significantly different between sexes: *$P < 0·05$.

Table 5 Percentage contribution of the main macronutrients to total energy intake at each follow-up among Italian infants in the Trieste Infants Food cohort

Nutrient	6 months	9 months	12 months	Italian DRV[11]	WHO (RDI)[12]
Total lipids (%)	40·2	36·0	33·8	40%E (AI)	30–40%E
SFA (%)	15·5	12·8	13·7	< 10%E (STD)	–
MUFA (%)	15·4	13·5	12·4	for difference†	–
PUFA (%)	5·1	4·1	3·4	5–10%E (RI)	–
Available carbohydrates (%)	49·6	49·7	50·1	–	55–65%E
Soluble carbohydrates (%)	32·7	22·1	22·0	–	–
Starch (%)	7·7	15·0	18·1	–	–

DRV, Dietary Reference Value; RDI, Recommended Daily Intake; %E, percentage of energy intake; AI, Adequate Intake; STD, Suggested Dietary Target; RI, Reference Interval.
†The quantity of MUFA is calculated by difference between total lipids and the sum of SFA and PUFA.

6 months to 12·4 % at 12 months, while that of PUFA ranged from 5·1 % at 6 months to 3·4 % at 12 months.

A comparison of the distribution of 'real' protein intakes in our cohort with the estimated protein requirements of our cohort from the Italian DRV[11] at each follow-up is shown in Fig. 2, 3 and 4, respectively. The DRV are expressed as the Average Requirement, equal to 1·11 g/kg weight per d, and the estimated protein requirements were calculated using the infants' weights reported in the questionnaires at 6, 9 and 12 months.

The distribution of protein intake, expressed as median and IQR, was greater in our cohort, when compared with estimated data using the Italian DRV[11], at each follow-up: 13·1 (IQR 8·0) g/d *v.* 8·4 (IQR 1·3) g/d at 6 months; 26·4 (IQR 12·4) g/d *v.* 9·5 (IQR 1·5) g/d at 9 months; and 31·3 (IQR 12·7) g/d *v.* 10·8 (IQR 1·4) g/d at 12 months.

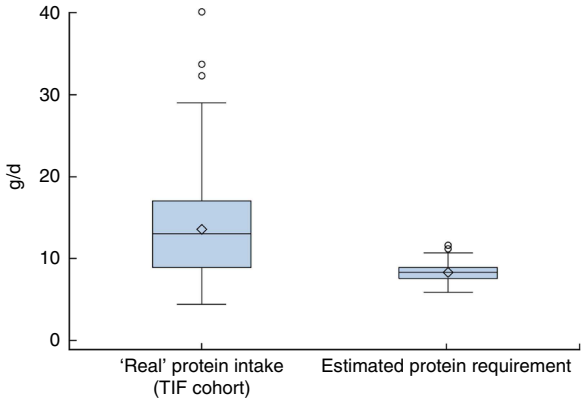

Fig. 2 (colour online) Box-and-whisker plots comparing the 'real' protein intakes of Italian infants at 6 months of age in the Trieste Infants Food (TIF) cohort and their estimated protein requirements from the Italian Dietary Reference Values[11] (calculated using the Average Requirement, equal to 1·11 g/kg weight per d, and the infants' reported weights at 6 months). The bottom and top edge of the box represent the first and third quartiles (interquartile range); the line within the box represents the median; the open diamond represents the mean value; the ends of the bottom and top whiskers represent the minimum and maximum values; and the circles represent outliers

Discussion

The present detailed nutritional evaluation, carried out in a cohort specifically recruited for this purpose, shows that energy and nutrient intakes were greater in male than in female infants, except for Ca and thiamin. This in line with reports from other studies[8] but further analysis of food sources of nutrients may be needed to better explain these differences between sexes regarding dietary patterns and dietary choices in infants.

The contribution of total lipids to total energy intake (Table 5) appears to be in line with both DRV[11] and WHO recommendations[12], amounting to ~40 % in infants aged 6–12 months. However, the energy contribution from PUFA appears to be lower than the DRV (5–10 % of energy intake) at 9 and 12 months of age, while that of SFA appears to be higher than the DRV (<10 % of energy intake) at all follow-ups. This could reflect an unbalanced diet with a low intake of essential fatty acids (*n*-3), in particular DHA and EPA that are important for neurodevelopment of infants. Regarding available carbohydrate intakes, values are lower than WHO recommendations[12]. Unfortunately, for this age range, no Italian reference data are available that can be used as a comparison for the analyses[11]. Moreover, the 'real' protein intakes of the TIF cohort (Figs 2, 3 and 4) were greater than the estimated protein requirements of the TIF cohort calculated using the Italian DRV[11]. The 'real' protein intakes cover the Average Requirement established using the Italian DRV but the values are two to three times greater. This could lead to short- and long-term negative health effects such as anticipation of the adiposity rebound, which normally occurs between 6 and 7 years of age, and an increased risk of kidney problems. The mineral consumption of the TIF cohort is very close to what is recommended, except for Na intake at 12 months (721·6 mg/d *v.* DRV of 400 mg/d), Fe intake at all follow-ups (from 3·5 mg/d at 6 months to 4·9 mg/d at 12 months *v.* DRV of 11 mg/d) and Ca intake at 6 months (383·5 mg/d *v.* WHO recommendation of 500 mg/d or DRV of 700 mg/d). Finally, the daily vitamin requirements are satisfied; the only exception is vitamin D whose daily intake decreases continually at the three follow-ups reaching a value that, at 12 months, is much lower than the reference value (1·3 mg/d *v.* DRV of 10 mg/d). The relative deficiency of Fe, Ca and

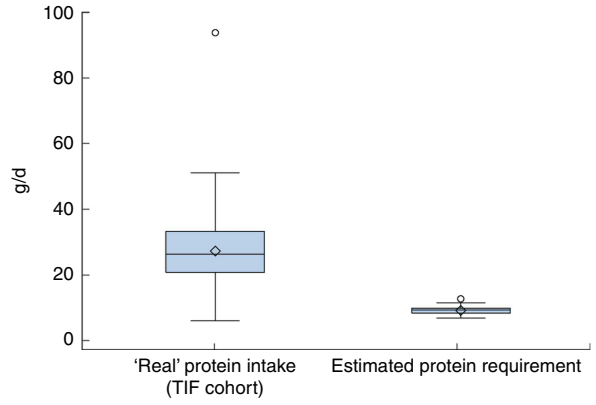

Fig. 3 (colour online) Box-and-whisker plots comparing the 'real' protein intakes of Italian infants at 9 months of age in the Trieste Infants Food (TIF) cohort and their estimated protein requirements from the Italian Dietary Reference Values[11] (calculated using the Average Requirement, equal to 1·11 g/kg weight per d, and the infants' reported weights at 9 months). The bottom and top edge of the box represent the first and third quartiles (interquartile range); the line within the box represents the median; the open diamond represents the mean value; the ends of the bottom and top whiskers represent the minimum and maximum values; and the circles represent outliers

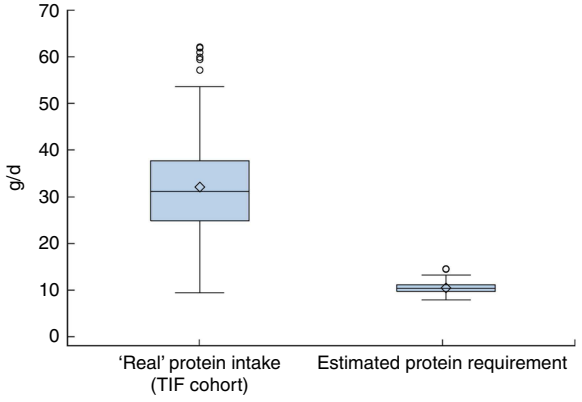

Fig. 4 (colour online) Box-and-whisker plots comparing the 'real' protein intakes at of Italian infants 12 months of age in the Trieste Infants Food (TIF) cohort and their estimated protein requirements from the Italian Dietary Reference Values[11] (calculated using the Average Requirement, equal to 1·11 g/kg weight per d, and the infants' reported weights at 12 months). The bottom and top edge of the box represent the first and third quartiles (interquartile range); the line within the box represents the median; the open diamond represents the mean value; the ends of the bottom and top whiskers represent the minimum and maximum values; and the circles represent outliers

vitamin D in the diet of infants in our cohort may be balanced by their higher bioavailability in breast milk and by exposure to sunshine as far as vitamin D is concerned[18]. This is another reason why it is important to prolong breast-feeding up to 2 years of life, as recommended[4], and to include foods rich in Ca and Fe as first complementary foods.

To our knowledge, the present study is the first to provide a detailed description of the dietary intakes of energy, macro- and micronutrients in an Italian population of infants at different points during the first year of life and when complementary feeding is established. The main strengths of our study are the adoption of a rigorous design (prospective cohort study), which allowed the accuracy of data collection, the use of a 3 d dietary record to collect dietary data and the extraction of food composition and nutritional data using Microdiet, a specifically dedicated software. Microdiet contains the Italian food composition database for epidemiological studies, that was compiled using standard methods established by the EuroFIR project (www.eurofir.org)[13]. These data were integrated with information from nutritional labels (e.g. baby food) and, in the case of human milk, from the literature[14,15]. This is an important tool for epidemiological research, public health nutrition and education, clinical practice and nutrition declaration of food labels.

The main study limitation was the loss to follow-up. However, no difference was seen in the main characteristics of women lost to follow-up compared with those who remained in the study, suggesting a random loss. Furthermore, the loss is comparable to that reported in other studies involving infants[8]. Moreover, the widespread use of baby foods, which are generally characterized by poor nutritional labelling, may have limited the precision with regard to the intakes of micronutrients such as vitamins and some minerals, while data concerning energy components (total proteins, available carbohydrates and total lipids) were completely covered[17]. The methodology adopted to estimate the consumption of breast milk (frequency of feeds, perceived length of each feed by mothers) and the use of a literature-derived composition constitute a weakness because it does not take into consideration inter- and intrasubject variability and may therefore contribute inaccuracy in the assessment of nutrient intakes[17]. Nevertheless, we decided not use the double weighing methodology (best method) because we think it is an invasive methodology and it goes against the main principle of breast-feeding. Our method has been used by other international infant cohort studies[6,8]. Finally, the nutritional comparison between our cohort's nutrient intakes and the reference values[11,12] may be hampered by the fact that the latter cover a wider age range (infants: 0·6–1 years). The mean daily energy and nutrient intake data reported for infants in the third Italian National Consumption Survey 2005–2006[19] and in the National Health and Nutrition Examination Survey (NHANES) 2009–2012[1] are comparable to the values we obtained for all infants, despite the fact that the two surveys cover wider age ranges: 0–2·9 years, and 6–11 months and 12–23 months, respectively. In 2014, an Italian group conducted a cross-sectional study (the Nutrintake 636 study) to compare the energy and nutrient intakes and anthropometric status of infants and toddlers living in North and South Italy, at different follow-ups[20]. Since they did not take into account the breast milk intake, we decided not to compare our results with theirs.

Conclusions

The present descriptive paper provides detailed information on energy and nutrient intakes in an Italian population of infants during the first year of life. Understanding the dietary habits and the nutritional intake of infants during the first year of life is important because this period has a high impact on the development of food preferences that act on metabolic and chronic risk factors[1,2]. The results highlight a substantial adherence with Italian DRV and WHO recommendations[11,12] concerning nutrient intakes, except the excessive protein intake, low vitamin D intake and the unbalanced intake of SFA and PUFA, that could lead to negative short- and long-term health consequences (e.g. obesity, rickets, kidney diseases, delays in neurodevelopment). However, further analyses will be carried out to identify dietary patterns and evaluate the adequacy of food choices in relation to national and international recommendations[4,5,21].

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Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S136898001800201X

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nutrients

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Article

Age-related trends in the diet of an infant's cohort in the northeast of Italy from six to twelve months of age

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Abstract: Complementary feeding is recognized as an important predictor of health later in life and is likely to affect the development of food preferences. This paper describes age-related trends in terms of energy, nutrients intake and dietary habits of an Italian infant subcohort (n=152), enrolled in Trieste. Infant dietary data, collected using a food diary at 6, 9 and 12 months of age, were used to estimate energy and nutrients intake using the Italian food composition database. Age-related trends were calculated using Page's trend test. An increasing age-trend was observed in the percentages of contribution of macronutrients to total energy intake, with the exception of total lipids, which instead decreased over time. Most of the infants shared a low varied diet especially with regards to protein intake sources, represented mainly by dairy and meat products rather than pulses and fish. This could also account for the low intake of essential fatty acids (ω3) that play an important role in infant neurodevelopment. Moreover, non-commercial baby foods contributed more in terms of quantity, energy and macronutrients intake, compared with commercial products. Healthy eating habits should be encouraged during the first year of life, promoting a varied and well balanced diet at family level.

Keywords: prospective infant cohort study; age-related trends; commercial baby foods and non-commercial products; energy and nutrients intake

1. Introduction

The transition from exclusive breastfeeding or formula feeding to family foods, which is referred to as complementary feeding (CF), typically covers the period from 6 to 18-24 months of age of infants. This period has been shown to lay the foundations for health and growth [1] and could have an impact on the development of food preferences [2]. During the early years of life, the consumption of health promoting foods, such as fruits and vegetables, reduces the risk of cardiovascular disease, obesity, hypertension, stroke and some cancers in adulthood [3]. Although it has been shown that humans, from birth, tend to prefer sweet, salty and umami tastes over those that are bitter or sour, some studies have suggested that food preferences are acquired through experience. Infants explore new and different flavors from maternal diet, during intra and extra uterine life, through amniotic

liquid and breast milk [4]. A varied diet, rich in fruits and vegetables, allows to meet macro and micronutrient requirements and provides adequate and well-distributed energy intake [5]. On the other hand, a CF approach based on the consumption of commercial baby foods may hamper the nutritional education inherent in this learning process, because infants may be less open to accept new flavors that they perceived as unfamiliar [6]. However, extremely few studies exist on the consumption of commercial and home-made baby foods in industrialized countries [7]. Furthermore, only few researches explore infant feeding habits and determine nutrient intakes in detail during the CF period. Among international studies, the most relevant are four large cohort studies: the Norwegian Mother and Child Cohort Study (MoBa) [8], the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD) [9], the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) [10] and the Public health impact of long-term, low-level mixed element exposure in susceptible population strata study (PHIME) [11]. However, all of these studies investigate how dietary habits combined with genetic and/or environmental aspects, influence the health and development of children. Moreover, very few data are available at national level [12,13].

The aim of this paper is to describe age-related trends in energy, macro and micronutrients intake and Body Mass Index (BMI), during the first year of life with a special focus on whether the dietary habits of this subcohort privileged home-made vs commercial baby foods. The present study offers the opportunity to better understand how eating patterns change during this sensitive time, and to identify critical aspects of feeding practices during CF, which should be monitored closely and addressed by recommendations, targeted to parents.

2. Materials and Methods

We investigated age-related trends in nutritional and anthropometric data obtained from an ongoing prospective infant cohort study conducted in the north east of Italy (Trieste Infant Food cohort – TIF cohort). In this study, detailed data were collected from 400 healthy infants recruited at birth at the Institute for Maternal and Child Health – IRCCS "Burlo Garofolo" Trieste, between July 2007 and July 2008. The study, which started in 2006 as a part of the PHIME European research project [14], was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the ethics committee of the Institute. All participating subjects gave their informed consent for inclusion, before they were enrolled in the study. The study design, methods, and sampling procedures have already been reported [15].

In brief, infants were eligible if their birth weight was 2,000 g or more, gestational age was 36 completed weeks or more, if they were not affected by severe diseases or congenital malformations that required hospital admission, and if their mothers were resident in the province of Trieste. Dietary data were collected using a 3-day dietary (3-DD) record (food diary) provided to mothers during the first contact, with instructions on how to record type, quantity and method of feeding over a 24 h period on three separate non-consecutive days, including one at the weekend at 3, 6, 9, 12, 18, 24 and 36 months of age of the infants. Mothers were asked to report weight and length of their children as measured by their community pediatrician during periodic health checks at 1, 3, 5–6, 8, 12, 18, 24 and 36 months of age. In addition, demographic, educational, social and anthropometric data of the mothers and fathers were obtained from a validated questionnaire designed for the PHIME study [14].

The present paper focuses on only 3 (6, 9, 12 months) of the 7 collection points, because this is the period in which CF starts and the changes in the infant's diet are greater and most rapid [16]. Only subjects who completed the food diary at all three collection points were considered for data analysis (TIF subcohort). The complete nutritional analysis of our cohort (TIF cohort) at the same collection points considered in this paper has been fully described in a prevoius paper [17].

Data extracted from food diaries were analyzed using the Microdiet V2.8.6. software (Microdiet software - Downlee Systems Ltd., High, Peak, UK), which contains the Italian Food Composition database for Epidemiological Studies [18], integrated with nutritional data from food labels in the case of commercial products and formula milk, and from literature for human milk [19,20]. Full details of the methodology are published elsewhere [21].

The nutritional analysis was performed on 28 food components: proteins (total), carbohydrates (available and soluble, starch, fiber), lipids (total, saturated, monounsaturated and polyunsaturated fatty acids; oleic, linoleic and linolenic acid; cholesterol), minerals (sodium, calcium, potassium, iron, zinc) and vitamins (vitamin B1, vitamin B2, vitamin B6, vitamin C, vitamin D, vitamin E α-TE, retinol, retinol eq., niacin, folate).

Foods were classified into 21 food groups based on the methodology proposed by Talamini et al. (2006) [22] and modified as follows: cereals, milk and dairy products, eggs, vegetables, fruit, seeds and nuts, herbs and spices (also salt), fish, meat, fats and oils, beverages (water and beverage without sugar), sugars, sweets and desserts, soft drinks, tubers, sauces, cured meats.

Based on the methodology used by Noble and colleagues in the ALSPAC study [23], we also classified foods into 4 food types: commercial products (CP; data from food labels), non-commercial products (NCP; data already present in the Italian food composition database), breast-feeding (BF) and formula feeding (FF). Slight methodological differences did exist between the two classifications, since our study did not consider rusks and fruit juices/beverages as separate categories from the other CP [23].

For the analysis of CF practices and of the contribution of each food type in terms of energy and macronutrients intake, we considered three separate categories, two relating to complementary feeding (NCP and CP), and one to milk based feeding (BF plus FF).

Furthermore, in order to highlight the age-related trends in daily intake of different food groups, we performed the analysis on the food groups that contributed the most to macronutrients intake: cereals, milk and dairy products, eggs, fruit and vegetables, pulses, fish and meat and cured meat.

Categorical data are presented as absolute frequencies and percentages, while continuous data as medians and interquartile ranges (IQR: 1st-3rd quartiles). The mean daily intake of macro-, micronutrients and energy was calculated, for each infant, on a 3-day observation basis, excluding the use of supplements.

Differences in continuous variables were assessed using non-parametric rank-sum Mann–Whitney tests or signed-rank Wilcoxon test as appropriate, while differences between categorical variables were analyzed with two-tailed Fisher exact tests. Trend analysis was performed using Page's trend test. With regards to the repeated measures (quantities of nutrients at 6, 9 and 12 months for the same subjects), the signed-rank test by Wilcoxon allowed us to test the significance of the difference between measures at different time points (i.e. 6 months vs. 9 months and 9 months vs. 12 months). Page's trend test, instead, was used to verify if there was a significant trend (decreasing or increasing) taking into account the three measures simultaneously. Both tests (Wilcoxon's and Page's) are non-parametric rank tests, not imposing hypothesis on the distribution of the data analysed and on their residuals. Statistical significance for all tests was set at a p-value below 0.05.

Anthropometric measures were compared with the WHO growth standards using WHO Anthro software, and are reported as BMI z-scores [24,25].

All the analyses were carried out using Stata/IC 14.2 (StataCorp LLC, College Station, TX, USA).

3. Results

The number of food diaries available were 268 at 6 months, 179 at 9 months and 176 at 12 months of age, but only for 152 infants completed food diaries were available at each collection points. The characteristics of the mothers and infants of the TIF subcohort (n = 152) are described in Table 1. The differences in sample size reported in the table for some variables are due to missing data in the questionnaires.

Table 1. Characteristics of mothers at enrolment and children at birth (n = 152).

Characteristic	n	%
Infant sex (n=151)		
Male	78	52
Female	73	48
Birth weight (n = 151)		

< 2500 g	0	0
2500-4199 g	142	94
≥ 4200 g	9	6
Birth length (n = 150)		
< 46 cm	1	1
46-52.9 cm	125	83
≥ 53 cm	24	16
Birth maternal age (n = 152)		
< 30 years	26	17
30-34 years	57	38
≥ 35 years	69	45
Maternal nationality (n = 152)		
Italian	140	92
Foreigner	12	8
Maternal marital status (n = 152)		
Married/living with partner	138	91
Separated/divorced	5	3
Single/not living with partner	9	6
Maternal education (n = 151)		
≤ Secondary school	18	12
Completed high school or equivalent	58	38
Bachelor degree or higher	75	50
Employment (n=137)		
Yes	131	96
No	6	4
Maternal pre-pregnancy BMI (n = 151)		
<18.5	14	9
18.5-25	102	68
25-30	25	17
≥30	10	7
Gestational age at birth (n = 150)		
36-37 weeks	9	6
38-42 weeks	141	94
Type of birth (n = 151)		
Vaginal	125	83
Cesarean	26	17

Abbreviation: n = number of subjects; % = percentage of subjects.

Forty-five percent of mothers were 35 years old or more (mean age 34.4±4.2), 92% were born in Italy, 91% were married or living with the father of the child, 88% had a medium-high level of education, 96% were in employment and 68% had a pre-pregnancy BMI that fell within the normal range. Most infants (94%) were born between 38 and 42 weeks of gestation, 83% by vaginal delivery. Infants were equally distributed by sex: 78 (52%) males and 73 (48%) females. The majority (94%) weighed between 2500 g and 4199 g at birth (mean weight 3432±442 g), and 83% were between 46.0 and 52.9 cm long (mean length 50.5±1.9 cm).

A comparison between the mothers belonging to the TIF subcohort (n=152) and the remaining TIF group (n=248) shown a statistically significant difference in terms of level of education (p=0.001), nationality (p=0.041) and mean age at delivery (p=0.004). More specifically, mothers belonging to the TIF subcohort had a higher level of education (88% vs. 79%), were more likely to be Italian (92 vs. 85%) and had higher mean age at delivery (34.4±4.2 vs. 33.0±4.9), as compared with the remaining TIF cohort.

At 6 months of age, 91% of infants were already on complementary feeding, with 63% of these still being breastfed, while only 9% were still exclusively breastfed. At 9 months of age, 99% of infants were receiving complementary foods, alone (7%) or in combination with breast milk (56%) or infant formula (36%) and only one infant (1%) was still exclusively breastfed. At 12 months of age, 39% of

infants were still breastfeeding. A complete overview of the feeding practices in the TIF cohort, during the first 24 months of life of the infants is published elsewhere [15].

Daily energy, macro- and micronutrients intake are shown in Table 2.

Table 2. Daily energy, macronutrient and micronutrients intake from 6- to 12 months of age (n = 152)

Nutrient	6 months Median (IQR)	9 months Median (IQR)	12 months Median (IQR)	p-value 6 vs. 9	p-value 9 vs. 12	p-value trend
Energy (kJ)	2246.0 (1881.5; 2808.7)	3254.7 (2619.6; 3631.3)	3274.0 (2851.8; 3825.0)	<0.001	0.0357	<0.001 ↑
Total proteins (g)	13.0 (8.9; 17.0)	27.0 (21.5; 33.7)	31.9 (25.1; 39.4)	<0.001	<0.001	<0.001 ↑
Total proteins (%E)	*9.7*	*13.9*	*16.3*			
Total lipids (g)	23.7 (20.0; 29.1)	29.8 (23.8; 36.8)	29.3 (24.1; 35.6)	<0.001	0.7171	<0.001 ↑
Total lipids (%E)	*39.7*	*34.5*	*33.7*			
Satured fatty acids (g)	8.9 (7.6; 10.5)	10.3 (8.3; 12.4)	11.3 (8.7; 15.3)	<0.001	<0.001	<0.001 ↑
Monounsatured fatty acids (g)	9.0 (7.5; 11.2)	10.6 (8.4; 13.9)	11.4 (8.1; 13.4)	<0.001	0.9121	0.0058 ↑
Oleic acid (g)	8.6 (7.3; 10.9)	10.1 (7.8; 13.3)	10.1 (7.3; 12.2)	0.0014	0.7461	0.0224 ↑
Polyunsatured fatty acids (g)	2.9 (2.1; 4.0)	3.2 (2.5; 4.3)	2.7 (2.0; 3.7)	0.0183	<0.001	0.8965
Linoleic acid (g)	2.4 (1.8; 3.3)	2.6 (1.9; 3.5)	2.0 (1.5; 3.0)	0.2110	<0.001	0.0054 ↓
Linolenic acid (g)	0.8 (0.6; 1.1)	0.4 (0.3; 0.6)	0.4 (0.3; 0.6)	<0.001	0.7336	<0.001 ↓
Cholesterol (mg)	68.3 (21.0; 96.2)	63.2 (35.4; 91.8)	88.1 (62.9; 118.4)	0.4178	<0.001	<0.001 ↑
Available carbohydrates (g)	67.0 (54.6; 87.6)	98.3 (82.6; 117.8)	103.6 (84.8; 128.0)	<0.001	0.0960	<0.001 ↑
Available carbohydrates (%E)	*49.9*	*50.5*	*53*			
Soluble carbohydrates (g)	46.5 (38.2; 54.7)	42.9 (33.3; 52.1)	45.4 (37.5; 53.7)	<0.001	0.0222	0.8352
Soluble carbohydrates (%E)	*32.5*	*20.7*	*21.8*			
Starch (g)	7.3 (0.7; 14.7)	24.9 (15.8; 40.1)	32.6 (18.3; 49.7)	<0.001	<0.001	<0.001 ↑
Fiber (g)	2.8 (1.0; 5.4)	6.8 (4.5; 9.0)	6.8 (4.7; 8.7)	<0.001	0.4290	<0.001 ↑
Iron (mg)	2.1 (0.6; 5.6)	4.9 (2.8; 7.3)	4.6 (3.2; 6.1)	<0.001	0.1343	<0.001 ↑
Calcium (mg)	313.5 (228.4; 467.9)	526.7 (367.0; 678.8)	587.1 (416.1; 773.6)	<0.001	<0.001	<0.001 ↑
Sodium (mg)	232.9 (167.2; 464.4)	517.1 (371.1; 865.2)	658.3 (422.9; 896.1)	<0.001	0.0632	<0.001 ↑
Potassium (mg)	610.3 (466.9; 829.5)	1007.3 (731.7; 1356.4)	1232.7 (912.6; 1621.2)	<0.001	<0.001	<0.001 ↑
Zinc (mg)	3.1 (2.5; 4.1)	3.5 (2.2; 4.6)	4.0 (3.1; 4.9)	0.3215	<0.001	<0.001 ↑
Vitamin B1 (mg)	0.4 (0.2; 0.6)	0.6 (0.4; 0.8)	0.6 (0.4; 0.7)	<0.001	0.5061	<0.001 ↑
Vitamin B2 (mg)	0.5 (0.3; 0.9)	0.8 (0.5; 1.0)	0.9 (0.6; 1.2)	<0.001	<0.001	<0.001 ↑
Vitamin B6 (mg)	0.4 (0.1; 0.6)	0.8 (0.6; 1.0)	0.9 (0.7; 1.2)	<0.001	<0.001	<0.001 ↑
Vitamin C (mg)	52.1 (35.5; 84.0)	63.0 (45.6; 88.9)	50.8 (36.2; 70.1)	<0.001	<0.001	0.7720
Vitamin D (μg)	0.3 (0.2; 5.2)	0.7 (0.2; 4.3)	0.5 (0.2; 1.4)	0.6233	0.0017	0.7890
Vitamin E α-TE (mg)	0.9 (0.2; 1.7)	2.5 (1.7; 3.7)	2.7 (1.7; 3.9)	<0.001	0.0470	<0.001 ↑
Retinol (μg)	10.3 (0; 117.4)	49.0 (25.5; 95.8)	108.0 (65.4; 190.9)	0.1219	<0.001	<0.001 ↑
Retinol eq (μg)	100.3 (6.2; 318.6)	422.8 (223.0; 730.4)	460.7 (274.9; 680.9)	<0.001	0.5950	<0.001 ↑
Niacin (mg)	2.9 (1.5; 5.5)	6.0 (4.0; 8.2)	6.1 (4.6; 8.5)	<0.001	0.1416	<0.001 ↑
Folate (μg)	81.3 (63.4; 115.6)	111.1 (80.6; 145.6)	105.6 (84.6; 142.4)	<0.001	0.1008	<0.001 ↑

Significant differences between 6 and 9 months and 9 and 12 months were tested using Wilcoxon signed-rank test (P<0.05). Trends were tested using Page's trend test (P<0.05).

The intake of energy, total proteins, saturated fatty acids, starch, calcium, potassium, vitamin B2, vitamin B6 and vitamin E α-TE significantly increased between 6 and 12 months of age. In particular total proteins intake almost tripled, from 13.0 (8.9; 17.0) g/day at 6 months to 31.9 (25.1; 9.4) g/day at 12 months, while the intake of calcium and potassium almost doubled, from 313.5 (228.4; 467.9) mg/day and 610.3 (466.9; 829.5) mg/day at 6 months to 587.1 (416.1; 773.6) mg/day and 1232.7 (912.6; 1621.2) mg/day at 12 months of age, respectively.

Although the trend analysis shown a statistically significant increase in the intake of total lipids, monounsaturated fatty acids, oleic acid, cholesterol, available carbohydrates, fiber, iron, sodium, zinc, vitamin B1, retinol, retinol eq, niacin and folate between the ages of 6 and 12 months, this trend flattened between 9 and 12 months. The only exception was zinc intake for which no statistically significant difference was found between 6 and 9 months of age. Moreover, as shown in Table 2, there was a significant decrease in linoleic and linolenic acids intake, with a non-statistically significant difference, between the ages of 6 and 9 months for linoleic acid and 9 and 12 months for linolenic acid. Finally, no age-related trend was observed regarding polyunsaturated fatty acids, soluble carbohydrates, vitamin C and vitamin D intake, despite there being a statistically significant difference between consecutive collection points. This was not true for vitamin D when intakes at 6 and 9 months were compared.

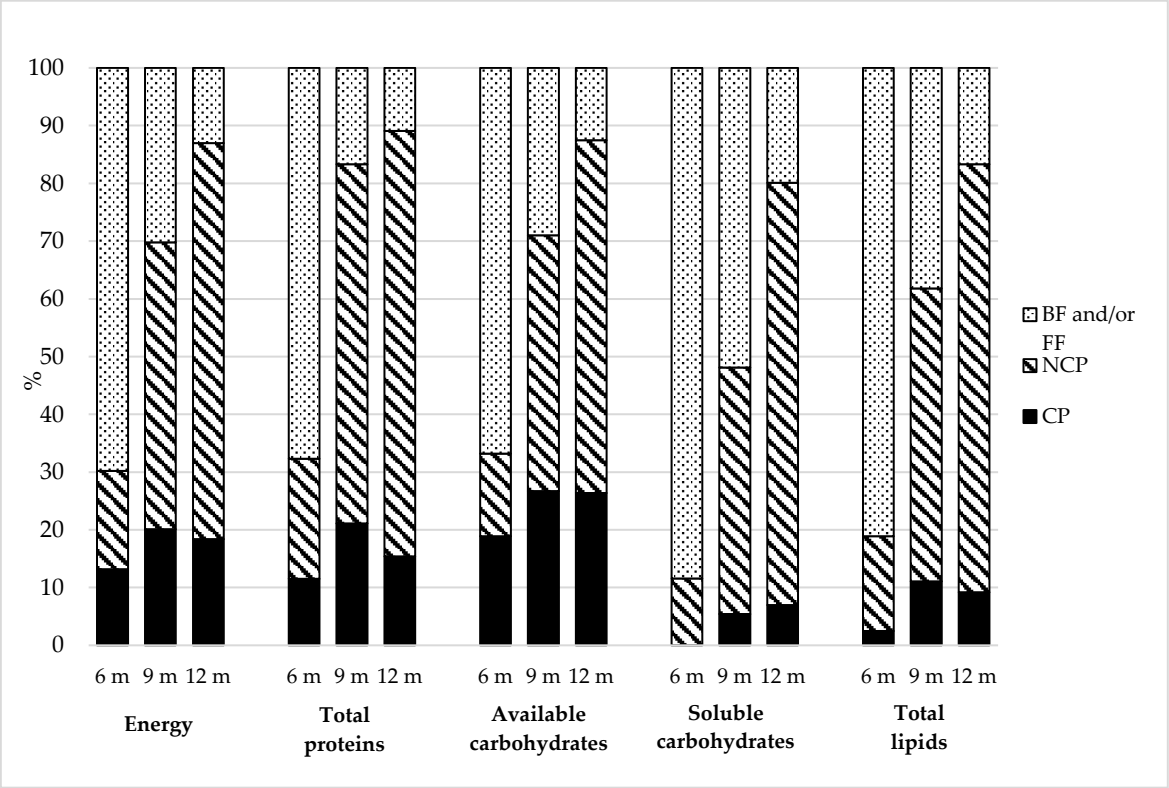

Figure 1. Contribution of different food types in terms of energy and macronutrients intake. **A**bbreviation: BF = Breastfeeding; FF = Formula feeding; NCP = Non-commercial products; CP = Commercial products; m = months of age.

Overall, NCP contributed more, in terms of energy and macronutrients, compared to CP at each of the three collection points, with the exception of available carbohydrates at 6 months which were mainly from CP. In particular, for NCP consumption, the trend analysis revealed a statistically significant increase in energy and nutrients intake between the ages of 6 and 12 months ($p<0.001$). Moreover, there was a statistically significant increase in CP consumption from 6 to 9 months ($p<0.001$) followed by a statistically significant decrease between 9 and 12 months ($p<0.001$). The only exceptions were soluble carbohydrates which continued to increase ($p<0.001$), available carbohydrates and proteins which showed a non-significant decrease between 9 and 12 months of

age (p=0.0787). At 6 months, soluble carbohydrates were found not to contribute to energy intake from CP (0%E), but this was probably due to missing data in baby food labels [21].

The age-related trends in daily intake of the selected food groups are reported in Table 3.

Table 3. Age-related trends in daily food group intake (g/day) between 6 and 12 months of age (n = 152).

Food group	6 months Median (IQR)	9 months Median (IQR)	12 months Median (IQR)	p-value 6 vs. 9	p-value 9 vs. 12	p-value trend
Cereals (g)	8.3 (0; 23.3)	31.8 (18.5; 47.6)	45.6 (28.9; 69.5)	<0.001	<0.001	<0.001 ↑
Milk and dairy products (g)	568.4 (446.7; 697.0)	416.1 (319.3; 530.9)	392.4 (309.9; 516.4)	<0.001	0.3596	<0.001 ↓
Eggs (g)	0 (0; 0)	0 (0; 0)	0 (0; 0.8)	0.0024	<0.001	<0.001 ↑
Fruits and vegetables (g)	107.7 (32.5; 193.2)	170.0 (83.4; 253.8)	173.8 (120.6; 276.4)	<0.001	0.0835	<0.001 ↑
Pulses (g)	0 (0; 0)	3.2 (0; 14.6)	4.6 (0; 16.6)	<0.001	0.5114	<0.001 ↑
Fish (g)	0 (0; 0)	0 (0; 26.7)	3.0 (0; 26.7)	<0.001	0.6556	<0.001 ↑
Meat and cured meat (g)	0 (0; 22.8)	28.3 (10.2; 53.3)	36.6 (15.6; 53.3)	<0.001	0.2014	<0.001 ↑

Abbreviation: IQR=InterQuartile Range. Significant differences between 6 and 9 months and 9 and 12 months were tested using Wilcoxon signed-rank test (P<0.05). Trends were tested using Page's trend test (P<0.05).

There was a statistically significant increase in the consumption of all food groups between the ages of 6 and 12 months, with the exception of milk and dairy products. The consumption of this food group decreased significantly, presumably as a result of the fact that, as reported in Figure 1, the consumption of breast milk and infant formula reduced between the ages of 6 and 12 months. The infants' diet was based on (in decreasing order of consumption): milk and dairy products (from 568.4 g/day at 6 months to 392.4 g/day at 12 months), fruits and vegetables (from 107.7 g/day at 6 months to 173.8 g/day at 12 months), cereals (from 8.3 g/day at 6 months to 45.6 g/day at 12 months), beef and cured meet (from 0 g/day at 6 months to 36.6 g/day at 12 months). The consumption of eggs (0 g/day at all three collection points), pulses (from 0 g/day at 6 months to 4.6 g/day at 12 months) and fish (from 0 g/day at 6 months to 3.0 g/day at 12 months) was negligible.

BMI z-score distribution at 6, 9 and 12 months of age is shown in Table 4. Anthropometric data were available only for 115 (76%) infants at 6 months and 117 (77%) infants at 9 and 12 months. Only one infant (1%) classified as overweight according to WHO Standards [24,25].

Table 4. BMI z-scores distribution at 6, 9 and 12 months of age (n = 115 at 6 months; n = 117 at 9 and 12 months).

	6 months n (%)	9 months n (%)	12 months n (%)
-2SD	8 (7)	1 (1)	-
-1SD	18 (17)	14 (12)	9 (8)
0SD	79 (69)	88 (75)	93 (79)
+1SD	9 (8)	13 (11)	14 (12)
+2SD	1 (1)	1 (1)	1 (1)

Abbreviation: n = number of subjects; % = percentage of subjects; SD = Standard Deviation.

4. Discussion

Our results show that, in the first year of life, the diet of the TIF subcohort infants was characterized by low variety, excessive intake of proteins, mainly from animal sources, and saturated fatty acids, and low intake of essential fatty acids (ω-3). This can lead to negative short and long-term health consequences, as already reported in a previous paper on the same cohort [17].

The assessment of the dietary intake of infants during the first year of life is generally recognized as difficult to accomplish. Only few national and international studies have analyzed the dietary habits of infants in a period that is so crucial for child growth, development and health, and is characterized by the transition from exclusive milk feeding to family foods. Among the few available studies, one of the most important is the DONALD study, a prospective cohort study conducted by the University of Bonn, Germany, to assess the relationship between diet, nutrition, and development during childhood and adolescence [9]. In particular, Foterek et al. (2016) reported significant time and age trends of energy and macronutrients intake between the ages of 3 months and 3 years in the DONALD cohort. With regards to the first year of life in particular, they observed a decrease in total lipids intake and a compensatory increase in total proteins and available carbohydrates intake [26]. The same trend in macronutrients intake was also observed in the TIF subcohort, with energy intake from total proteins increasing significantly from 10% E at 6 months to 16% E at 12 months (Table 2). Our results also reveal that, in contrast with national and international recommendations, total protein intake derived mainly from animal sources (dairy products and meat/cured meat). This was reflected in the significant increase in calcium and vitamin B intake which mainly relies on animal food sources. Substantiating the hypothesis of low food variety in our subcohort , at 12 months of age only few infants were consuming pulses, eggs and fish and, even then, only in small quantities (Table 3) [2,27]. The introduction of these food groups after 9 months of age may also explain the non-statistically significant difference observed in the intake of polyunsaturated fatty acids, oleic and linoleic acids, cholesterol and zinc between the ages of 6 and 9 months.

As shown in Table 2, there was a significant linear trend increase in saturated fatty acids intake and a significant linear trend decrease for linoleic and linolenic acids. This is probably due the combination of reduced breast and/or formula feeding and low consumption of fish, which are the main sources of polyunsaturated fatty acids, and increased consumption of meat/cured meat and dairy products, which are rich in saturated fatty acids (Table 3).

Two studies have analyzed the contribution of CP and NCP in terms of energy and macronutrients intake [7,28]. In the first study, the authors found significant differences between the two food types in terms of energy and macro and micronutrients content, but the sizes of the effect were small throughout the study period and therefore scarcely relevant for dietary practice [7]. The second study showed that an infants' diet based mainly on NCP is associated with a more varied diet during the first year of life and with reduced adiposity [28]. Unfortunately, these results cannot be compared with ours because of the different criteria used to classify the categories in question. Hilbig and colleagues estimated the energy and nutrients content of CP and NCP meals instead of single CP and NCP foods, as in our classification. We observed that the consumption of NCP was prevalent in our infants' diet at all three collection points. In case of CP, only soluble carbohydrates displayed a significant trend increase from 0% (0 g/day) of total soluble carbohydrates intake at 6 months to 7% (3.2 g/day) at 12 months, probably due to increased consumption of sweets and/or desserts and sweet beverages. Also, the intake of soluble carbohydrates from NCP increased from 12% (5.4 g/day) of total soluble carbohydrates intake at 6 months to 73% (33.2 g/day) at 12 months, however, this percentage includes the contribution of homemade desserts and sweets and fruits (Figure 1).

Since, only one infant in the TIF subcohort classified as overweight, we decided not to study the influence of different complementary feeding habits (i.e. prevalent use of CP vs. NCP) on infant BMI, as reported in another study [28]. As reported by other authors, evidence regarding the influence of CF choices (type of foods and timing of introduction) on growth and body composition remain inconclusive [28,29].

The strengths and limitations of our study need to be discussed. Among the major strengths are the frequent and detailed measurements of nutrition and growth, the adoption of a rigorous design (prospective cohort study) that allowed for accurate data collection, the use of a 3-day dietary (3-DD) record which is the gold standard for the assessment of dietary intake during infancy [30], the extraction of food composition and nutritional data using an opportunely integrated version of the Microdiet software. However, some limitations must be considered when interpreting the results. First, the widespread use of commercial baby foods, generally characterized by poor nutritional

labelling, may have limited the precision of the analysis with regards to the intake of micronutrients such as vitamins and some minerals. Instead, the data on energy components (total proteins, available carbohydrates and total lipids) can safely be considered complete [21]. Second, the method employed to assess the consumption of breast milk (frequency of feeds, perceived length of each feed by mothers) and the use of a literature-derived composition that does not take inter- and intrasubject variability into consideration, may have led to underestimation of nutrients intake. Nevertheless, we decided not to use the double weighing methodology, which is considered the most rigorous method, because we feel it is an invasive procedure that goes against the main principle of breastfeeding. The methodology we adopted in this study has been used before in other international infants cohort studies [8,10]. Finally, the results shown that a certain degree of auto-selection may have occurred among mother/child pairs who completed the food diaries at all three collection points, compared to the rest of the TIF cohort, because of the food diaries being quite lengthy and complex. That a bias might be present is suggested by the higher duration of breastfeeding (mean 12.3±8.6 months) and higher prevalence of breastfeeding at 12 months of age (39%), compared to the rates reported in previous regional and national surveys [31]. This auto-selection may have affected the rate of reduction of the sampling size by 62% (152/400). However, as already reported elsewhere, the comparison between the mothers of the TIF subcohort and the rest of the TIF group shows they shared a similar approach to infant feeding practices [17].

5. Conclusions

The data from our study demonstrate an increasing age-trend in the percentages of contribution of the main macronutrients to total energy intake, except for total lipids, during the first year of life. Most of the infants shared a low varied diet, as already reported in a previous paper [32], with energy intake from total proteins deriving mainly from selected animal sources (dairy products and meat/cured meat). This might be reflected in the unbalanced intake of fatty acids, also as a consequence of reduced breast/formula feeding, that favors saturated over polyunsaturated fatty acids that play an important role in infant neurodevelopment.

Overall, NCP contributed more, in terms of energy and macronutrients intake, compared to CP, during the CF period. However, there was a linear increase in CP consumption between 6 and 9 months of age.

Our results suggest that, in order to promote healthy eating habits during the first year of life and encourage a varied and well-balanced diet at family level, a number of public health strategies should be implemented at national level. Among these the protection, promotion and support of breastfeeding during the first year of life and the development of evidence-based guidelines on infant feeding, endorsed by all health professionals and free from commercial interests.

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over 24 h was positively correlated with the amount of flavan-3-ols consumed with the cranberry juice.
Conclusions: A high inter-individual variability in circulating and urinary metabolite levels was observed.

CAN REMOTE MONITORING BY E-MAIL BE A USEFUL SUPPORT TOOL FOR HOME MANAGEMENT OF THE KETOGENIC DIET?

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Introduction: The ketogenic diet (KD) is an effective therapeutic option for patients with drug-resistant epilepsy (DRE) and is the only therapy currently available for Glucose Transporter Type 1 Deficiency Syndrome (GLUT1-DS). Careful monitoring by the nutritional team is required, especially in the first few months of therapy, to check adherence to the diet, adjust the menus to nutritional requirements and investigate possible side effects. Nutritional and dietetic monitoring is usually conducted during control visits, but can be integrated by e-mail. The actual use of remote monitoring by e-mail has never been investigated before.
Objectives: The aim is to evaluate the use of remote monitoring by e-mail during 1 year of KD.
Methods: This is a retrospective study on 34 patients (20 GLUT1/14 DRE) with a mean age of 7.4±4.3 years, in therapy with KD for at least 1 year. All the e-mails exchanged between the nutritional team and the patient's family were evaluated, analyzing the frequency and content of the e-mails at 3-6-9 and 12 months (diet modification or suspension, ketonemia values and blood tests, side effects, drugs and supplements).
Results: 3 families never sent e-mails. 31 families have sent 43.3±29.7 e-mails/year, mainly concerning ketonemia values, diet modification, supplements, and side effects.
The GLUT1's families, have sent more e-mail that DRE's ones in relation to the diet change ($p=0.002$) and to the taking of supplements ($p=0.016$) in the first 3 months of thearpy.
The number of e-mails sent by DRE families to report the presence of side effects was double than those sent by GLUT1 families, albeit with no significant difference.
Among the main side effects reported were crisis persistence, nausea and asthenia.
The 30% of GLUT1' families used a software for self-management of the KD, which however was not decisive on the number of e-mails sent.
Conclusions: The majority of the patients analysed took advantage of remote monitoring by e-mail. Remote monitoring by e-mail use was prevalent in GLUT 1 patients' families because KD is currently the only therapy available for this disease and must be followed throughout life.

UPDATE OF "BABYFOODS" AND "SNACKS" CATEGORIES FROM THE FOOD COMPOSITION DATABASE USED FOR THE ANALYSIS OF INFANTS DIET AT 18 MONTHS OF AGE IN PHIME STUDY

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Introduction: Food Composition Databases (FCDBs) are important tools used in nutritional epidemiology to determine dietary adequacy. In particular, composition data of many foods commonly consumed during the first years of life are limited to nutritional labels.
Objectives: To describe the methodology adopted to update "baby-foods" and "snacks" categories from the FCDB used in PHIME study (Valent et al, 2013). Secondarily, to compare infants' nutritional intakes from food diaries collected at 18 months of age, using original FCDB and updated FCDB.
Methods: Food composition data were drawn from Italian Food Composition Database for Epidemiological Studies – IFCDES (BDA, 2008), from food labels and, in the case of human milk, from literature. The analysis was performed on 10 food diaries considering 29 nutrients. The missing food composition data from food labels were assessed using a standardised methodology, based on ingredients list and recipe simulation.
Results: Our work concerned 190 foods, 171 (90%) from IFCDES and 19 (10%) from food labels. The mean percentages of missing data in the original FCDB were 9% for vitamins, 7% for minerals and 10% for fatty acids. In the updated FCDB, human milk was the only item presenting missing data. The comparison of nutritional intakes between original and updated FCDBs highlighted an underestimation up to 70% for vitamins (meanly 1% for vit. C, 2% for vit. B2 and B12, and 33% for vit. E), up to 16% for minerals (meanly 6% for K, Fe and Zn, and 2% for Ca and Na) and up to 80% for fatty acids (meanly 20% for MUFA, 18% for PUFA, and 28% for linolenic acid).
Conclusions: The estimation of the missing nutrients, based on the ingredients list and recipe simulation, led to a complete FCDB. Our analysis highlighted an underestimation of fatty acids intake while the underestimation of most vitamins and minerals was negligible. Moreover, it was observed that prior the implementation of Reg. (UE) 1169/2011, food labels included more composition data concerning Ca, vit. B2, B12 and C content.

THE ENERGYKIDS PROJECT: PILOT STUDY ON THE ENERGY BALANCE OF PRIMARY SCHOOL CHILDREN DURING SCHOOL DAYS AND SUMMER CAMP DAYS

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Introduction: Specific nutritional requirements must be cover during childhood to ensure an adequate growth. The meals served in the schools of the Municipality of Parma and in the Giocampus Summer Camp are prepared considering the national guidelines for school catering, taking into account the seasonality and the variety of the served food. However, children tend to be more active during the summer camp than at school, and their energy requirements may increase.
Objectives: The objective of this study was to evaluate the energy balance during school days and summer camp days of primary school children living in the city of Parma (Italy).
Methods: Participants were asked to complete a 3-day weighed food diary and to wear an activity-tracker for the same 3 consecutive days, during both school days and summer camp days. Weight and height were measured at the beginning of each week to calculate the BMI and to define the weight status through the specific IOTF gender- and age-related cuts-offs. The mean energy balance was estimated as the difference between the mean total energy expenditure (calculated multiplying the daily physical activity level by the basal metabolic rate from the Schofield's predictive equation) and the mean daily energy intakes (obtained through the Italian food database of the European Institute of Oncology).
Results: In total 105 children (45% F and 55% M, 9.0 1.1 years) were enrolled in the study. From the analysis of the first 55 subjects, the average BMI corresponded to a normal weight status, during both periods, without significant differences between sexes. During the summer camp period, the minutes of light, moderate and vigorous activities were higher than during school days and, consequently, also the physical activity level (School: 1.5±0.8; Summer Camp: 1.7±0.2) and the daily energy expenditure (School: 1726.8±281.4; Summer Camp: 2002.8±325.6), whose increase was significant for both sexes. Although the children's diet was nutritionally balanced, the energy intake did not change significantly between the two periods. Therefore, the energy balance was negative during both periods (School: -94.1±352.4; Summer Camp: -354.1±343.6) and it changed significantly from the school to the summer camp, especially in boys.
Conclusions: These findings underline the importance of providing children with nutritionally adequate meals, suggesting that the energy

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