EARLY LIFE FACTORS AND CHILDHOOD OBESITY DEVELOPMENT

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INTERNATIONAL DOCTORAL THESIS

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UNIVERSITAT ROVIRA I VIRGILI

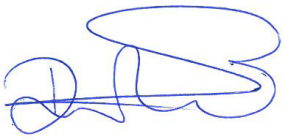
Reus, 2016

UNIVERSITAT ROVIRA I VIRGILI

I STATE that the present study, entitled "Early life factors and childhood obesity development" presented by Sílvia Fernández Barrés for the award of the degree of Doctor, has been carried out under my supervision at the Basic Medical Sciences Department of this university.

Reus, 5th September 2016

Doctoral Thesis Supervisor/s



Victoria Arija Val

Dora Romaguera Bosch

To Lluís

Acknowledgments

I would like to thank all the people who helped and gave me support on my PhD during the last 3 years and contributed in some way to this thesis.

First, I would like to thank Dr Victoria Arija and Dr Dora Romaguera for being my thesis directors and guide me through this hard work.
To Dr Victoria Arija for giving me the opportunity to start my research and teaching career in her Unit and for being a supportive mentor and trust on me. To Dr Dora Romaguera for giving me the chance to collaborate with CREAL, for showing me how interesting and exciting nutritional epidemiology can be, and for her kindness, support and patience.
This doctoral thesis would not have been possible without economic support. I would like to thank the Universitat Rovira I Virgili for my PhD and mobility grant. Furthermore, I would like to thank all the funding institutions, the staff of the INMA project and Project Viva and all the families who made our research possible.

During my PhD I had the great opportunity to work in 3 research groups:

Universitat Rovira i Virgili

Thanks to all my current and former colleagues of our research group Nutrition and Mental Health (NUTRISAM) lead by Dr Victoria Arija and Dr Josepa Canals: Amal, Carla, Carmen, Carmina, Eva, Felipe, Gemma, Irene, Lucía, Magally, Marcela, Núria A, Núria V, Patrícia, Paula, Sabina, Sílvia and Vicky. All of them contributed to my teaching and research experience. They were always willing to share their knowledge, and also coffees and good moments.
Special mention for my NUTRISAM colleagues who have become some of my closest friends and they gave me their professional and personal support even from distance during the most stressful period of my thesis: Estefania Aparicio, Cristina Bedmar, José Cándido Fernández, Cristina Jardí and Blanca Ribot.

Despite my research group, I would like to express my gratitude to other colleagues: my "lunch time" colleagues in the *ground floor* (Alba, Eli, Lucia and Maga) and the ones in the *second floor* (Anna, Laura, Rosa Mari and Sara). To my colleagues during my years in the dean's office (including Rosa Albadalejo).

Finally, the ones who were my classmates and are now good friends: Cristina, Mònica, Patrícia and Santi.

ISGLOBAL CREAL

I am sincerely grateful to Dr Jordi Sunyer and Dr Martine Vrijheid for giving me the opportunity to collaborate in the INMA project and treat me like a member of their team. They are both excellent researchers, I always learned from their comments and suggestions; in fact, being part of CREAL is a huge learning opportunity. Thanks to all the member of CREAL, especially to my "working team" who contributed directly to my research and to this thesis: Cyntia, Dania, David, Glòria, Jordi, Lourdes C, Mònica, Susana, and special mention to Dora Romaguera who has led my research and has been a source of inspiration and motivation.
To my closest colleagues, the ones with whom I shared the volley beach party and the biking trips: Albert, Anna, Ariadna, Ceci, Gabi, José, Judit C, Judit G, Lourdes A, Lourdes C, Marta, Mònica, Montse, Patty, Tania, and the rest of the *ground floor*.
To my CREAL friends who were incredible patient in the last months, and with whom I share laughs and gossips: Glòria and Mariona.

Harvard Pilgrim Health Care Institute / Harvard Medical School

I am extremely grateful to Dr Matthew W Gillman for his guidance and for being one of the best mentors I have ever had, for being a kind and good listener, and for our fruitful discussions.
I would like to express my gratitude to Dr Emily Oken for giving me the opportunity to stay at their research group, and also for her valuables contributions and her immense knowledge. Thanks to all the members of the Obesity Prevention Program and Project Viva. I would like to thank Sheryl Rifas-Barba for her contribution to my work, and for being so efficient and helpful. I would like also to thank Julie McGrath for making everything possible and caring about me like a mother when I was in Boston. To Dania Valvi to trust on me, facilitate my stay and becoming a good neighbor and friend.
To my "special" colleagues who became my lunch time friends: Fuying, Maria, Milly, Mitzi and Sandra.

From other Harvard departments, hospitals and MIT, to the ones that made my stay in Boston a wonderful and unforgettable experience: Aurore, Francesca, Lucia, Lukas, Marta and Jordi, Mathieu, Natalia, Sandra and Charlotte and the French troupe.
To Darko and Jason for making me feel at home, and sharing laughs and stimulating chats with me.

Despite the members of the academia, I would like to thank my beloved ones for their support.
I am sincerely grateful to my parents, Félix and Conchita, for working really hard to allow their children to have better chances in life. "Muchísimas gracias por todo, estoy orgullosa de vosotros y os quiero".
Thanks to Carlos, my little brother, for his support and company, especially during the last lonely days. "Pesadilla, te quiero!". To my other siblings (Alícia and Félix), nieces (Yvette, Clara and Martina), and other members of the family (including Anastasia, my godparents, Leo, Nette, Otto, Rustik, Kira and Chico).
To Lluís, who has "suffered" this thesis process as much as me, and has been supportive and patience. He has always been there for the last 8 year, and shares my project of life. "Em sento tan afortunada de tenir-te al meu costat. A partir d'ara nova vida… i alabanza, amor, … T'amoro molt!!!".
To my family in law, for being so supportive in the hard moments, especially to Anna and Pere "Gràcies per aquest immens amor que despreneu i per fer-me sentir com una filla!". To Isa and Jesus who helped me so much when I decided to move to Boston.
To my best friends who are always there no matter what, Lourdes, Meri and Natalia. Without them, I would not have the strength to keep going.
To all my friends Montse, Irene, Elena, Luis, Claudia, Rachida, Mar, Mireia, Eric, Maria, Eva, Coco, Eva, Víctor, Meri, Lluís, Puça, Bea and my goddaughter Caterina.
To my "Icelandic" friends who after 10 years are still there, especially Paolo and Celia who have shared important moments of my life, an even from far away I feel them very close. To my friends from Mexico and Australia, especially to Ashanti and Lulu.

To all of you, thanks

Barcelona, 5th September 2016

ABSTRACT

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Background: Childhood obesity has become a major public health problem because its prevalence is increasing in the entire world and it is associated with adulthood obesity and other chronic diseases. As childhood obesity may start in prenatal periods, identifying modifiable early life risk factors could help prevent development of childhood obesity. Early prevention is crucial, because once obesity is established it is harder to reverse it.

Main objective: The main objective of this thesis was to investigate the prospective association between potentially modifiable early life factors in pregnancy and infancy, and offspring development of childhood obesity.

Methodology: We used data from two birth cohort studies, the INMA project (Spain) and Project Viva (USA). We included women at their 1st prenatal visit and follow them and their offspring until the 7-year visit.
The prenatal risk factors included were maternal smoking in pregnancy, maternal sugar-sweetened beverage intake in pregnancy, adherence to the Mediterranean diet (measured using relative Mediterranean Diet score (rMED)) in pregnancy, gestational weight gain and gestational diabetes. We also included perinatal and postnatal risk factors: cesarean section, infant breastfeeding duration, age at introduction of solid foods, sleep duration in infancy and infant rapid weight gain in the first 6 months. The outcomes evaluated at 4 years were: age- and sex-specific body mass index (BMI) z-scores, waist circumference, risk of overweight and abdominal obesity, cardiometabolic risk score (including HDL, triglycerides, systolic and diastolic blood pressure and waist circumference), lipid score (including HDL, triglycerides and BMI z-score) and other age, sex and cohort specific biomarkers: leptin, C-peptide, adiponectin, Apo A-1, Apo B, C-reactive protein and interleukin 6. We also assessed the longitudinal child growth trajectories from birth to 4 years. At 7-year assessment, the outcomes were BMI z-scores, waist-to-height ratio (WHtR), and fat mass index and fat-free mass Index.

Multivariable linear, logistic and multinomial analyses were performed to assess the association of modifiable early-life risk factors and childhood obesity

development. In addition, we used prediction models to assess to what extent the combination of early-life risk factors were associated with childhood obesity.

Results: Excessive gestational weight gain, short infant sleep duration and rapid infant weight gain were associated with offspring BMI z-score in both cohorts. Gestational diabetes mellitus and rapid infant weight gain were associated with offspring WHtR at age 7.
Rapid infant weight gain from birth to 6 months was the strongest early life risk factor of BMI, WHtR, fat mass index and fat-free mass index with estimates ranging from 0.27 to 1.57.
Prediction models suggested that only 6-7 of the 9 prenatal and postnatal risk factors of childhood obesity in the USA cohort also predicted adiposity in the Spanish setting. Discrepancies were found in cesarean section, sugar-sweetened beverage intake, short breastfeeding duration and early introduction of solid foods.
Maternal adherence to the Mediterranean Diet during pregnancy was not associated with BMI z-score at 4 years, but was inversely associated with offspring waist circumference (β: -0.57; 95%CI:-1.07, -0.07; p for trend = 0.024), a marker of abdominal obesity.
Maternal rMED showed no association with cardiometabolic risk, with blood pressure or other biomarkers at 4 years of age. However, rMED was positively associated with Apo A-1 (β 0.30; 95%CI: 0.08, 0.51; p for trend = 0.007).
Maternal rMED at 3rd trimester was associated with lower risk of developing a detrimental longitudinal growth pattern in childhood (Relative Risk 0.65; 95%CI: 0.43, 0.98; p for trend = 0.055), characterized by higher birth size and accelerated growth compared with the reference growth pattern (average birth size and slower growth).

Conclusions: During the prenatal and postnatal periods, modifiable early life risk factors play an important role in the development of childhood obesity. According to our findings, the combination of modifiable risk factors that predicted higher obesity differed across settings and populations. However, rapid infant weight gain was a common risk factor for general and abdominal obesity in childhood. Another of the risk factors studied, the Mediterranean Diet during pregnancy, may have a protective effect on childhood health because it decreased the risk of higher birth size and accelerated growth, and waist circumference. It also increased the circulating Apo A-1 in 4-year-old children. However, this dietary

pattern did not show an association with body mass index, cardiometabolic risk, or other biomarkers.

Early life interventions focused on pregnant women, including modifiable risk factors, may be effective to prevent the development of childhood obesity. Further research in this field is needed with a longer follow-up and direct measures of adiposity.

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ABBREVIATIONS

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In alphabetical order

aMED	Alternate Mediterranean Diet Score
Apo A-1	Apolipoprotein A-1
Apo B	Apolipoprotein B
BMI	Body Mass Index
CDC	Centers for Diseases Control and Prevention
CRP	C-reactive protein
DOHaD	Developmental Origins of Health and Disease
DXA	Dual-energy X-ray absorptiometry
FMI	Fat Mass Index
FFMI	Fat-Free Mass Index
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment index
IDF	International Diabetes Federation
IGF-1	Insulin-like growth factor 1
IL-6	Interleukin 6
IOM	Institute of Medicine
IOTF	International Obesity Task Force
LDL	Low-density lipoprotein
LGA	Large for gestational age
OR	Odds Ratio
PUFA	Polyunsaturated fatty acid
METS	Metabolic equivalents
rMED	relative Mediterranean diet Score
RR	Relative Risk
SD	Standard Deviation
SFA	Saturated fatty acids
SGA	Small for gestational age
SMD	Standardized mean difference
SSB	Sugar-sweetened beverages
TG	Triglycerides
T2DM	Type 2 diabetes mellitus
WHO	World Health Organization
WHtR	Waist-to-height ratio

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INTRODUCTION

1. INTRODUCTION

1.1 Epidemiology of childhood obesity

Obesity is defined as a chronic disorder and represents an abnormal or excessive fat accumulation that can impair health [1].
Obesity is a complex disorder, with several causes, and it was often attributed to genetic causes and the imbalance between the energy intake and the energy expended with physical activity. However, it has been lately considered caused by a combination of exposure to an obesogenic environment and inadequate biological and behavioral responses to that obesogenic environment [2, 3].

Childhood overweight and obesity has been increasing dramatically in the last decades, and even though the rise in the prevalence seems to be slowing down in the last years, obesity has become a serious public health burden in developed and developing countries. According to the World Health Organization (WHO) in 2014, more than 1.9 billion adults and 41 million children under 5 years old were overweight (including obesity) [4]. If the current trend continues, the expected prevalence of overweight or obesity for children under 5 years old will be 70 million for 2025 [4].

Childhood obesity has a big impact on the quality of life of children, on their physical and mental health, given that obesity causes gastrointestinal, musculoskeletal, cardiovascular, metabolic and orthopedic morbidities and also mental disorders such as depression, eating disorders, and social problems and stigmatization [4, 5]. Furthermore, obesity generates significant direct and indirect costs, for the individual, the family and the whole society [6].

Some authors are starting to identify the metabolic syndrome as a pediatric condition, when it has been traditionally considered an exclusive condition of the adulthood. This syndrome is characterized by the presence of glucose intolerance, insulin resistance, dyslipidemia, central obesity and hypertension. A study conducted by Friend et al. estimated a prevalence of metabolic syndrome in childhood of 3.3%, and this prevalence was higher in overweight children (11.9%) and obese children (29.2%) [7].

The increase of the prevalence of childhood obesity and the metabolic syndrome is likely to be the cause of the 30% increase of type 2 diabetes prevalence among children and youth in the last 2 decades, disease that was rarely seen in children before [8, 9].

Moreover, childhood obesity represents a significant predictor of later obesity, because childhood obesity tracks to adolescence and then into adulthood [10, 11]. Childhood obesity is also a predictor of other disorders and diseases in adulthood, such as hypertension, dyslipidemia, insulin resistance and type 2 diabetes, asthma, musculoskeletal disorders, some cancer and cardiovascular disease (mostly coronary heart disease and stroke), and premature death [4, 5, 12-16].

1.2 Measures and definition of childhood growth, obesity and metabolic syndrome

In the following sections, we present the measures and definitions of childhood growth, childhood obesity and metabolic syndrome.

1.2.1 Childhood growth

Two periods of childhood growth are differentiated, the prenatal and postnatal period.

In the prenatal period, fetal growth is measured usually by ultrasound methods, and biometric measurements are taken and compared to expected measures for gestational age. These methods depend of an accurate pregnancy dating.

To detect abnormal fetal growth, estimated fetal weight or abdominal circumference are commonly used and values below to a certain percentile (for instance 5^{th} or 10^{th}) are considered as indicators of "fetal growth restriction". There are other index and methods, like estimated fetal ponderal index, or 3D ultrasonopgraphy, but biometry remains the most common method and the gold standard [17].

In the postnatal period there are 2 periods of interest for childhood growth, at birth and during the first months of life.

During decades, birth weight has attracted most of the attention, this is the most common measure at birth and it is usually correlated with the birth weight of the mother.

Birth weight can vary between populations, in Spain the average birth weight in boys at term is 3,300.41 (Standard Deviation (SD) 396.85) g and in girls 3,185.00 (SD 372.08) g, whereas in UK the average birth weight among white infants was 3,420 g in the Millennium Cohort [18, 19].

Furthermore, birth weight may vary due to ethnic reasons, maternal height, weight and age and also due to other modifiable factors, such as smoking during pregnancy. There are also variations due to the season of delivery; the babies born in autumn are heaviest and the ones in winter are lightest.

There are different classifications of babies depending of their birth weight (Table 1).

Table 1. Classification system for describing infant size at birth

Classification	Birth weight
Normal birth weight	2500-4000 g
Low birth weight	< 2500 g at term
High birth weight	> 4000 g at term
Appropriate for gestational age	> 10^{th} and < 90^{th} centiles for gestational age
Small for gestational age (SGA)	<10^{th} for gestational age
Intrauterine growth restriction	< 2 SD for gestational age
Large for gestational age (LGA)	> 90^{th} or >97^{th} for gestational age
Macrosomia	> 4000 g or > 90^{th}
Adapted from McArdle et al. [20]	

As observed in Table 1, some of the definitions do not have into account the gestational age at birth, such as the WHO definition of low birth weight (< 2500 g). That may be a problem, due to the heterogeneity of the definition because it includes babies born preterm and babies at term with small for gestational age (SGA). It is recommendable to use birth weight for gestational age [17]. In this sense there are growth charts available, international and national, that have into account gestational age. From these growth charts, percentiles can be used. In the table we included definitions that used the percentile 10^{th}, but some authors use other percentiles (for instance the 3^{rd} and the 5^{th}).

There international fetal growth standards were created with information at birth of healthy children by the WHO, based on data from 6 different countries [21]. There are also national growth reference, for instance in Spain, that are based in a cross

sectional study including 9362 babies born between the 26th and the 42nd weeks of gestation [18, 22].

Lower birth weight occurs usually due to inadequate intrauterine conditions that lead to abnormal fetal development.

On the other hand, higher birth weight usually indicates a more favorable environment *in utero*, except in some cases, like macrosomic babies born of diabetic mothers [23].

Birth weight may not be the best method to assess adiposity in the neonate, because with the same birth weight different populations may differ in their percentage of fat mass and fat-free mass. Therefore, birth weight is considered as a crude estimate of adiposity in children. For this reason some researches use other measures of adiposity at birth, like abdominal, chest and thigh circumferences, and skinfold thickness [24-26]. Some studies include a direct measure of adiposity, such as air displacement plethysmography [27, 28].

Measures of birth size have been widely used as a surrogate of nutritional environment and the conditions experienced *in utero*, especially birth weight. It has usually been considered as an outcome for assessment of several factors during pregnancy, such as gestational diabetes. Some authors suggest that birth weight may be a mediator between pregnancy and childhood obesity. It has been considered also a surrogate of obesity and also a risk factor of later cardiovascular disease (for example the works by Barker et al.). The role of birth weight as a risk factor of childhood obesity will be discussed in the section 1.6.2.

After birth, there is a period called infancy (from birth to 24 months) considered of rapid growth compared with other periods of life. The growth involves length, weight gain and also brain growth (and other physiological processes and organ growth), but not all the babies develop at the same pace.

Linear growth is influenced mostly by genetic reasons, for instance height is determined by the gens, with a small variation due to nutrition and disease. During infancy, body length increases steadily to reach about the 75% of half the final adult height [29]. However, weight increases above the 2.5-fold, and this represents approximately the 20% of the final adult weight. The brain size increases from 25% at birth to reach the 80% of adult brain size [30].

Growth during infancy also implies an increment of body fat; babies have around 15% of body fat at birth, increasing up to 25% at 6 months and 30% at 12 months [31].

If this growth is restricted (in weight or/and length) or it is excessive is considered of concern, because it may lead to health consequences in later life (see section 1.6.3). Regarding the monitoring of infancy growth, growth charts are used. The rate and pattern of growth over time is more relevant that the exact weight or height at a particular time. The pattern is expected to be steady following a line curving in the same way, and within the centile lines in the chart.

As mentioned before, it is of interest to detect the presence of excessive infant weight gain (also called "catch-up"), for this purpose, weight-for-length is an adequate measurement. Furthermore, the Centers for Diseases Control and Prevention (CDC) 2000 growth standards and the WHO growth standards (more detail about growth charts in the following section) can be used to assess the linear and ponderal growth. Experts discourage the use of 'overweight' and/or 'obesity' in this age [32].

Instead of centiles, age and sex- specific z-scores can also be used. The z-score is an index that is calculated as it follows:

$$Z - score = \frac{(observed\ values) - (median\ reference\ value\ of\ a\ population)}{Standard\ deviation\ of\ a\ reference\ \ population}$$

A z-score of 0 is equivalent to the median (or 50th centile); a z-score of +1.00 SD is approximately equivalent to the 84th percentile; a z-score of +2.00 SD to the 98th percentile and a z-score +2.85 SD to >99th percentile [33].

Other measures of rapid growth used in infancy are the change of z-score of weight-for-age and height-for-age during a period of time. In this sense, there is a lack of consensus of the definition of rapid growth, but several authors adopted the definition of rapid growth in infancy suggested by Ong et al, that considers rapid growth as a gain in weight greater of 0.67 z-score between two separate evaluations [34].

1.2.2 Obesity

Obesity is defined as the accumulation and storage of excess body fat that can impair health [1, 35]. In the following sections we differentiate between general obesity and central obesity.

1.2.1.1 General obesity

Body Mass Index (BMI, kg/m^2), also called Quetelet's index, is commonly used as a measure of excessive body fat in clinical and epidemiological settings. This classification based on weight and height is used because of the lack of a consensus criterion to define childhood obesity based on an excessive body adipose tissue and also for its simplicity.

Body composition changes with normal growth. The BMI usually decreases after birth, and it is followed by a rapid increase during the first year of life. Then, the BMI decreases until five to seven years of age, where it reached the minimum, and then increases gradually until adolescence and adulthood [36]. The decrease of BMI also is a decrease of subcutaneous fat and percentage of body fat. This second period when the BMI curve increase is called adiposity rebound [32].

In adults (>18 years old) BMI is categorized based on international standards cut-offs that are age- and sex- independent (table 2) [37].

Table 2. International classification of Body Mass Index (BMI) in adults according to World Health Organization

Classification	BMI (kg/m^2)
Underweight	<18.50
Normal weight	18.50-24.99
Overweight	≥25.00
Obese	≥30.00

In children, BMI distribution changes with age and sex; therefore, age- and sex-specific values of BMI (percentiles and z-scores) are used, rather than raw values of BMI [35].

There are several reference standards for evaluating childhood BMI and different cut offs for classification of BMI into 'normal weight', 'overweight' and 'obesity'. These definitions are based on anthropometric measurements for practical reasons.

The US **Centers for Diseases Control and Prevention (CDC)** developed growth charts with specific age- and sex- references for ages 2 to 18. These growth charts were not developed as health-related standards; instead, these are percentiles developed based on data from nationally representative surveys conducted in United States between 1963 and 1980. These growth charts are the most commonly used in United States. The cut-offs to classify overweight and obesity are ≥85th and ≥95th percentiles, respectively. Before 2010, the cut offs were ≥85th 'at risk of overweight' and ≥95th 'overweight'. Currently the CDC recommends the use of the WHO growth standards for infants and children from 0 to 2 years, and the CDC growth charts from 2 to 18 years for the American population [38].

In other countries, the standards of the **World Health Organization (WHO)** are more common. Previously the WHO recommended the use internationally of the CDC reference growth charts (1995); between 1997 and 2003 a study of approximately 8500 children in different locations with different ethnic backgrounds was conducted (Brazil, Ghana, India, Norway, Oman and the USA). These growth standards provided international standards for all children from 0 to 5 years old and have become a model for growth, since they are based on a sample of healthy breastfed children [21]. Since their release many countries have implemented them [39]. The cut-offs for obesity classification are based on sex and age-specific centiles (overweight > 85th percentile and obesity >95th percentile) and also on standard deviations: BMI > 2SD (overweight) and BMI > 3SD (obesity) [40].

The WHO recommends the use of the WHO growth reference 2007 for children from 5 to 19 years old, which are a reconstruction of the 1977 CDC/WHO references. They are the original values supplemented with data from the WHO standards (from 0 to 5 years). The definition of overweight and obesity according these reference are overweight (BMI > 1SD) and obesity (BMI>2 SD) [41].

In 1999, an expert committee of the **International Obesity Task Force (IOTF)** recommended the use of BMI to define overweight and obesity. Due to the lack of a specific cut-off in children related to an increase health risk, this committee recommended age- specific cut-offs based on the adult cut-offs (≥25 kg/m^2 for overweight and ≥30 kg/m^2 for obesity). Cole et al. used data from 6 reference populations (Brazil, Great Britain, Netherlands, Hong Kong, Singapore and the USA) and derived percentile curves that pass through the points of 25 and 30 kg/m^2 at age 18 and centiles and z-scores are also available [40]. These references are

international; however, some authors argue that they not represent adequately the non-Western populations.

Some countries, including UK, France, and Denmark have developed their own age- and sex- specific BMI reference charts using local data [42-45].

In Spain, there are also specific growth curves and cut-off points; these were published by the **Faustino Orbegozo Foundation**. They consist in 3 different tables, the first was by Hernandez et al. (1988), and included children from 6 months to 9 years of age. The cut-offs to establish overweight and obesity were ≥90-91th and ≥97-98th percentiles, respectively. In 2004, Sobradillo et. used data from 2 studies, and proposed the percentile 85th for overweight and the 95th for obesity. Few years after (2008), Carrascosa et al. used data from a greater sample size (approximately 32,000 children and young adults from birth to 24 years). In the latest tables, they used the following cut-offs: overweight (>85th percentile) and obesity (>90th percentile) [18]. In 2011, they included the definition of overweight and obesity following the methodology proposed by IOTF, the new cut-offs are based on the curves that pass through an equivalent BMI of 25 and 30 at 18 years of age, specific for sex [22].

Some researchers have compared the prevalence of overweight and obesity when using different growth charts and cut-offs. When assessing overweight, the prevalence are similar, for the prevalence of obesity, the IOTF gives lower prevalence than the use of CDC [33]. One study conducted in Canada reported 19% of overweight/obesity among boys between 2 and 5 years old when using the IOTF reference, whereas the prevalence was 37% with the WHO definition [46]. Another study compared the prevalence of childhood overweight and obesity using French national reference, the WHO standards and the IOTF, and found that there were a moderate agreement between the 3 references, but the WHO reference reported higher prevalence [47]. To face this disparity, more authors include the prevalence with both international standards in their works.

There are also some limitations on the use of BMI charts, because in some cases the charts are derived from a single population, and when using them there is the assumption that the individual is comparable to the reference population. Another limitation is to identify the charts as a representation of an ideal population, and not a reference population. This may be particularly problematic if in order to

develop the growth charts, the population of reference chosen has a high prevalence of obesity [33].

BMI has been validated against other methods of direct measurement of body adiposity and, although it is not an ideal method, it is recommended in the use of screening for childhood and adolescent obesity [33].

BMI is a simple measurement to identify overweight or obese children, but it does not identify children with abdominal obesity. Also it has its limitations to identify body fat, and discriminate between lean and fat mass. For example within the same category of BMI there is a trend for people to have more adiposity and less lean mass than previous generations [4, 48], and also BMI may reflect different body fat content in different racial and ethnic groups. This measure shows some limitations, and although excess body fatness cannot be measured directly from weight and height, and BMI may be particularly inaccurate for children with other ethnic characteristics like Asian (for the given body fat composition there may be difference in BMI), there is a high correlation between fat mass and BMI among children, and BMI is recommended as a first screening tool.

Other indirect methods to assess adiposity are weight-for-height and skin-fold thickness. Weight-for-height is commonly used to assess undernutrition in developing countries, and this measure is not useful in adolescents. Even though it has into account weight and height as BMI, an expert committee (with members from the American Medical Association, the CDC, and the Maternal and Child Health Bureau from US government) recommends the use of BMI over weight-for-height, because BMI correlates better with body fat with acceptable accuracy.

To assess adiposity, the use of skin-fold thickness is more common. There are different skin-folds (such as triceps and subscapular), and from them we can estimate fat mass and percentage of fat mass with prediction equations. This method has been widely used because it is noninvasive and can measure subcutaneous fat [32]. However, one of the limitations of this method is related to the equations used, since these equations need to be validated for different age, sex and ethnic groups. Furthermore, it needs a trained technician, and the intra and inter-observer reliability is low [33, 49].

There are other methods to measure adiposity, which are direct: Dual-energy X-ray absorptiometry (DXA), computerized tomography, bioelectrical impedance analyses and magnetic resonance imaging.

We can derive Fat Mass Index (FMI) and Fat-Free Mass Index (FFMI) from DXA. This method is based on the principle that transmitted X-rays are differentiated by bone tissue and soft tissue. With the use of equations the soft tissue can be divided in fat and lean tissue. Although DXA is an expensive method and needs an experienced technician, it is preferred than the computerized tomography because delivers lower radiation.

Computerized tomography uses X-ray, and allows the identification of total, abdominal and subcutaneous body fat and the calculation of percentage body fat mass. As mentioned before, this method deliver radiation, and it is not suitable for children.

A less invasive method is bioelectrical impedance. This method is based on the conductivity, and assumes that it reflects the fat-free mass. With the use of equations, fat mass and fat-free mass can be calculated. This method has some limitations, there are different equations used, it relies on hydration status and it may vary with different ethnic status [33].

Few studies use magnetic resonance imaging because total body fat, fat mass, and percentage fat mass can be estimated. This method can differentiate abdominal and subcutaneous fat. However, this method is very expensive and it is not adequate for younger children, because the child need to lie still for a prolonged time.

Other methods exist but are less common and/or not adequate for children, because they take time and need the cooperation of the child, such as hydro-densitometry and air-displacement plethysmography.

1.2.2.2 Abdominal obesity

Central obesity is also called central or visceral obesity, and it is characterized by the presence of excessive abdominal fat around the stomach and abdomen.

There are different indirect measurements to evaluate the presence of abdominal obesity. The most common is waist circumference (in cm or inches), but there are others like waist-to-height ratio (waist circumference in cm/height in cm), and waist to hip ratio (waist circumference in cm/hip in cm).

Waist circumference is measured at the minimum circumference between the iliac crest and the rib cage using a non elastic anthropometric tape. It an easy, non expensive with good reliability and validity method commonly used in clinical

practice, and in epidemiology. There is a lack of consensus about cut-offs to classify the children at low or high risk of abdominal obesity according their waist circumference. There are national reference charts developed for waist circumference classification (in the UK), but non international charts or cut-offs. Most of the researchers use internal sex–specific percentiles to dichotomize their population at risk and without risk, for example the 90th percentile of the sample [33].

Some authors suggest that waist circumference depends not only on the sex, but also on the ethnicity and height, and for this reason the waist-to-height ratio was proposed for adults and children. A cut-off of 0.50 seems to discriminate the adults with central obesity, independently of their sex, ethnicity or height. In children, this cut-off has been proposed, but has some limitations, and its capacity to identify children with higher cardiovascular risk is not clear [50].

Another measure proposed is waist-to-hip ratio. But some authors argue that the use of this ratio may not be appropriate because it is age dependent, and may attenuated some of the associations that are stronger with waist and hip circumference separately [51].

A study conducted in children aged 3-19 years that evaluated and compared the use of these measurements as screening tools for fat mass, found that waist circumference is a good screening tool of central obesity [51]. When comparing these tools with direct measures of abdominal adiposity, waist circumference is more correlated with abdominal fat, than waist-to-hip ratio [33]. However, a study conducted in 2339 children and adolescents (from 8 to 19 years) from USA found that waist-to-height ratio was a better predictor of adiposity than BMI or waist circumference [52].

When comparing with BMI some studies show that waist circumference in children provides a better estimate of visceral adipose tissue, and also that it is more efficient in its ability to predict insulin resistance, blood pressure, cholesterol and triglycerides levels [32].

Other direct methods exist that are able to identify central obesity, such as computerized tomography and magnetic resonance imaging. These methods are described in the previous section (1.2.2.1). These methods are considered the gold standard for assessing central fat distribution in adults, but they are expensive, need the collaboration of the participant and computer tomography involve radiation, therefore they are not adequate for young children [51].

DXA is able to measure trunk fat mass, and it has a strong correlation with abdominal fat measured with the previous mentioned methods. Some authors used trunk fat mass as an estimate of abdominal obesity [51].

Central obesity is highly correlated with cardiovascular biomarkers, adverse lipoprotein concentrations and blood pressure, even in childhood and adolescence. Visceral obesity is also correlated with hyperinsulinemia in children [33]. When comparing BMI and waist circumference, the latest is more efficient in predicting insulin resistance, blood pressure, serum cholesterol and triglycerides [53-55]. However, one study conducted in Spain that found similar ability of BMI, waist circumference and skinfold thickness to identify children with metabolic syndrome [56].

In this sense, abdominal obesity is considered one of the earliest signs of metabolic dysregulation. If abdominal adiposity appears early in childhood (before 10 years of age) is strongly associated with the development of glucose intolerance and dyslipidemia and later risk of developing type 2 diabetes mellitus [57]. The mechanism behind may be related to the proinflammatory status conferred by excess visceral adipose tissue. This process seems to be involved in the development of insulin resistance [58].

1.2.3 Metabolic syndrome

Childhood overweight and obesity may be relevant promoters of independent risk factors for cardiovascular disease, as recently shown in a study published in the *New England Journal of Medicine* [5]. Some researchers suggest that beyond general obesity, the increase body fat percentage or central obesity may play an important role in increasing the prevalence of the metabolic syndrome cardio-metabolic biomarkers.
In this section a selection of the cardio-metabolic biomarkers related to obesity and the metabolic syndrome is presented:

1.2.3.1 Dyslipidemia

Dyslipidemia is defined as an abnormal amount of lipids in the blood. The lipid fraction is constituted mostly by triglycerides, cholesterol and lipoproteins. The lipoproteins more common are low-density lipoprotein (LDL), very low-density lipoprotein and high-density lipoprotein (HDL) [59]. The increase levels of these lipids are considered hyperlipidemia, this can be due for an increase of cholesterol

(hypercholesterolemia), triglycerides (hypertriglyceridemia) or lipoproteins (hyperlipoproteinemia (usually LDL)). Low levels of HDL are considered also dyslipidemia. In adults there are standards cut-offs, but there is not agreement in children and adolescents. Some guidelines recommend 10 years of age, as a stable time for lipid assessment, because during puberty there is a normal decrease of triglycerides and LDL cholesterol (about 10%) [60].

Values for plasma lipids and lipoproteins according the National Cholesterol Education Program Expert Panel on Cholesterol Levels in Children are presented in the table 3 [60].

Table 3. Plasma lipid, lipoprotein and apolipoprotein concentrations for children and adolescents

Note: Values given are in mg/dL. To convert to SI units, divide the results for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and non-HDL-C by 38.6; for triglycerides (TG), divide by 88.6.

Category	Acceptable	Borderline	High+
TC	< 170	170-199	≥ 200
LDL-C	< 110	110-129	≥ 130
Non-HDL-C	< 120	120-144	≥ 145
ApoB	< 90	90-109	≥ 110
TG			
0-9 years	< 75	75-99	≥ 100
10-19 years	< 90	90-129	≥ 130

Category	Acceptable	Borderline	Low+
HDL-C	> 45	40-45	< 40
ApoA-1	> 120	115-120	< 115

* Values for plasma lipid and lipoprotein levels are from the National Cholesterol Education Program (NCEP) Expert Panel on Cholesterol Levels in Children. Non-HDL-C values from the Bogalusa Heart Study are equivalent to the NCEP Pediatric Panel cutpoints for LDL-C. Values for plasma ApoB and ApoA-1 are from the National Health and Nutrition Examination Survey III.

\+ The cutpoints for high and borderline high represent approximately the 95th and 75th%iles, respectively. Low cutpoints for HDL-C and ApoA-1 represent approximately the 10th%ile.

Adapted from Expert panel on Cholesterol [60]

Overweight and obese children have more risk of having dyslipidemia, especially if they have a high body fat percentage or central obesity [36]. This phenotype usually presents high triglyceride levels and low HDL levels. In some cases also present a normal-to-mild elevation in LDL cholesterol level.

Moreover, elevated lipid levels tracks from childhood and adulthood, and also some studies have found relationship between LDL cholesterol and apolipoproteins measured in children and youths with measures of atherosclerosis in adulthood.

The atherosclerosis is a process that begins with the accumulation of abnormal lipids in the vascular intima, and can lead to thrombosis, vascular rupture or acute ischemic syndromes [60].

Other lipoproteins are measured but some studies suggest that the screening of apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) not give additional advantage, comparing with cholesterol HDL, LDL and triglycerides screening [60, 61]. However, other studies suggested that are good indicators of cardiovascular disease in adults [62].

1.2.3.2 Blood pressure

Some epidemiological studies show that the levels of blood pressure have increased in the last 20 years, and also the prevalence of hypertension among children and adolescents [60]. This increase could be explained partially by the increase of obesity prevalence, since obese children are three times more likely to have hypertension when compared to non-obese children. Furthermore, prehypertension and hypertension in childhood usually tracks into adulthood [36, 60].

For the screening of high blood pressure, an expert committee suggested an algorithm based on age, sex and height, and the presence of other risk factors. The most common practice is the definition of high blood pressure when is above of a specific percentile, for example above of the 95th percentile of the reference tables for age, sex and height [5, 63]. In some epidemiological studies, they used intern cut-offs instead of reference tables.

1.2.3.3 Glucose homeostasis

Higher BMI is associated with higher insulin levels as seen in the Bogalusa Heart Study [64]. Hyperinsulinemia and insulin resistance may lead to Type 2 diabetes mellitus (T2DM) [36].

T2DM is characterized by hyperglycemia caused by insulin resistance and/or defects in insulin secretion. T2DM is a risk factor for early cardiovascular disease. There is an increasing prevalence among children and adolescents, usually asymptomatic and with mild-to-moderate hyperglycemia [60].

The American Diabetes Association recommended screening children for T2DM and Prediabetes when they are overweight and have 2 or more risk factors such as family history of T2DM, maternal history of gestational diabetes mellitus or signs of

insulin resistance and when they are older than 10 years old (or at onset of puberty if occurs at a younger age) [65, 66].

There are various methods used in children to define disturbed glucose metabolism such as the oral glucose tolerance test, with a good reliability when compared with intravenous glucose tolerance test, and more commonly the fasting insulin level, the fasting glucose/insulin ratio and the quantitative insulin-sensitivity check index. Some studies use the glycated hemoglobin levels and the fasting glucose to consider the children at risk of diabetes. The American Diabetes Association recommends the following definitions for abnormal values >5.7% for glycated hemoglobin and ≥5.6 mmol per liter for fasting glucose [67]. One of the methods more used in pediatric population with a high reliability is the HOMA-IR index (homeostasis model assessment), this is a validated formula that gives an estimate of insulin resistance, following this equation: HOMA−IR=Fasting insulin (μU/mL) x Fasting glucose (mg/dL) /405 [68].

1.2.3.4 Definition of metabolic syndrome

Usually the metabolic syndrome definition is the clustering of several risk factors, some of them previously mentioned in this section: dyslipidemia, high blood pressure and disturbed glucose metabolism. Obesity, and especially abdominal obesity, is also one of the considered items of the metabolic syndrome. This syndrome increases the risk of cardiovascular disease and type 2 diabetes mellitus, also when the metabolic syndrome is present in childhood, as shown by some longitudinal studies with a follow-up of 25 years [69].

The definition and criteria of the included items differed according different organizations, for adults there are these main definitions shown in Table 4.

Table 4. Components of the metabolic syndrome in adults

	WHO	IDF	ATTPIII	AACE
Triglycerides ≥ 150 mg/dL	X	X	X	X
HDL < 40 mg/dL in men and < 50 mg/dL in women	X	X	X	X
Blood pressure > 130/85 mmHg	X	X	X	X
Insulin resistance (IR)	X			
Fasting glucose > 100 mg/dL		X	X	X
Glucose 2 hours: 140 mg/dL				X
Abdominal adiposity		X	X	
High Body Mass Index	X			X
Microalbuminuria	X			
Risk factors and diagnostic	> 2	Abdominal adiposity	3 plus IR	Clinical criteria

WHO: World Health Organization; IDF: International Diabetes Federation; ATTPIII: National Cholesterol Education Program Adult Treatment Panel III; ACCE: American Association of Clinical Endocrinologists; HDL: High Density Lipoprotein cholesterol

Adapted from Lizarzaburu [70]

There is a lack of consensus for a definition of metabolic syndrome in children and youth. In the table 5 there is a summary of different definitions of metabolic syndrome in children used in epidemiologic studies.

Table 5. Differing criteria used to define metabolic syndrome in children and adolescents

	WHO criteria	IDF criteria (10-16 years old)	NCEP ATP IIII criteria
Glucose (mmol/L)	Insulin resistance or diabetes	Fasting ≤5.6	Fasting ≤6.1
Triglycerides (mmol/L)	≥1.7	≥1.7	≥1.7
HDL-C (mmol/L)	<0.91 boys, 1.0 girls	<1.03	<1.0
Systolic blood pressure (mmHg)	≥140	≥130	≥130
BMI (kg/m^2)	>30		-
Waist circumference (cm)	Waist-to-hip ratio >0.9 (boys), >0.85 (girls)	≥90th centile	Boys 102 cm, girls 88 cm
Insulin (pmol/L)	Insulin resistance		-

BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; IDF: International Diabetes Federation; NCEP ATP IIII: National Cholesterol Education Program's Adult Treatment Panel; WHO: Word Health Organization.
Adapted from Titmuss and Srinivasan [71].

Table 5 includes the new definition of metabolic children of The International Diabetes Federation (IDF) for children and adolescents, this definition is available for children 6 years old or older, but the IDF recommends not to use the diagnosis of metabolic syndrome in children younger than 10 [72]. For children older than 16 years old, IDF recommends the use of the existing IDF criteria for adults.

Even thought the IDF definition exists, it is considered that theirs is a lack of unified definition of the pediatric metabolic syndrome. For this reason, many authors are still confused about which definition is the most appropriate. Some authors preferred the use of internal cut-offs (quantiles), or a score, to evaluate the risk of cardiometabolic disease. Moreover, screening for metabolic syndrome is not widely used clinically in young ages, for its complexity, since there is the need of having measures for all the components of the metabolic syndrome [58]. For these reasons and the lack of a clear definition, a panel of expert committee recommended "not to considered the metabolic syndrome as a separate risk factors", and to screen and prevent the single components of these syndrome [60].

The etiology of the metabolic syndrome is not fully understood, but it is likely to be caused by the expression of genotypes modified by environmental interactions, and it may be mediated through abdominal obesity and insulin resistance [60].

Even thought there is a vast evidence of the role of obesity on cardio-metabolic risk, research suggests that general obesity is not essential for a higher cardio-metabolic risk, since there is also a metabolically obese but normal-weight phenotype in pediatric age, and as mention earlier, abdominal obesity may have a more relevant role in this association [73].

1.2.3.5 Other cardiometabolic biomarkers

Other obesity related biomarkers have been proposed but they are not widely used, such as C-reactive protein, adiponectin and leptin [74]. C-reactive protein (CRP) has been suggested because in adults, it seems clear the association between obesity and inflammation, and C-reactive protein is an unspecific biomarker of low-grade chronic inflammation. In adults CRP levels >3 mg/L suggest low chronic inflammation and >10 mg/L acute infection. However, there are at not specific cut-offs for children. CRP levels in adulthood and childhood are slightly increased when there is a higher BMI, higher waist circumference, and adiposity [58, 75, 76].

Furthermore, higher levels of C - reactive protein have been associated with the risk for cardiovascular disease and type 2 diabetes mellitus in adults. But the utility of this protein for screening in children and adolescents is not clear yet, because only few studies have explored the association between this protein and clinical outcomes, and this biomarker presents some limitations [58, 77].

Another novel biomarker is adiponectin. This hormone can increase the insulin sensitivity. Some studies show that lower levels of adiponectin are associated with higher BMI and fat mass percentage, and also can predict the risk of developing metabolic syndrome over a 6 years period [58].

Another biomarker related to several cardiometabolic risk factors in adults and children is leptin. This hormone controls metabolism, controlling food intake and stimulating energy expenditure and obese children tend to have higher leptin levels. Some authors suggest that leptin may be used as a marker of metabolic syndrome in children and adolescents [74].

1.3 Developmental Origins of Health and Disease (DOHaD)

The *fetal origins hypothesis* suggests that one of the causes of childhood obesity and cardiometabolic risk may be the exposure in early life to environmental factors. This hypothesis also called the *Barker's hypothesis* started with articles published in The Lancet by Barker and colleagues [78-80].

In the first of these studies, Barker studied the geographic distribution of diseases across England and Wales and showed a positive geographic correlation for infant mortality (between 1921 and 1925) and ischemic heart disease from 1968 to 1978. This correlation suggested that the origins of cardiovascular disease are in early life. Barker et al was the first to report an association between low birth-weight for gestational age (considered "as a surrogate marker for an adverse intrauterine environment") and increased susceptibilities for metabolic and other complex diseases later in life [81]. This hypothesis was tested in a second study carried out in Hertfordshire, with participants born between 1911 and 1930, and death rates from coronary heart disease decreased when increasing birth weight. This association was also found in several studies conducted in Europe, North America, China and in developing areas in Latin America and India [57, 82].

One of the most famous studies supporting this hypothesis is based on the Dutch famine: a cohort study of 2000 participants who were born in Netherlands between 1943 and 1947. During the II World Word, pregnant women had available 400-800 kcal/day during the winter of 1944-1945 due to an embargo and the problems for the food supply. The researchers could study the effect of starvation in the first, second and third trimesters of pregnancy, and compare the data with the children born before and after this critical period. The offspring of women who suffered the famine during pregnancy had higher risk of glucose intolerance, impaired insulin secretion and hypertension; among them, the offspring exposed to famine during early gestation had the worst effects, because they had also more risk of other conditions such as atherogenic lipid profile, obesity or coronary heart disease [57, 83-85]. Other famines showed similar patterns, for example people born in Nigeria during the Biafra famine (1968-1970) had a substantial higher risk for obesity and other diseases [86].

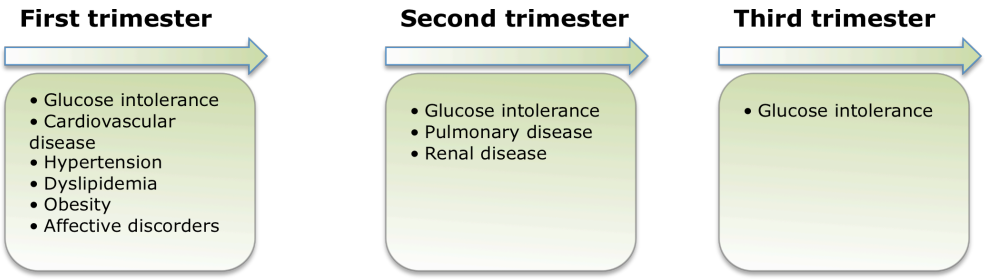

Figure 1. Timing of *in utero* nutritional deprivation and different later-life disease outcomes. Based on the Dutch famine birth cohorts. Adapted from Boekelheide et al. [87]

One of the components of the Fetal Origins hypothesis is the "thrifty phenotype" hypothesis proposed in 1992 by Hales & Barker that suggests that undernutrition in intrauterine period program an adaptation of the fetal metabolism for potential adverse postnatal environments [81].

Gillman in 2005 reported about the paradigm of "Developmental origins of health and disease (DOHaD)". This paradigm gave a greater perspective of prenatal origins of adult disease, beyond the previous studies focused on birth weight and later cardiovascular health [88]. Currently, this paradigm is the name of a Society and a Journal, and what is commonly agreed between its members is that there are sensitive time windows during early life for development of specific tissues, in preconception, *in utero* and in postnatal life, and that during this period environmental factors can have long-lasting effects by the programming effect, and can include changes in vascular structure and function, insulin secretion, renal development, and glucose and cholesterol metabolism [89]. The effect may increase the prevalence of chronic diseases o non-communicable diseases, such as obesity, cardiovascular and metabolic diseases and also respiratory diseases and neurodevelopmental disorders.

1.4 Biological Mechanism

This section describes potential biological mechanisms that could support the paradigm of DOHaD and specifically explain the association between early life exposures to environmental factors and childhood obesity and cardiometabolic risk.

There are different "critical windows" of development, which are periods of development when particular organs are more sensitive to nutritional, hormonal

and metabolic environment [90]. The periods of differentiation and proliferation of cells are organ-specific. These periods of development may be sensitive to environmental exposures, these may interfere with organogenesis and as a result the morphology and functionality of the organ may be altered for the rest of the life because the exposures alter normal tissue and organ development [81]. Some animals models have been used to study this theory and few studies in humans [91-93].

The mechanisms by which fetal development is influenced by environmental factors, for instance nutrition, are not completely understood. Different biological mechanism have been suggested, some mechanism are based on the concept of "developmental plasticity" [94, 95]. The plasticity is the ability of a single genotype to produce more than one alternative form of structure (several phenotypes), physiological state or behavior in response to environmental conditions. These several phenotypes depending of the environmental exposures can be beneficial for the offspring, because they enable a better match of the individual to his postnatal environment than would be possible if there would be the same phenotype for all the environments. Plasticity can be also described as the ability of the fetus to adapt tissue structure when environmental changes occur. When this adaptation occurs and has long term consequences, it is described as a "programming" or "metabolic imprinting". These processes are defined as any situation "where a stimulus during a critical period of development, results in long-term changes in the structure or function of the organism" [96].

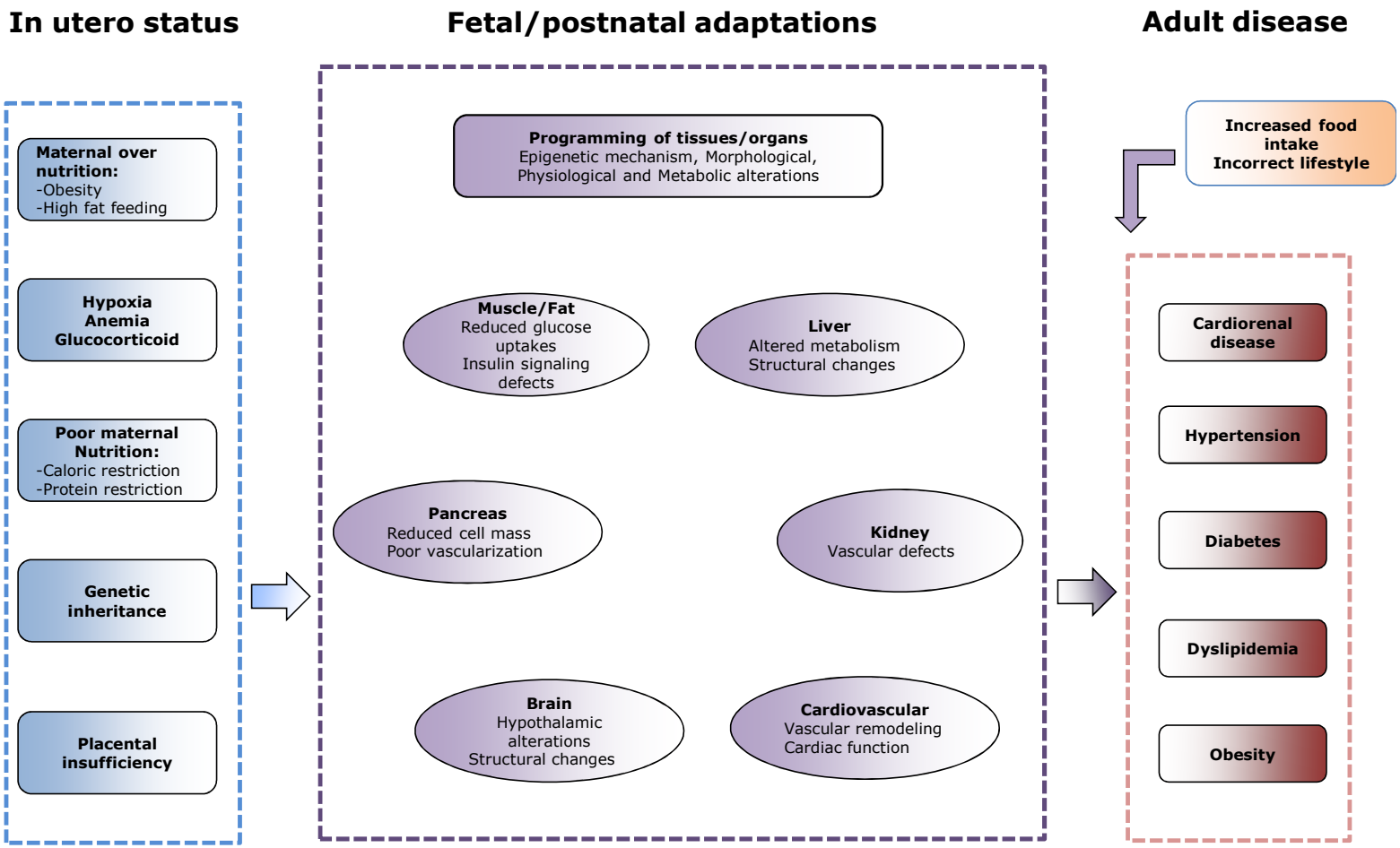

Figure 2.Programming factors of adult cardiovascular disease. Adapted from Visentin et al [88]

This programming effect could be caused by epigenetic modifications of the gene expression; these are heritable changes in the gene expression not caused by changes in the DNA sequences. The epigenetic modifications can result in persistent changes in gene regulation and pathways [97, 98]. These changes are heritable but not fixed through all the life, since they can be altered during different life stages: embryogenesis, fetal development, and also later in life. Sometimes these effects can be reversible, but usually they conduct to irreversible processes affecting the differentiation and organogenesis, and also may affect the homeostatic process. The epigenetic mechanisms include methylation, modification of histone proteins and microRNAs [99]. It is generally assumed that epigenetic modifications may mediate the gene-environment interaction. Therefore by epigenetic modifications, individuals with similar DNA but exposed to different environmental signal, express different phenotypes, and may have different predisposition to chronic diseases [81, 82].
Few transgenerational studies with animal models support this epigenetic hypothesis, such as a study conducted by Dunn and Bale, that showed that *in utero* exposure to a high-fat diet resulted in increased body size in the 3rd generation female offspring [100].

The telomere attrition has been also proposed as a potential mechanism of fetal programming of cardiovascular disease [101]. Telomeres are nucleoproteins included in the chromosomes, they are repetitive DNA sequences and they protect the chromosome from fusion and degradation [102]. The length of telomeres can be decreased by oxidative stress.

Another potential biological mechanism is based on the disruption of the endocrine environment, for instance alteration of the supply of glucocorticoids, which can lead to changes in gene regulation [103].

Some studies suggest that hormones are involved in this process, and they are environment-dependent organizers of the endocrine system, this system has a special role regulating all the fundamental processes of life. One hypothesis is that high concentrations of hormones, which are non-physiological and are caused by an altered early life environmental exposure, can malprogram the neuroendocrine system and it may lead to developmental disorders and chronic diseases in later life. The hormones identified are cortisol, leptin, insulin and ghrelin, but there may be other hormones involved in this process [96].

Insulin may play an important role in obesity development and other metabolic disorders, as proposed by Freinkel et al. (1980) that suggested the concept "fuel-mediated teratogenesis". This theory proposed that overnutrition ("increased nutrients"), specifically glucose levels and insulin have a positive correlation with increase body weight and impaired glucose tolerance in the offspring of diabetic mothers and also of mother with mild glucose intolerance [96]. To face this maternal hyperglycemia, the fetal pancreas and liver are stimulated to segregate insulin and insulin-like growth factors, leading to a macrosomic infant [104]. Another effect could be that the pancreas can be affected and the number of functional beta-cells could be reduced [105]. Insulin may also have an effect on hypothalamic controllers, thus high concentration of insulin in the immature hypothalamus may lead to a permanent dysplasia of central nervous regulating metabolism and body weight. In animal models, insulin in neonates induces to morphological alterations in hypothalamic structures that can lead to the development of obesity and adult hyperinsulinemia [81, 96].

There is another theory link to insulin, the fetal insulin hypothesis that was proposed by Hattersley and Tooke, and it describes a process where poor fetal nutrition induces metabolic adaptations. These adaptations are beneficial for the fetus during pregnancy but have lasting negative effects that can lead to obesity and metabolic diseases in later life [57, 106].

1.5 Prenatal factors influencing the risk of later obesity in childhood

Some of the prenatal environmental factors that have been considered potential predictors of childhood obesity are presented in the following sections. We focus our attention on risk factors that are potentially modifiable and related to lifestyle.

1.5.6 Maternal obesity

One of the prenatal factors of childhood obesity more studied is maternal obesity. Several cohort studies have found a positive association between maternal obesity and childhood obesity and cardiometabolic risk. A recent meta-analyses by Yu et al. showed that pre-pregnancy overweight/obesity had an increased risk of offspring obesity (Odds Ratio (OR) 3.06; 95%CI: 2.68, 3.49; P<0.001) compared to pre-pregnancy normal weight [107].

Some authors suggest that maternal obesity per se may not be the primary cause of childhood obesity; rather, other factors related to maternal obesity during pregnancy like hyperglycemia, dyslipidemia and hypertension are more likely to be the causal factors [57]. Some hypothesized mechanisms are that the high intrauterine glucose concentrations, may lead to an excessive insulin secretion by fetal pancreatic B cells. This process may result in a stimulation of fetal growth, because insulin is a growth hormone [57]. Some studies have explored this hypothesis, for example a study conducted in the Greek cohort RHEA that showed that pre-pregnancy overweight/obesity was associated with a higher risk of offspring overweight (Relative Risk (RR): 1.83; 95%CI: 1.19, 2.81), central adiposity (RR: 1.97; 95%CI: 1.11, 3.49) and greater fat mass compared with offspring of normoweight mothers [108]. These associations were not mediated by gestational diabetes.

The association of maternal obesity with childhood obesity may be explained by shared environmental, lifestyle and genetic characteristics, and also by intrauterine mechanisms (some of them are described in Figure 3, adapted from Gaillard [109]), or a combination of all of them [108]. Some studies have tried to disentangle the effect of maternal obesity on childhood obesity, studying the maternal and also paternal BMI and their relation with childhood obesity. For instance, in the Cohort study ALPAC they hypothesized that "a stronger association between maternal-offspring than paternal-offspring pairs would imply an intrauterine effect". They argued that their lack of difference in strength between the maternal and paternal associations with the childhood obesity

suggested that maternal obesity may be caused by shared genetic and environmental characteristics rather than intrauterine effects [110]. Few other studies support this argument, as reported in the 2013 review by Patro et al. and and also from other studies not included in this review conducted in Norway and India. They suggest that diet may be one of the plausible contributors [111-114].

Although shared environment seems to be the major predictor of childhood obesity, some of the association between parental obesity and offspring obesity may be driven by genetic variation [115]. One of the genes associated with obesity development that has given rise to more interest is FTO [116, 117]. This gene is related to regulation of energy consumption, and some studies suggest that children with specific alleles of FTO may have greater food consumption and reduced satiety [35].

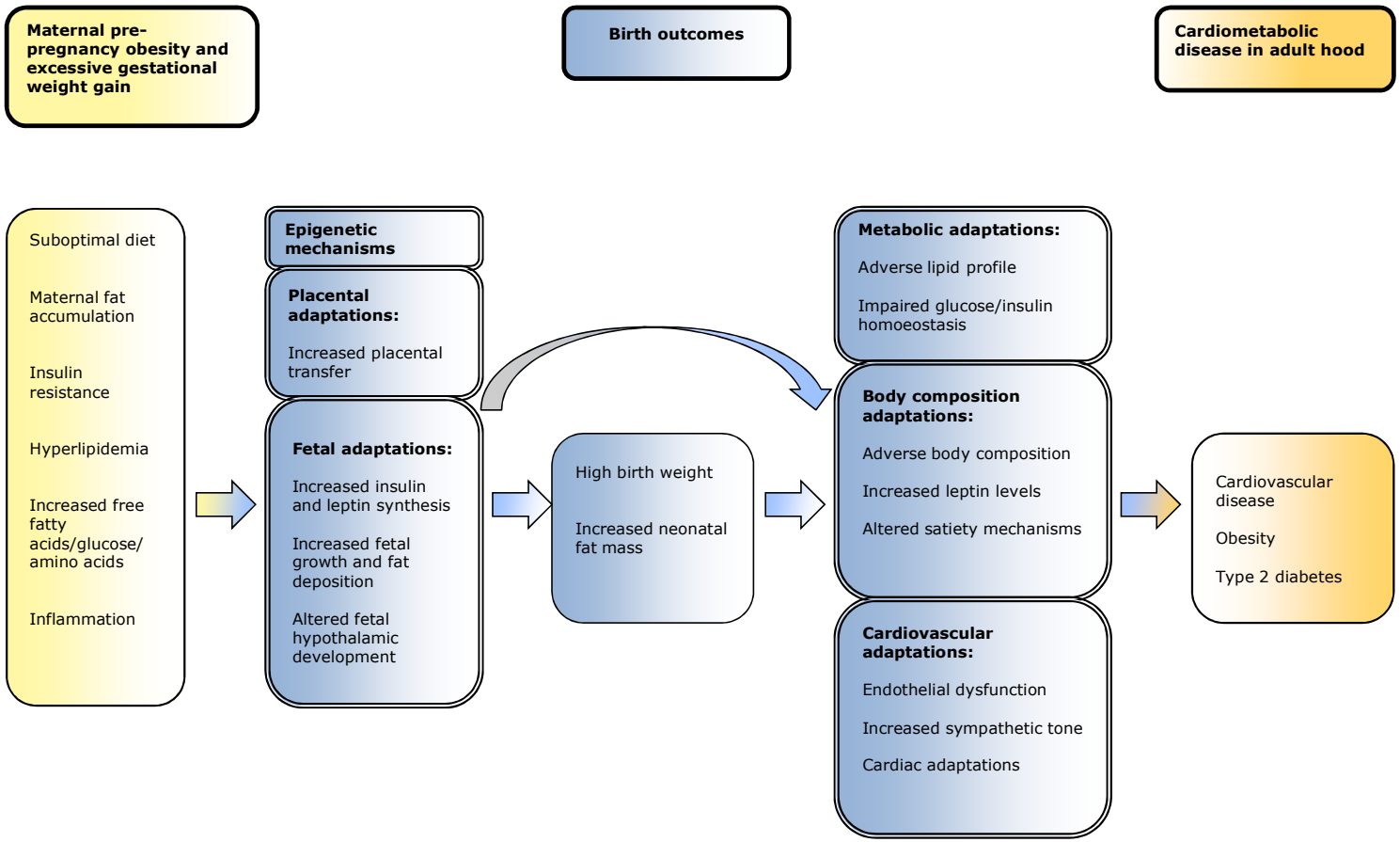

Figure 3. Potential mechanims for the association of maternal obestiy during pregnancy and offspring developmental adaptations. Adapted from Gaillard

1.5.2 Gestational weight gain

Another maternal factor that has attracted much attention is gestational weight gain. The Institute of Medicine (IOM) defines excessive gestational weight gain as shown in table 6, taking into account the pre-pregnancy BMI [118].

Table 6 Institute of Medicine (IOM) recommendations for Total and Rate of weight gain during pregnancy, by pre-pregnancy Body Mass Index (BMI)

		Total weight gain	Rate of weight gain
Classification	**BMI (kg/m^2)**	**Range in kg**	**Mean (range) in kg /week**
Underweight	<18.50	12.5 – 18	0.51 (0.44 – 0.58)
Normal weight	18.50-24.99	11.5 – 16	0.42 (0.35 – 0.50)
Overweight	≥25.00	7 – 11.5	0.28 (0.23 – 0.33)
Obese	≥30.00	5 - 9	0.22 (0.17 – 0.27)

Based on IOM recommendations [118]

The IOM recommends gaining optimal weight during pregnancy because gaining excessive gestational weight may have short and long term effects on the maternal health and also on the infant health, such as gestational diabetes, preeclampsia and cesarean delivery [118]. Furthermore, the offspring of women who gained excessive weight may have higher risk of developing obesity as shown by several studies [119, 120]. In this sense, a meta-analysis conducted by Mamun et al. (2014) found that offspring of women who gained excessive weight during pregnancy had a 40 % higher risk of childhood obesity, when compared with offspring of adequate gestational weight gainers [121].

Moreover, some studies showed that excess gestational weight gain is also associated with higher fat mass and central adiposity in the offspring [122-124].

The mechanism involved in this relationship are not fully understood, some of the hypothesized mechanisms are that the offspring may inherit the maternal genetic predisposition to gain weight, and for this reason they would have higher risk of obesity, and another mechanism would be the shared lifestyle between mother and child. There is also the hypothesis that high gestational weight gain could lead to excessive fat deposition that could cause a high transmission of free fatty acids to the fetus and causes higher offspring adiposity. These free fatty acids could be transformed into glucose, and the exposure to a maternal hyperglycemia could lead to a higher fetus development, as suggested by the fetal insulin hypothesis [125, 126]. It has also been proposed that the excessive

free fatty acid and glucose supply could lead to an alteration of the development of fetal fat cells, and to a long term increment of adipose tissue [120].

1.5.3 Gestational diabetes

Gestational diabetes screening is a common practice worldwide, since gestational diabetes is a well known risk factor of developing diabetes in later life for the pregnant women [127]. In the last decades, the evidence of the detrimental impact of gestational diabetes also on the infant has further supported this screening practice [128].

Animal models showed that the presence of maternal gestational diabetes can lead to a higher risk of gestational diabetes, T2DM, obesity and cardiovascular disease in the offspring [129, 130]. Human studies supported these findings that offspring exposed to maternal gestational diabetes have an increased risk of developing general obesity, central adiposity, metabolic syndrome and also T2DM [119, 131-136]. However, some authors argue that the effect of gestational diabetes on childhood obesity risk, especially general obesity, is highly driven by maternal pre-pregnancy obesity. In this sense, the substantially attenuation of the association between gestational diabetes and obesity when controlling by maternal pre-pregnancy BMI could support this hypothesis [137, 138].

The mechanism of action follows the hyperinsulinemia hypothesis suggested by Pedersen, that a maternal hyperglycemia status could lead to intrauterine over nutrition and fetal hyperinsulinemia [139]. This could cause excessive fetal growth and metabolic dysregulation [140]. Some longitudinal studies explored this potential mechanism, assessing whether maternal glucose was associated with childhood BMI. Some of them found that there was a positive association in gestational diabetic women and non diabetic women, and this association remained significant after adjusting for maternal BMI [140].

Few studies have assessed the effect of an optimal treatment of maternal hyperglycemia on childhood obesity, and have found that treatment improved offspring insulin sensitivity but not childhood obesity risk [141, 142]. According the current evidence, the effects of maternal gestational diabetes could be considered a vicious cycle given that the offspring of a diabetic mother has a high risk of obesity and T2DM. Offspring will have a higher risk of perpetuating the cycle if they become obese and develop Diabetes Mellitus. The interaction between the phenotype and the later exposure to an unhealthy lifestyle (for

instance: sedentary and high-fat diet) may modulate the risk of the offspring and can contribute to maintain this vicious cycle [143].

1.5.4 Smoking

Smoking during pregnancy is a lifestyle factor that it is potentially modifiable and it has been associated with adverse pregnancy outcomes including childhood obesity. Several longitudinal cohort studies have assessed the effect of smoking during pregnancy and childhood obesity, and they found a positive association between them [119, 144-146].

In fact, two meta-analyses found that children exposed to maternal smoking *in utero* had an increased odds of 50 % of having obesity, even after adjusting for other lifestyle factors and sociodemographic characteristics [145, 147].

The potential detrimental effect of smoking on central obesity and metabolic syndrome in childhood is not clear. Some animal studies indicate that animals exposed to nicotine *in utero* have a decreased B cell function, impaired glucose tolerance and increased metabolic syndrome risk [148-151]. Evidence from human studies is scarce, and further longitudinal studies are needed [115, 152].

The mechanisms that could explain this association are not fully understood, one of the most common hypothesis is that smoking lead to low birth weight and rapid infant growth could lead to childhood obesity [145]. However, some studies have controlled their analyses by birth weight and rapid infant growth and still found an independent effect of smoking on obesity risk. Another plausible hypothesis is that smoking increases body fat accumulation. This increase could be caused by nicotine that can cross the placenta, and cause fetal hypoxia and long term cardiovascular consequences [147].

1.5.5 Diet

Many studies have explored the potential influence of maternal diet on offspring obesity development. Different approaches have been used, like the study of macro- and micronutrient intake, or the consumption of single foods and dietary patterns. In the following sections we will review the current literature on this topic.

1.5.5.1 Macro and micronutrients

Several studies have focused their interest on the study of macro and micronutrient intake during pregnancy and childhood obesity development.

Some animal models have been developed to explore the association between maternal diet and childhood obesity. The most common model has tested the effect of a high-fat diet (western diet) on the offspring obesity risk, and has found a positive association in rats, mice and ewes [153-156]. Traditionally, maternal obesity was considered necessary in this association, but some studies suggest that maternal obesity is not a requirement, and a high-fat diet may have an effect per se [157-159]. Other models have been used, like high-sugar diet; in fact a study showed that male offspring from dams fed with high-sugar diet had increased adiposity, triglyceride, and LDL concentrations, when compared to standard fed rodents [160].

There are few studies in humans that explored the association between macronutrient intake and childhood obesity with disparities in their findings. One of these studies, conducted in Ireland on 585 pregnant women, found that mothers with higher intakes of sugar and saturated fatty acids (SFA) had an increase OR of having overweight/obese children at 5 years of age (OR: 4.57; 95%CI: 1.01, 20.69 and OR: 3.35; 95%CI: 0.97, 11.57, respectively) compared to mothers with low intakes. However, total fat and total carbohydrate intake did not show any effect [161].

Another cohort study in the UK investigated the association between maternal energy intake and grams of carbohydrates, proteins and fats during pregnancy and offspring fat mass and fat-free mass at 10 years of age. There was no significant association between the maternal and paternal energy and macronutrient intake and childhood adiposity [162]. However, they found an interesting association between maternal protein and fat intakes and the offspring intakes, even when adjusting by paternal intakes, and this association was stronger than the paternal-offspring association, suggesting that maternal diet may influence the offspring diet via intrauterine mechanisms, and not for shared environment. According to this hypothesis, maternal diet during pregnancy could program the development of dietary preference and appetite regulation of the offspring. That is of special importance, because child dietary intakes of protein and fat were also associated with adiposity [162].

Maslova et al. also found that protein intake during pregnancy was associated with offspring overweight at 20 years of age, especially when proteins were from animal sources. In this study conducted in Denmark with a sample of 684 mother-child pairs, they also explored the association between protein intake and waist circumference and biomarkers (concentrations of adiponectin, leptin, IGF-1, insulin, glucose, glycated hemoglobin, cholesterol and triglycerides). They found no significant associations [163]. However, maternal protein intake may play a role in the development of blood pressure, as found by researchers of the Women and Their Children's Health Study. They observed that maternal low protein-to-carbohydrate ratio was associated with higher systolic blood pressure in their offspring at age four [164]. These authors suggested that an optimal ratio of macronutrients intake may exist.

Regarding fatty acids, Vidakovic et al. in 2016 explored the association between polyunsaturated fatty acid (PUFA) concentrations during pregnancy and offspring general and abdominal adiposity at 6 years of age in the Generation R study. Lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy were associated with a higher body fat percentage, and abdominal adiposity in the offspring [165]. There was no association between PUFA during pregnancy and offspring BMI and results from previous cohort studies have been inconsistent [166-169].

In this line, one meta-analysis that included 6 randomized controlled trials found no association between supplemental n-3 PUFA during pregnancy and offspring BMI in preschool age (Standardized mean difference (SMD): 0.07; 95%CI: -0.22, 0.36) and school age (SMD: 0.12; 95%CI:-0.06, 0.30) [170].

Like with proteins, PUFA intake may have an impact on cardiovascular health, especially on blood pressure. That is what a study conducted in 129 Australian mother-child pairs found, i.e. higher intake of PUFA and Omega 6 in pregnancy were associated with higher systolic blood pressure of the offspring (at age 4) [164].

Some researchers have used the micronutrient approach to study their influence on childhood obesity development. One of the micronutrient that has generated more interest in the last years is Vitamin D. Several studies conducted in children and adolescents have shown an inverse association between vitamin D status (defined by serum concentration of 25-hydroxyvitamin D (25(OH)D) and BMI and other adiposity outcomes [171, 172]. In this line, a study conducted in the

Spanish cohort INMA found that maternal deficit of vitamin D concentrations increased the offspring risk of overweight and the BMI at 1 year of age. However, the association did not remain at 4 years of age [173].

A study conducted in the birth cohort study Southampton Women's Survey used repeated direct measured of adiposity as child fat mass by DXA. This study showed an association between a low Vitamin D status during pregnancy and offspring higher fat mass at birth and at age 6. Similar to INMA cohort, the association was attenuated at age 4 [174].

In contrast, other recent studies are in disagreement because they report a lack of association of vitamin D and offspring obesity development (neonatal adiposity, at 5 years and 9.5 years old) [175, 176].

Fewer studies have investigated the intake of other micronutrients during pregnancy and their impact on childhood obesity, and they found null associations for folate intake and supplements of multivitamins [177, 178]. Yaknik et al. found that in the Pune Maternal Nutrition cohort conducted in India, mothers with low vitamin B12 and high folic acid levels had children with more insulin resistance, higher body fat percentage and higher abdominal fat at age 6 [179].

One of the cohorts with more publications about maternal diet and its influence on the offspring is the British ALPAC. Although their lack of studies focused on obesity in the offspring, they have studied the influence of several maternal intakes of micronutrients and their influence in growth and cardiometabolic factors. They report a null association between maternal intake of any micronutrient and blood pressure at age 7 and 15, a weak association between iron and magnesium intake and height and between magnesium, potassium, and folate with the bone density at 9.9 years of age [111].

1.5.5.2 Foods

Some studies have used another approach, and have focused their attention on specific food intake. They explored the potential influence of maternal food intake during pregnancy on the development of childhood obesity, in order to design guidelines with food specific recommendations. The number of studies in humans is low, but there is some evidence that certain foods consumed during pregnancy may increase the risk of the offspring for obesity.

One of the food items that could increase obesity risk is meat and meat products. Maslova et al. found that these diet components increased the risk of overweight in the female offspring (Q4 of meat intake compared to Q1, Relative Risk (RR): 2.47; 95%CI: 1.09, 5.60; p = 0.03). In the same publication, they also found that a high consumption of cereals and cereal product decreased risk of overweight in offspring at 20 years of age (Q4 compared to Q1, RR: 0.27; 95%CI: 0.12, 0.62; p = 0.002). These associations were observed only in female offspring. No other food group included (fish, milk, vegetables and fruits) was associated with BMI or overweight risk at 20 years [163].

Other animal products, like fish intake, have been associated with increased risk of childhood obesity, as found in a multicohort study [180].

Other studies have taken into account milk intake, but they have not explored yet the association with obesity development, instead they focused their attention on birth outcomes like abdominal adiposity, finding a positive association [181].

Similar situation happens with vegetable and fruit intake during pregnancy, the evidence is limited to birth outcomes and no study has yet explored their influence in obesity development [182].

Interest has risen recently to study the effect of artificial sweeteners intake during pregnancy and childhood obesity. There is evidence on animal models that these sweeteners may influence the development of obesity and metabolic syndrome in the offspring. Moreover, a recent study has been published that explores this association, and it has found that daily consumption of artificially sweetened beverages was associated with a higher risk of infant overweight at 1 year of age compared with offspring of mothers who not consumed these beverages (adjusted OR: 2.19; 95%CI: 1.23-3.99). Surprisingly, there was a null association with sugar-sweetened beverages [183]. In contrast with two previous studies that found a modest effect on childhood anthropometry in infancy [184, 185].

1.5.5.3 Dietary patterns

The study of dietary patterns gives a broader picture of the overall diet of a person, compared to the study of nutrients or single foods. Furthermore, dietary patterns may be more useful to study the association between diet and disease [186].

Since foods and nutrient interact between each other, and they are highly interrelated, it is difficult to separate out the specific effect of single foods or nutrients in relation to childhood obesity.

Few studies have assessed the influence of dietary patterns and later risk of obesity; most of the studies have focused their interest in pregnancy and birth outcomes [187-194].

One of the dietary patterns considered a healthy dietary pattern is the Mediterranean Diet. This diet is characterized by a high content of fiber, mono- and poly-unsaturated fatty acids and antioxidants. The low adherence to the Mediterranean Diet during pregnancy has shown an association with risk factors of childhood obesity, such as neonatal insulin resistance [195].

Some studies have explored the association between the glycemic index and glycemic load during pregnancy and offspring outcomes. The glycemic index is defined as "a measure of the change in blood glucose following ingestion of carbohydrate-containing foods (…). It is a mean of quantifying the relative blood glucose response to carbohydrates in individual foods, comparing them on a weight-for-weight basis (i.e., per gram of carbohydrate)". While the glycemic load "of a particular food is the product of the glycemic index of the food and the amount of carbohydrate in a serving. By summing the glycemic load contributed by individual foods, the overall glycemic load of a meal or the whole diet can be calculated" [196].

Some cohort studies showed a positive association between the glycemic load and the glycemic index during pregnancy and offspring adiposity measures. A study conducted in the prospective cohort Southampton Women's Survey found that per each 10-unit increment of glycemic index in early pregnancy there was an increase of 0.43 of child fat mass (95%CI: 0.06, 0.80) at 4 years of age, and per 50-unit glycemic load increase β: 0.43 (95%CI: 0.19, 0.67). These estimates were slightly attenuated at 6 years of age [197]. These findings were at early pregnancy, no association was found when the glycemic index and load was

assessed in the late pregnancy. There was no association with neonatal adiposity. In agreement with a previous randomized controlled trial the ROLO study, after the intervention with a low glycemic index diet, there were no difference between the groups, except for a moderate difference in the thigh circumference (15.9 ± 1.7 cm vs. 16.6 ± 1.5 cm; P = 0.04) [198].

In contrast, a pilot randomized controlled trial explored the effect of a low-glycemic index diet during pregnancy in women at risk of gestational Diabetes Mellitus on the offspring adiposity, and found that adherence to a low-glycemic index was associated with a lower birth weight and length, but not with adiposity at birth or at one year of age [199].

Moreover, it has been hypothesize that the glycemic index and glycemic load could be associated with abdominal adiposity and cardiometabolic risk. One study conducted in Denmark explored these associations and found that maternal glycemic index (not glycemic load), was positive associated with HOMA-IR, insulin and leptin concentrations in the offspring at 20 years of age [200].

This study found also a positive association between glycemic load and with waist circumference, but this difference was only found in girls. In contrast, there was no association between glycemic index and load on other biomarkers, such as total cholesterol, HDL, LDL, triglycerides and other outcomes as systolic and diastolic blood pressure [200].

The mechanism that underlies the associations is not fully understood, but it may be related to the exposure to a maternal hyperglycemia and the consequences that this exposure produces, like fetal hyperinsulinemia, appetite control dysregulation, and change in the endocrine system.

1.6 Postnatal influence on childhood obesity

Recently, not only the gestation period but also the 2 years after delivery has been considered key moments of development, based mostly on animal experiments.

In the following sections we exposed some risk factors that are suspected to be associated with childhood obesity development.

1.6.1 Birth Type

Birth type is a perinatal factor that has raised much attention in the last years. One proof of that, it is the publication of several studies exploring the relation between birth type and child health.

A meta-analyses including 19 cohort and case-control studies observed that cesarean section was associated with a 34 % increased risk of childhood obesity [201]. This finding is in line with two reviews and meta-analyses conducted previously that found similar positive associations [202, 203].

Even thought cesarean section was suspected to influence metabolic risk factor in the offspring, a recent cohort study that included 2063 young Brazilians, found that cesarean section was associated with higher BMI but not with metabolic risk factors [204].

The potential biological mechanism is not fully known, some theories indicate that cesarean delivery may affect the normal developing of gut microbiota in infants, and microbiota is associated with inflammatory status that could lead to childhood obesity [205]. Another theory suggests that cesarean delivery may affect childhood obesity by DNA methylation [206]. Another potential mechanism could be related to maternal obesity, since obese women are more likely to have a cesarean section, and their babies are more likely to be obese [207].

1.6.2 Birth weight

In the following section we will discuss the role of birth weight as a postnatal risk factor of childhood obesity and cardiometabolic risk.

Several studies have found an association between low birth weight and higher body mass index later in life in UK cohorts [208-210]. In a Spanish study, lower birth weight was associated with lower lean body mass and greater central obesity (waist-to-hip ratio and skinfold thickness) in adults [211]. However, other studies focused on the association between low birth weight and other measures of fat mass, have shown no association or a weaker association [212-214]. This lack of association is also supported by a systematic review and meta-analysis that found that low birth weight (< 2500g) was not associated with increased risk of obesity.

Low birth weight may have an effect on the cardiometabolic risk factors. In fact, several longitudinal studies have observed associations between birth weight and blood pressure, and coronary heart disease in adulthood. As shown in previous section 1.3, the Barker hypothesis suggests that birth weight is an indicator of fetal programming *in utero*, and it is associated with later disease. However, some authors suggest that women who delivery low birth weight babies, themselves have a higher risk of coronary heart disease. This fact may contradict the fetal programming theory, and support that there is a genetic cardiovascular susceptibility that increase the risk of vascular complications during pregnancy. Furthermore, it increases the risk of having preterm or low birth weight infants that inherit a susceptibility to heart disease [17].

Furthermore, low birth weight has been considered historically as a surrogate for poor intrauterine growth, but some authors considered that birth weight is a poor index of intrauterine growth, because it does not consider genetic growth potential and does not differentiate fat mass and fat-free mass [215]. To face this limitation, using direct measures of fat and fat-free mass would be more adequate.

On the other hand, high birth weight, especially macrosomic deliveries (birth weight ≥ 90thcentile) are positive associated with a higher risk of later BMI in childhood and adulthood [23]. This finding and also several authors suggest that the association between birth weight and obesity have a J or U shape, with a higher prevalence of obesity observed for the lowest and the highest birth weight (Figure 4) [216, 217].

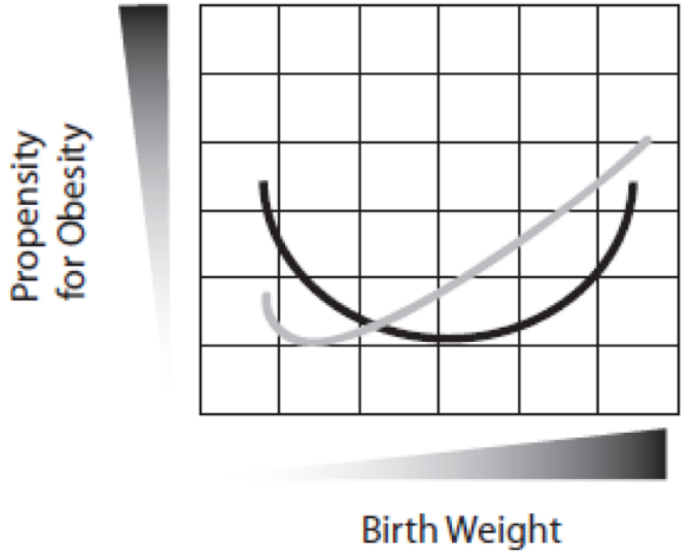

Figure 4. J- or U- shaped relationship between birth weight and the propensity to develop obesity in adulthood. Adapted from Parlee and MacDougald [218]

Some reviews have supported this hypothesis of higher risk in both ends of the birth weight spectrum [219, 220], but the few studies that used direct measures of adiposity found that birth weight was not only positively associated with higher BMI, but also with higher fat-free mass. This indicates that bigger babies will have more lean mass in the future, rather than fat mass [221, 222]. This theory was not supported by a study conducted in the UK cohort ALSPAC that found that higher birth weight was associated with higher fat mass [223]. For these reasons direct measurement of fatness at birth are necessary.

Despite the effect on childhood obesity, a study conducted by Boney et al. found that children born large for gestational age had an increased risk of metabolic syndrome during childhood when exposed to an adverse intrauterine environment (maternal obesity or gestational diabetes mellitus). This suggests an interaction between *in utero* environment, birth weight and later development of disease [224]. Other studies found that higher birth weight was associated with later risk of T2DM, hypertension, coronary heart disease, stroke and even some types of cancers [23, 225-232].

The potential mechanism behind this association is that maternal overnutrition leads to fetal hyperglycemia and hyperinsulinemia that may promote excessive

fat deposition during the third trimester. Another explanation is related to the increased levels of growth factors *in utero* (insulin and IGF) [23].

1.6.3 Rapid infant weight gain

As argued in the previous section (1.2.1) sometimes infant weight gain has been considered an outcome and also a mediator factor in the relation between maternal exposures and offspring obesity. In this section, we present the evidence supporting the role of rapid infant weight gain as an independent risk factor of later general and abdominal obesity, and also its role in the onset of cardiovascular disease.

Several definitions and measurements of infant weight gain are used in epidemiological studies and they are described in the previous section (1.2.1).

Rapid weight gain during infancy has been found to be predictive of later obesity in several observational studies. One study that assessed the influence of rapid weight gain during the first 3 years of life found that it was associated with higher BMI, fat mass and waist circumference at 17 years of age [32, 233]. These findings are in line with other studies that found that rapid infant weight gain from birth to 6 months was associated with later childhood obesity [145]. A review conducted by Mendez et al. found that out of the 46 studies included, 45 showed a positive association between rapid weight gain and childhood obesity [146]. Other reviews and meta-analyses conducted later are in agreement with these findings.

Low birth weight (and/or SGA) and rapid postnatal growth may play together a detrimental role in childhood obesity development and also for other metabolic conditions, such as Type 1 diabetes mellitus and metabolic syndrome [234-238].

This growing evidence that infants who rapidly grow are more likely to be obese children and obese adults, and also to have higher risk of metabolic disorders exists even in subjects who are born with optimal birth weight [36, 239].

1.6.4 Breastfeeding

The role of breastfeeding on childhood health has raised a lot of interest in the last decades. Some researchers include in their analyses type of breastfeeding and duration of breastfeeding.

Despite the growing number of publications, there are inconclusive results about the protective effect of breastfeeding on childhood obesity. There are two meta-analyses that tried to address these questions: a meta-analyses of 17 studies conducted by Harder et al. found that there was a dose-response effect, each month of breastfeeding was associated with a 4% decrease in risk of obesity [240]; a recent meta-analysis of 18 studies found that children breastfed for more than 7 months were less likely to have obesity (OR: 0.79; 95%CI: 0.70, 0.88) compared to children breastfeed less time [241]; there are other studies that found null associations between breastfeeding and childhood obesity [242, 243]. Some authors argue that these discrepancies may respond to residual confounding, because other sociodemographic and lifestyle factors may play a role in these associations.

Some researchers suggest that people breastfed had cardiovascular benefits, like lower cholesterol levels and lower prevalence of T2DM, based on some long-term follow-up studies [244]. Furthermore, it may have an effect lowering the risk of blood pressure, and metabolic syndrome [245].

Moreover, formula feeding may play a role in the development of childhood obesity, specifically, the types of formula milk [246]. One trial compared the growing patterns of infants fed lower protein formula and higher protein cow's milk formula and, and they found that the last grew more rapidly, and the low-protein formula had a lower BMI later in life [247].

The mechanism underlying the association between breastfeeding and formula feeding and childhood obesity could be related to the protein intake, as suggested by the *Early Protein Hypothesis* [248]. This theory suggests that a high-protein intake in early stages of life may increase the levels of insulin-realizing amino acids; these may lead to an increased secretion of IGF-1, which stimulates growth and activity of adipogenes. That could be translated to a rapid weight gain and a later risk of obesity development.

1.6.5 Introduction of solid foods and infant feeding practices

Related to breastfeeding there are other postnatal factors that could have an impact on childhood obesity development: timing of solid foods introduction and infant feeding practices.

Few studies have assessed the association of early introduction of solid foods and childhood obesity with inconsistent results. Huh et at conducted an analyses in the Viva cohort study and found that early introduction of solids (before 4 months of age) increased the odds of obesity at 3 years of age (OR: 6.3; 95%CI: 2.3, 6.9) among the children who were formula-fed compared with later introduction of solids [249]. Other studies found similar results [146]; on the other hand, there are other studies that found null associations between timing of introduction of solids and childhood obesity [250, 251].

Few studies have taken into account quality of infant feeding and its association with obesity development. One of these studies was conducted in 536 children, and found that children with higher adherence to a "infant guideline score" (characterized by high consumption of fruit, vegetables and home-prepared foods) had lower fat mass at 4 years of age (assess by DXA) compared to infants with lower adherence. However, there was no association with BMI at 4 years of age. Similar results were described by Okubo et al., children with higher quality of diet at 6 and 12 months of life, had lower fat mass at 6 months of age but not difference in BMI.

A review conducted by Pearce et al. tried to explore this question, and found that high intakes of energy and protein (especially animal and milk protein) could lead to an increased BMI and body fat [252].

The mechanism underlying these associations are uncertain, one explanation could be that the quality of the infant diet is an indicator of the quality of his diet in later periods, since dietary preferences could track intro childhood and adulthood. Other explanation is that a higher quality of diet may be related with a healthier parental lifestyle and also with other sociodemographic characteristics that may act as confounders in these associations.

1.6.6 Sleep duration

There is a growing interest in the role of sleep duration as a predictor of health and disease outcomes, both in adults and children. Recently, infant sleep duration has been considered a potential determinant of later childhood obesity risk.

Several reviews and meta-analyses have reported an inverse association between sleep duration and obesity [253-255]. The latest meta-analyses published, included 22 longitudinal studies and showed that children and adolescent who slept fewer hours had an OR of 2.51 (95%CI: 1.64, 2.81) for being overweight or obese compared to subjects who slept enough hours [256].

The proposed mechanism for this association is that short duration of sleep may increase energy intake and decrease its expenditure by altering hormonal responses, mostly leptin and ghrelin. These hormones play an important role on satiation and food intake, reducing food intake and stimulating it, respectively [257-259]. Another potential explanation is that sleeping is part of the lifestyle, and short sleeping can be associated with other lifestyle related to obesity (low physical activity for instance) [256, 260].

RATIONALE

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2. RATIONALE

Childhood obesity prevalence is increasing in the entire world, and given that it is associated with adulthood obesity and other chronic diseases, it has become one of the main public health problems. Despite this, the reasons for the increase in childhood obesity prevalence are uncertain. Some of the attributed causes are the greater availability and affordability of food, the decrease of physical activity and also a genetic predisposition to obesity. However, these causes would not fully explain the rapid increase in childhood obesity, and other environmental causes may have a role in the early stages of the disease. It is believed that childhood obesity starts in prenatal periods, thus identifying early life risk factors could help to prevent childhood obesity. Early prevention of childhood obesity is crucial, because once obesity is established is very unlikely to reverse it, given the modest effect observed after many interventions. And even if the obesity is addressed through weight reduction, the presence of childhood obesity may leave an imprint in the adult health, and may maintain an increased risk of other comorbidities [4].

Some of the risk factors associated with childhood obesity are non-modifiable, but others are potentially modifiable such as smoking during pregnancy or gestational weight gain or in infancy like sleeping duration. One of the modifiable factors that has been poorly studied is maternal dietary pattern during pregnancy and its impact on childhood obesity development. Only few longitudinal birth cohort studies have focused their attention on maternal diet, and this type of studies enable us to investigate potential causal associations for their prospective design.

This doctoral thesis describes a study of the early life modifiable factors involved in the development of childhood general and abdominal obesity and cardiometabolic risk based on longitudinal prospective birth cohort studies. The aim of this thesis is that the results could contribute to the knowledge of childhood obesity etiology, and they might be helpful in the design of public health programs and interventions for the prevention of childhood obesity.

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HYPOTHESIS AND OBJECTIVES

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3. HYPOTHESIS AND OBJECTIVES

Hypothesis

Modifiable maternal and infant early life factors could influence childhood general and abdominal obesity development. Specifically maternal dietary patterns could be associated with general and abdominal obesity development in childhood and with related cardiometabolic biomarkers.

Objectives

The main objective of this thesis was to investigate the prospective association between potentially modifiable early life factors and offspring childhood obesity development.

The specific objectives of this study were:

1. To identify potential modifiable early life risk factors for offspring childhood general and abdominal obesity at mid-childhood.
2. To examine the extent to which combination of modifiable prenatal and postnatal risk factors predict childhood general and abdominal obesity at mid-childhood in two different populations in USA and Spain.
3. To examine the association between maternal adherence to the Mediterranean Diet during pregnancy and risk of general and abdominal obesity at pre-school age.
4. To evaluate the association between maternal adherence to the Mediterranean Diet during pregnancy and cardiometabolic biomarkers a at pre-school age.
5. To evaluate the association between maternal Mediterranean Diet during pregnancy and child longitudinal growth trajectories from birth to up to 4 years.

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MATERIAL AND METHODS

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4. MATERIAL AND METHODS

n this section, we provide description of the different methods used to assess the aims of the doctoral thesis.

4.1 Study design

Data from two birth cohort studies were used in the present thesis:

INMA Project

The population-based INMA Project ("INfancia y Medio Ambiente"-Environment and Childhood) is a network of 7 prospective birth cohorts in Spain:
There are three old cohorts, already ongoing when the network was created, in the region of Ribera d'Ebre (n=102), Menorca (n=530) and Granada (n=668) and four new cohorts with a common protocol in Sabadell (n=777), Valencia (n=855), Asturias (n=494) and Gipuzkoa (n=638) (Figure 5). The main aim of the INMA Project is to study the role of environmental pollutants in air, water and diet during pregnancy and early childhood in relation to child growth and development. Details of the study design are described by Guxens et al. [261].

Figure 5. Cohorts included in the INMA Project.

The inclusion criteria for pregnant women were being ≥16 years, intention to deliver at the reference hospital, ability to communicate in Spanish or regional

languages, singleton pregnancy, no assisted conception. Pregnant women were recruited during prenatal visits in the first trimester of pregnancy at public health care centers or hospitals. From 45% to 98% of the eligible women agreed to participate in the study.

All procedures were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki and all women provided written informed consent, and institutional review boards of participating institutions in each region approved the studies.

The mother and their children have been followed up from pregnancy to adolescence. Each cohort had a different period of recruitment, and follow-up, but they all performed the follow-up assessment at the main time-points (pregnancy, birth, and 4 years) (Figure 6).

For the present thesis we used data from the new cohorts, Sabadell, Valencia, Asturias and Gipuzkoa. The recruitment of pregnant women in Sabadell was from July 2004 to July 2006, in Valencia from November 2003 to June 2005. Recruitment in Asturias took place from May 2004 to July 2007 and in Gipuzkoa from April 2006 to January 2008. Most women from all the new cohorts were recruited at their first prenatal visit (10 – 13 weeks of gestation), except for 120 women of Sabadell cohort who were recruited at delivery.

We conducted in-person study visits with participants in the 1st and 3rd trimesters of pregnancy. Trained INMA staff followed mother-child pairs at birth, and at child ages 6, 14 months, 4 and 7 years. They performed interviews, physical examinations, ultrasound scans, and they obtained measurements and biological samples from the mother- child pairs, and collect data from medical records.

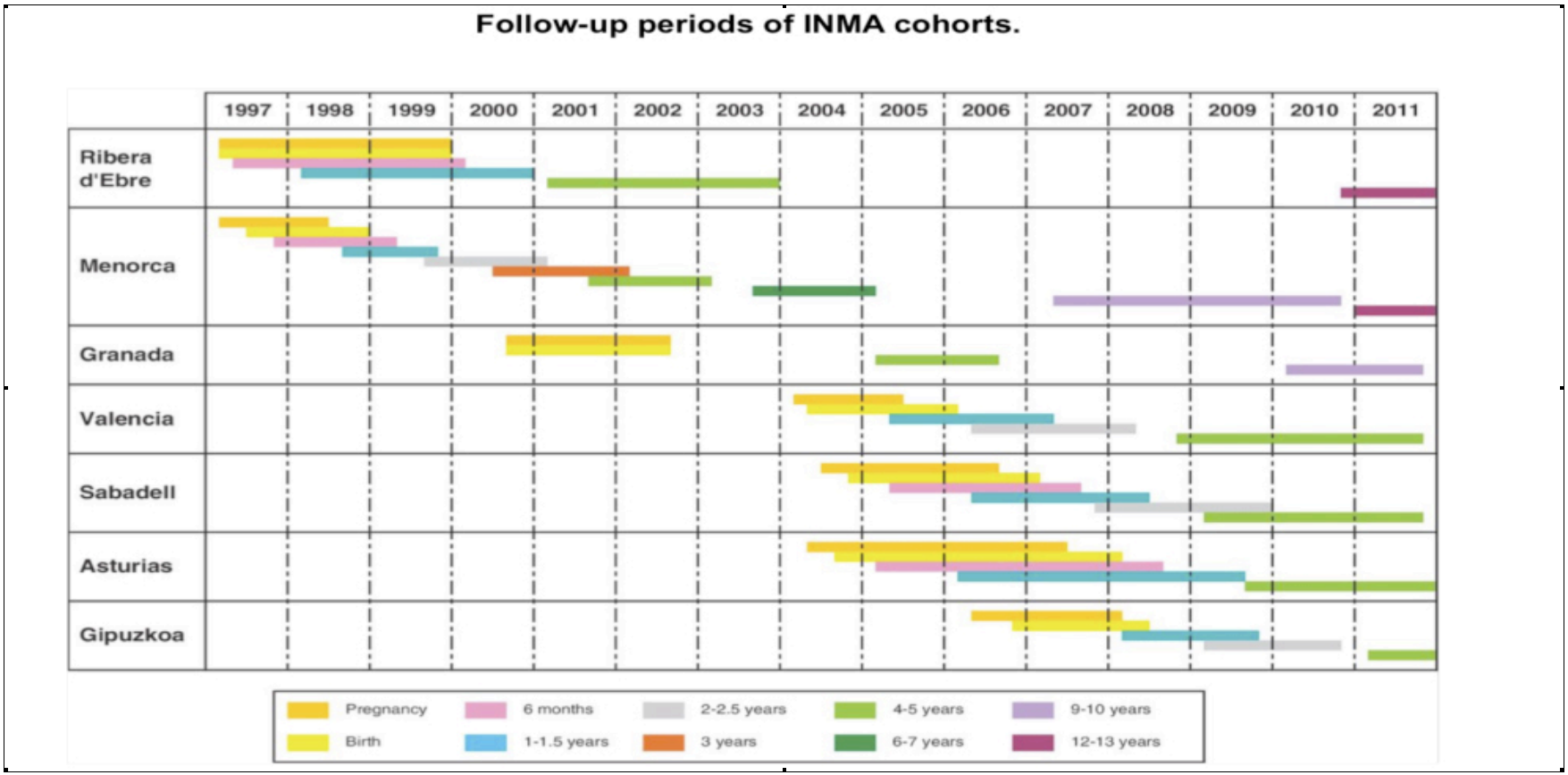

Figure 6. Follow-up periods of INMA cohorts. Adapted from Guxens et al. [261]

Material and methods

Project Viva

Project Viva is a longitudinal pre-birth cohort aimed to examine the extent to which events during early development affect health outcomes over a lifetime.
Recruitment of women took place at their first prenatal visit (median 9.9 weeks of gestation) from April 1999 to November 2002 from a multi-specialty group practice in eastern Massachusetts (Atrius Harvard Vanguard Medical Center in Massachusetts (USA)).

Eligibility criteria included fluency in English, gestational age < 22 weeks at the initial prenatal clinical appointment, and singleton pregnancy. Full details of the study are described by Oken et al.[262].

All procedures were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki and all participants provided written informed consent, and institutional review boards of participating institutions approved the study protocol.

We recruited 2670 women (64% of those who were asked for participation), of those 2128 were still enrolled at birth and deliveried a live infant.
The mother and their children have been followed up from pregnancy to adolescence. Viva trained staff performed in-person study visits with participants at 1^{st} and 2^{nd} trimesters of pregnancy, and with mothers and children the first days after delivery, in infancy at 6 months, in early childhood (median 3.2 years) and mid-childhood (median 7.7 years). Mothers completed mailed questionnaires at 1, 2, 4, 5 and 6 years. Viva trained staff performed interviews and collected samples, and assessed anthropometry, blood pressure and cognitive development.

Participant flow of Project Viva from pregnancy to mid-childhood is described in Figure 7.

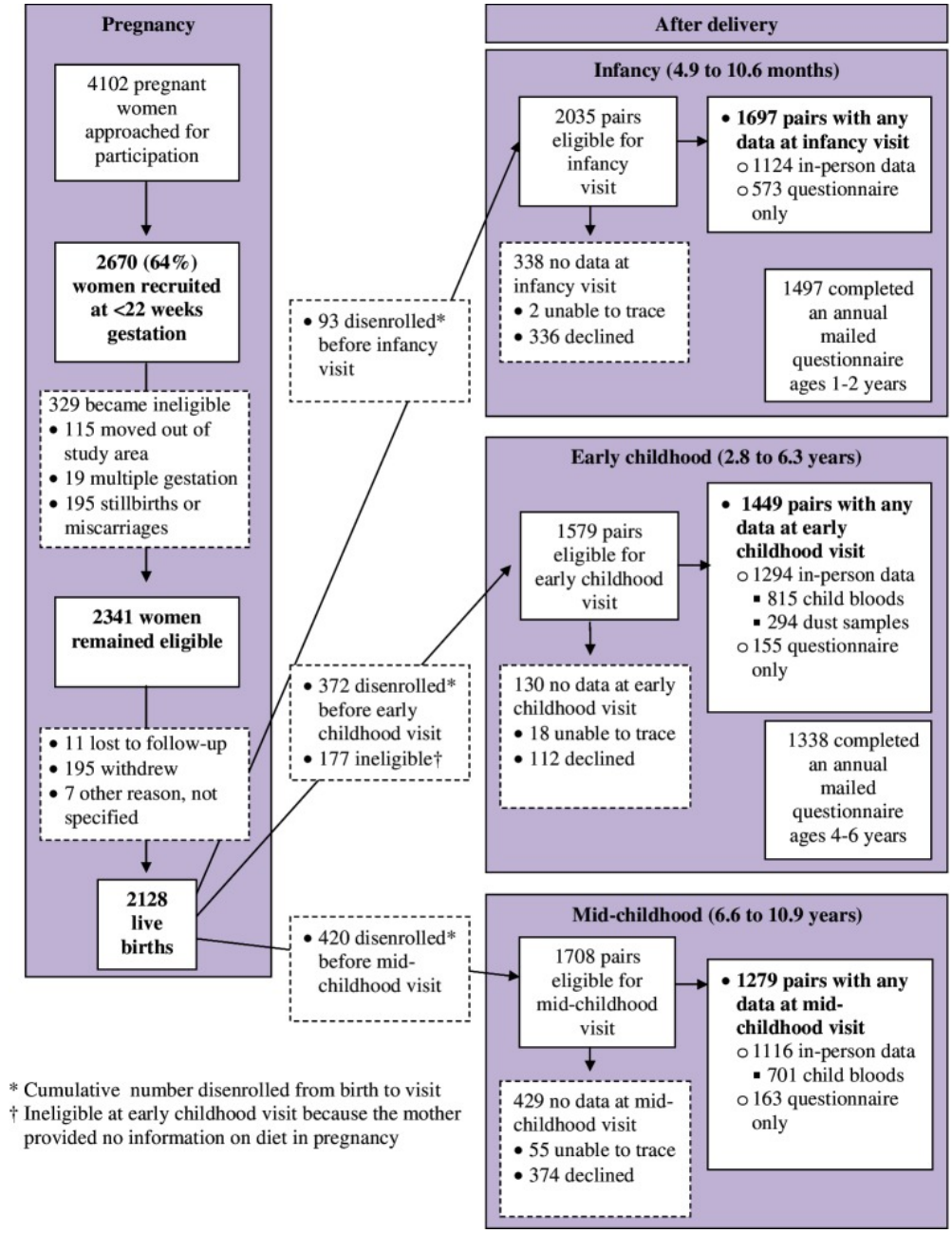

Figure 7. Participant flow from recruitment through mid-childhood in the Project Viva cohort. Adapted from Oken et al. [262]

4.2 Participants

We used different study samples to assess the objectives of this thesis and they are described as follow:

In order to identify potential modifiable early life risk factors for offspring childhood general and abdominal obesity at mid-childhood (**1st objective**) and to examine the extent to which combination of modifiable prenatal and postnatal risk factors predict childhood general and abdominal obesity at mid-childhood (**2nd objective**), we used data from participants in INMA project and Project Viva.

In INMA project, we used data from Sabadell (n=657, women recruited at birth were not included (n=120)) and Valencia (n=855), since they were the only INMA cohorts with available data on the exposure and outcome variables of interest. We included 981 mother-child pairs who attended pregnancy visits and the 7 year visit. We excluded participants diagnosed with diabetes mellitus prior pregnancy. The final analysis sample was 979 (64.7% of initially enrolled) pairs of mothers and children.

In Project Viva, we recruited 2128 women who delivered a live singleton infant; we included 1116 participants who provided information during pregnancy and at the 7-10 years visit. We excluded participants with diabetes mellitus prior pregnancy. The final analytic sample was 1108 (52.1% of initially enrolled) mother-child pairs.

We used only data from INMA project at 4 years for the 3 remaining objectives: to examine the association between maternal adherence to the Mediterranean Diet during pregnancy and risk of general and abdominal obesity (**3rd objective**), cardiometabolic risk score and biomarkers at pre-school age (**4th objective**) and early life growth trajectories (**5th objective**).

We included participants from 4 INMA cohorts Sabadell (n=777), Valencia (n=855), Asturias (n=494) and Gipuzkoa (n=638). Of those we limited our analyses to those who had data on the exposure variables (adherence to the Mediterranean Diet) and the outcome variables.

To accomplish our **3rd objective,** we included 1906 (69% of initially recruited) and further we excluded 79 babies who babies who were born preterm (< 37 weeks of gestation) because preterm babies may follow different catch-up growth curves. The final analytic sample was 1827 (66.1% of initially enrolled) pairs of mothers and children.

To accomplish our **4th objective**, we included 968 (35.0% of initially recruited) and we excluded mothers who were diagnosed of diabetes prior pregnancy. The final analytic population included was 964 (34.9% of initially recruited) pairs of mothers and children.

For the **5th objective** we included 2762 participants and we excluded the ones without available data on exposure or outcome variables. The final analytic population was 2244 (81.2% of initially recruited).

4.3 Instruments

4.3.1 Exposure assessment

4.3.1.1 Smoking during pregnancy

Data on smoking habits at the beginning of pregnancy was collected through questionnaires at 1st and 3rd trimesters in INMA Project and 1st and 2nd trimester in Project Viva. For the analyses, we categorized the variable as women who smoked during pregnancy versus women who never smoked or quit before pregnancy.

4.3.1.2 Gestational weight gain

We asked mothers to report their pre-pregnancy weight and we collected all weights measured during prenatal visits from medical records. We calculated gestational weight gain by subtracting the pre-pregnancy weigh from the last prenatal weight before delivery.
We classified women into excessive gestational weight gain versus inadequate or adequate gestational weight gain according to US Institute of Medicine guidelines [263].

4.3.1.3 Gestational diabetes mellitus

We obtained information about gestational diabetes mellitus from medical records, clinicians routinely screened women using a glycemic challenge test, that consisted in blood samples 1 hour after an oral glucose load of 50 g. If the blood glucose was >140 mg/dL, they conducted a fasting 3-h 100-g oral glucose tolerance test.

The cut-off for diagnoses of gestational diabetes mellitus differed in the two studies. In INMA Project, following the Spanish Society of Obstetrics and Gynecology recommendations women were considered with impaired glucose tolerance with one abnormal value and diagnosed with gestational diabetes mellitus if two or more of the oral glucose tolerance test glucose levels exceeded the National Diabetes Data Group criteria: fasting >105 mg/dL, 1-hour >190 mg/dL, 2-hour >165 mg/dL or 3-hour >145mg/dL [264]. For the analyses we

dichotomized the variable as gestational diabetes mellitus versus not gestational diabetes mellitus (including impaired glucose tolerance or normal glucose).

In Project Viva, abnormal oral glucose tolerance test results were blood glucose >95 mg/dL at baseline, >180 mg/dL at 1h, >155 mg/dL at 2h, or >140 mg/dL at 3h based on Carpenter and Coustan [265, 266]. We categorized women with 2 or more abnormal values as having gestational diabetes mellitus and one abnormal value as impaired glucose tolerance [267].

In a previous analysis conducted in Project Viva, male offspring of gestational diabetes mellitus mothers had increased adiposity, whereas female offspring of impaired glucose tolerance (but not gestational diabetes mellitus) mothers had increased adiposity [268]. For this reason in the current analyses for Viva participants we considered abnormal glucose as gestational diabetes mellitus for mothers of male offspring, and impaired glucose tolerance for mothers of female offspring.

4.3.1.4 Maternal Dietary assessment

Maternal diet was assessed using a food-frequency questionnaire specific for each cohort study. We provided a description of the food-frequency questionnaire for each study:

In INMA project, we used a 101-item food-frequency questionnaire. The questionnaire was an adapted version of Willett's questionnaire developed and validated for use among adults and pregnant women living in Spain with satisfactory coefficients for validity and reproducibility [269].
At the 1st trimester, we asked mother about their diet during the last 3 months (from last menstrual period to week 12 of pregnancy) and at the 3rd trimester about their diet from the 1st visit up to the 3rd trimester (from week 12 to week 32 of pregnancy). Standard units and serving sizes were specified for each food item. The questionnaire included 9 possible answers to determine frequency of intake, ranging from 'never or less than once per month' to 'six or more times per day'. The response to each food item was converted to average daily intake for each participant. Nutrient values and total energy intake were obtained from the US Department of Agriculture food composition tables and other published sources [270, 271]. We calculated nutrient intakes by multiplying the frequency of consumption for each food item by the nutrient composition of the portion size

specified on the food frequency questionnaire and by addition across all foods to obtain total intake of each nutrient for each individual.

In Project Viva, we used a semiquantitative food frequency questionnaire adapted for pregnancy from the same questionnaire from which INMA project adapted its questionnaire, calibrated during pregnancy by comparing dietary intakes obtained using the questionnaire against blood levels of several nutrients [272-274].
The food frequency questionnaire included 146 questions about the average frequency of consumption of specified foods. The food frequency questionnaire was administered in the 1st and 2nd trimester of pregnancy, to assess their intake in the 1st trimester, we asked women about their intake from the woman's last menstrual period until she completed the food frequency questionnaire, and for the 2nd trimester, we asked for the intake in the previous 3 months.
Standard units and serving sizes were specified for each food item. To calculate nutrient intakes, we multiplied the frequency of use of each food by the nutrient composition of specified portions. We then summed the nutrients across all foods to obtain a total nutrient intake for each participant. To obtain estimates of nutrients, we used our nutrient composition database that is based primarily on US Department of Agriculture food composition tables and is continually supplemented by other published sources and personal communications from laboratories and manufacturers [270].

4.3.1.4.1 Mediterranean Dietary pattern

Mediterranean Dietary pattern was derived using *a priori* technique. We used the **relative Mediterranean Diet Score (rMED),** a variation of the original Mediterranean Diet score [275, 276], developed in a Spanish population to assess the adherence to the Mediterranean Diet [277].
The rMED was constructed with the average dietary data from the 1st and 3rd trimester of pregnancy, in order to represent long term dietary habits in pregnancy. The score is calculated based on the consumption of 9 typical components of the Mediterranean Diet (Table 7). To adapt the score to pregnant women, the component on alcohol consumption was removed because the recommendation is to avoid alcohol consumption during pregnancy and most of the women did not consumed it in our study; hence only 8 food groups were considered. The consumption of vegetables (excluding potatoes), fruits & nuts, cereals, legumes, fish, olive oil, total meat (including red and processed meat)

and dairy products were measured as grams per 1,000kcal/day and were divided into tertiles. A value of 0, 1, and 2 was assigned to the intake tertiles, positively scoring higher intakes for the 6 components presumed to fit the Mediterranean Diet. The scoring was reversed for 2 components presumed not to fit the Mediterranean Diet (meat and dairy) positively scoring lower intakes. After summing-up scores of each component, the final score range was 0-16; It was further divided into tertiles to identify those with low, medium and high adherence to the Mediterranean Diet.

Table 7. Relative Mediterranean Diet Score (rMED) components and scoring

Components	Scoring
Vegetables	+
Fruits and nuts	+
Cereals	+
Legumes	+
Fish	+
Olive oil	+
Total meat	-
Dairy products	-

4.3.1.4.2 Sugar-sweetened beverages

We obtained information on sugar-sweetened beverages (SSB) from the previously described food frequency questionnaires. For the analyses, the definition and categorization of the variable of sugar-sweetened beverages differed between cohorts and they are described as follows:

In INMA Project, we defined sugar-sweetened beverages as sugar-sweetened soda. The food frequency questionnaire included questions about juice consumption including fruit drinks and 100% juice together, but we could not differentiate between them. Therefore, we included only soda intake in the definition of sugar-sweetened beverages. We categorized the variable as ≥1 versus <1 SSBs/day the mean between 1st and 3rd trimester.

In Project Viva, we defined sugar-sweetened beverages intake as sugar-sweetened soda and fruit drink intake. Since the sugar-sweetened beverage is more frequent in US population and we included fruit drinks in the definition, the

cut-off was higher in this cohort. We categorized the variable as ≥2 versus <2 SSBs/day in the 2nd trimester.

4.3.1.5 Cesarean section

We obtained information about type of delivery from electronic hospital records.
In these analyses, we categorized type of delivery as cesarean section versus vaginal delivery.

4.3.1.6 Breastfeeding duration

We collected information on breastfeeding duration through questionnaires administered at 6 and 14 months in INMA project and 6, 12 and 24 months in Project Viva. We asked mothers whether they were still breastfeeding, and if they had stopped, the children's age at cessation.
We categorized duration of any breastfeeding as <12 months versus ≥12 months, according the recommendations of the American Academy of Pediatrics [278].

4.3.1.7 Age at introduction of solid foods

We collected age at introduction of solid foods through questionnaires administered to the mothers at 14 months in INMA project, and 6 and 12 months in Project Viva. For the analyses the categorization of the variable of age at introduction of solid foods differed between cohorts and they are described as follows:

In INMA project, we categorized age at introduction of solid foods based on the WHO that recommended breastfeeding for at least 6 months. Therefore, short breastfeeding was considered < 6 months versus ≥ 6 months [279].

In Project Viva, we categorized early solid food introduction as <4 months (among the formula feed) vs. ≥4 months in Project Viva (including breastfeed infants), based on the findings of a previous study from Project Viva [249].

4.3.1.8 Daily infant sleep

We assessed infant sleep duration by parental questionnaires at 2 years of age (in INMA project) and 6 and 12 months (in Project Viva). We asked parents to quantify the average daily sleep hours of their children.

We categorized the variable of daily infant sleep at the 1 year: <12 hours/day versus ≥12 hours/day in Project Viva, and <11 hours/day versus ≥11 hours/day in INMA project at 2 years of age. These are the minimum hours recommended by the National Sleep Foundation (USA) for these ages [280].

4.3.1.9 Rapid infant weight gain

In INMA project, we used repeated weight measures from birth to 6 months of age extracted from medical records. For children without weight measurement available within±14 days of their exact 6-month anniversary (10.2% of children), we used preexisting sex-specific growth models developed to predict weight at 6 months of age [281]. We used age- and sex- specific z-scores for weight-for-age at birth and at 6 months of age using the WHO referent [21]. We calculated the difference between weight-for-age z-score at birth and at 6 months.

In Project Viva, trained staff weighed infants at 6 months with a digital scale (Seca Model 881; Seca Corporation, Hamburg, Germany) and measured length at birth and 6 months with a Shorr measuring board (Shorr Productions, Olney, MD). We calculated the difference between weight-for-age z-scores at birth and at 6 months from the US CDC growth chart data [282].

In these analyses, we ranked the variable infant weight gain in cohort-specific quartiles, and considered the highest quartile as rapid infant weight gain.

4.3.2 Outcome assessment

4.3.2.1 Child Anthropometry

4.3.2.1.1 Body Mass Index and obesity risk

Study trained staff measured child weight (nearest gram) and height (nearest 0.1 cm) at 4 and 7 years of age using standard protocols (without shoes and in light clothing), with an electronic scale and calibrated stadiometer.
We used 2 time points as an outcome for the different objectives:

At 4 years, we used data only in participants of INMA project. We used data on age and sex-specific child BMI (weight/lenght^2) based on the WHO referent [283]. Overweight was defined as a BMI equal to or above the 85th percentile, and obesity as BMI z-score equal or above the 95th percentile.

At 7 years, we used data of both cohorts (INMA project and Project Viva). We calculated age- and sex-specific BMI z-scores using CDC growth standards [282].

4.3.2.1.2 Waist circumference and abdominal obesity risk

Study trained staff measured waist circumference at the nearest 0.1 cm using an inelastic tape (SECA model 201; SECA, Hamburg, Germany in INMA project and Harpendem HSK-BI, CE-0120 in Project Viva), at the midpoint between the lowest rib margin and the iliac crest, in a standing position and after a gentle expiration, using standard protocols. This measurement was obtained at 4 years of age only in INMA, and at 7 years of age in both cohorts. This measurement was not obtained in the INMA-Gipuzkoa cohort, and hence Gipuzkoa was excluded from the analyses using waist circumference.
We used 2 time points as an outcome for the different objectives:

At 4 years, we used sex-specific z-scores of waist circumference based on our sample distribution. Waist circumference >90th sex-specific percentile (of the sample distribution) was considered increased risk for abdominal obesity [284]. In sensitivity analysis we used waist-to-height ratio (WHtR; i.e. waist circumference in cm/height in cm) and the cut-off WHtR >0.5 as increased risk for abdominal obesity [285].

At 7 years, we calculated WHtR multiplied by 100 in both cohorts, in order to better interpretation of the results.

4.3.2.1.3 Fat mass index and fat-free mass index

Trained staff measured total body fat with DXA (Hologic model Discovery A) in Project Viva at 7 years of age. In INMA project, no data on body composition (with DXA) were available.
We calculated fat mass index (FMI: kg/m^2) and fat-free mass index (FFMI; kg/m^2). We calculated sex-specific internal z-scores for FMI and FFMI.

4.3.2.2 Cardiometabolic risk and related biomarkers

We used data on cardiometabolic risk and related biomarkers only from INMA project. The methods are described as follows:

4.3.2.2.1 Blood pressure

Study trained personnel measured systolic and diastolic blood pressure at 4 years once after five minutes of rest at the primary health center, using a digital automatic monitor (OMRON CPII) and a special cuff adjusted to the upper right arm size of the children. We calculated the age, sex, height and cohort specific z-score systolic and diastolic blood pressure, since the measures may vary according to child age, sex and height. The measures were standardized for INMA specific cohorts (Sabadell, Valencia, Asturias, but not available in Gipuzkoa) to account for the regional differences in the INMA project [286]. We calculated blood pressure using the average of z-score diastolic and systolic blood pressure [287].

4.3.2.2.2 Lipids

Trained staff measured lipid levels using non-fasting blood samples at 4 years of age in INMA project. The lipids we measured were: high-density lipoprotein (HDL) and triglycerides (TG) using standard analytical techniques (ABX-Pentra 400; Horiba Medical; Japan). We calculated the age, sex and cohort specific z-scores for these biomarkers, to account for potential variations between age, sex and cohorts. We used these variables as continuous.

4.3.2.2.3 Other biomarkers

Trained staff measured other child biomarkers of interest at 4 years of age in specific INMA cohorts.

Leptin, a hormone secreted by adipocytes, was assessed in two INMA cohorts, in Sabadell by Luminex method and a subsample by ELISA methods and in Gipuzkoa by ELISA method. We found high correlations between samples analyzed by both methods in Sabadell ($r=0.84$; p-value<0.001). We calculated sex, age and cohort specific leptin z-scores in order to standardize the data.

C-peptide is a pancreatic peptide in the proinsulin molecule, and it was measured in two INMA cohorts, in Sabadell by Luminex and in Gipuzkoa by ELISA method. We calculated sex, age and cohort specific c-peptide z-scores.

We measured **adiponectin** (antidiabetic hormone) in Sabadell cohort using standard analytical techniques (Luminex). We calculated sex and age specific adiponectin z-scores.

The apolipoproteins **Apo A-1** and **Apo B** were measured in Sabadell by using standard analytical techniques (ABX-Pentra 400; Horiba Medical; Japan). We calculated sex and age specific z-scores.

The inflammatory biomarker **Interleukin 6** (IL-6) was measured in two cohorts: Sabadell by Luminex method and in Gipuzkoa by ELISA method. We calculated sex, age and cohort specific IL 6 z-scores.

We measured **C-reactive protein** (CRP), an inflammatory biomarker, in the four new INMA cohorts by using turbidimetry, except for Sabadell that we used ABX-Pentra 401. In all the cohorts we measured high sensitivity CRP, except for Valencia that was standard CRP. We calculated sex, age and cohort specific CRP z-scores.

4.3.2.2.4 Cardiometabolic risk score

We assessed the metabolic risk at 4 years of age by calculating a continuous **cardiometabolic risk score**. We calculated our cardiometabolic risk score using the IDEFICS study definition because they have a study population similar to ours

(i.e. European and in early and mid-childhood) [287]. To be as consistent as possible with Ahrens et al. 2014 we used the sum of the age, sex and cohort specific z-scores for the individual metabolic risk score components: waist circumference, blood pressure and lipids. In the risk score we used waist circumference as an indicator of central adiposity. The blood pressure z-score (additionally adjusted by height) was used as an indicator of blood pressure (see previous section 4.3.2.2.1) and the mean of TG and of the inverse of HDL (because a higher HDL is a favorable outcome) as an indicator of lipid levels. We did not have information on glucose or insulin levels so we could not include this parameter in our score. We only calculated the cardiometabolic risk score in the population with available data for waist circumference, blood pressure, TG and HDL at 4 years of age (n=958). Population from Gipuzkoa cohort was not included since measurements of waist circumference and blood pressure were not taken. Finally, a higher metabolic score indicated a higher metabolic risk at 4 years of age.

Table 8. Components of the Cardiometabolic risk score for children

Abdominal adiposity	z-score waist circumference[1]
Blood pressure	Average between z-score systolic blood pressure and z-score diastolic blood pressure[2]
Lipids	Average of TG and inverse HDL[1]
Glucose	Not available

Based on Ahrens et al. [287].
[1]Age, sex and cohort specific z-scores.
[2]Age, sex, height and cohort specific z-scores.

We created a continuous **lipid score**, which included 2 components: BMI and lipids. In the lipid score we used BMI z-score based on the WHO referents as an indicator of general obesity [288]. We used the average between TG and the inverse of HDL as an indicator of lipid levels.
We created this score for its simplicity and in order to include data from all the INMA-cohorts.

4.3.2.3 Child longitudinal growth trajectories

We used BMI z-scores based on the WHO referents [288]. Further, we developed child longitudinal BMI z-score trajectories from birth to 4 years of age using latent

class growth analysis only in INMA cohort. This method relaxes the assumption that a single growth curve can explain sufficiently an entire population and instead allows parameter differences to be captured across unobserved subpopulations by assuming a number of discrete latent classes.

Growth classes were constructed using these BMI z-scores, where each class corresponded to a different growth pattern. Each subject is classified to one latent class. Based on Akaike Information Criterion and Bayesian Information Criterion, a model with 5 growth classes was determined to have the best fit for our given data.

4.3.2.4 Covariates

We recorded other covariates that based on previous knowledge were considered *a priori* as a potential confounding factors, mediators or effect modificators in some of the associations studied in this thesis.

We collected information through interviews and questionnaires on maternal social class in INMA project (occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the International ISCO88 coding system; I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled) [289], household income in Project Viva (<$40000, $40000-$70000, >$70000), maternal educational level (primary or less, secondary, university degree), maternal physical activity during pregnancy collected at 1st an 3rd trimesters as self-perception (total and leisure-time physical activity in pregnancy in metabolic equivalent (METS) and also classified in sedentary, little active, moderately active and quite-very active), maternal age at delivery, breastfeeding duration and predominant breastfeeding (as continous in weeks and categorical: none, >0-16 weeks, 16-24 weeks, >24 weeks), child birth weight, INMA project parental country of origin, Project Viva child race/ethnicity and child age at anthropometry measurements and biomarkers collection. Information on child sex and gestational age at birth was obtained from clinical records

Table 9. Summary of the variables included in the study

	1st objective	2nd objective	3rd objective	4th objective	5th objective
Birth cohort	Viva (n=1108) and INMA-Sabadell and Valencia (n=979)	Viva (n=1108) and INMA-Sabadell and Valencia (n=979)	INMA-Sabadell, Valencia, Asturias and Gipuzkoa (n=1827)	INMA- Sabadell, Valencia, Asturias and Gipuzkoa (n=964)	INMA - Sabadell, Valencia, Asturias and Gipuzkoa (n=2244)
Exposure					
Gestational weight gain	√	√			
Gestational diabetes mellitus	√	√			
Smoking during pregnancy	√	√			
Sugar-sweetened beverages	√	√			
Cesarean section	√	√			
Breastfeeding duration	√	√			
Age at introduction of solids	√	√			
Daily infant sleep	√	√			
Rapid infant weight gain	√	√			
Mediterranean Diet rMED			√	√	√
Outcome (age)					
BMI / obesity	√ (7 years)	√ (7 years)	√ (4 years)		
Waist circumference / Waist-to-height ratio / abdominal adiposity	√ (7 years)	√ (7 years)	√ (4 years)		
Fat mass and fat-free mass	√ (7 years – Only Viva)	√ (7 years – Only Viva)			
Cardiometabolic risk score				√ (4 years)	
Lipid score				√ (4 years)	
SPB, DPB				√ (4 years)	
HDL, TG				√ (4 years)	
Leptin, C-peptide				√ (4 years)	
Adiponectin, Apo A-1, Apo B				√ (4 years)	
IL-6, CRP				√ (4 years)	
Growth trajectories					√ (birth to up to 4 years)

rMED: relative Mediterranean Diet score; BMI: Body Mass Index; SPB: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HDL: High Density Cholesterol; TG: Triglycerides; Apo: Apolipoprotein; IL-6: Interleukin 6; CRP: C-reactive protein

4.4 Statistical analysis

Descriptive statistics were used to compare sociodemographic characteristics of mothers and children included in this thesis. Differences in categorical variables were assessed using the X^2 test, differences in continuous variables were analyzed by ANOVA.

Moreover, we applied different statistical analyses for each objective:

-To identify potential modifiable early life risk factors for offspring childhood general and abdominal obesity at mid-childhood (1st objective):

In this analysis we included data from 2 INMA project cohorts (Sabadell and Valencia) and Project Viva. We included 9 risk factors during pre– and postnatal period that have been associated with higher childhood obesity and are potentially modifiable: gestational weight gain, gestational diabetes mellitus, smoking during pregnancy, sugar-sweetened beverages intake, cesarean section, breastfeeding duration, age of introduction of solid foods, daily infant sleep and rapid infant weight gain. We dichotomized each of them for analytic simplicity.
We used multivariable linear regression models to explore the associations between the 9 dichotomous risk factors and the outcomes (BMI z-score and WHtR (by 100)) at 7 years of age. We ran a regression model for each of the risk factors with the outcomes, and we further adjusted them by 5 confounders that are not modifiable once pregnancy commences: maternal pre-pregnancy BMI and education level, and child sex, race/ethnicity, as well as age at the outcome measurement and plus region in INMA. Then, we included the 9 risk factors in a single model with and without adjustment with the same 5 non-modifiable covariates. We also examined additional potential confounders: maternal age in both cohorts and maternal socio economic status (in INMA project) and household income (in Project Viva). Since their inclusion did not materially change the effect estimates for the 9 risk factors, we did not include them in the final models. In both cohorts, to account for missing data, we performed multiple imputation for all mother-child pairs. We then limited the analysis to the included participants. We imputed 50 values for each missing observation and combined multivariable modeling estimates using Proc MI ANALYZE (SAS).

-To examine the extent to which combination of modifiable prenatal and postnatal risk factors predict childhood general and abdominal obesity at mid-childhood in two different populations in USA and Spain (2nd objective):

In this analysis we included data from 2 INMA project cohorts (Sabadell and Valencia) and Project Viva. We created 512 combinations of the 9 dichotomous risk factors previously described and we used parameter estimates from the multivariable linear regression models to estimate the predicted mean of BMI z-score and WHtR, for each of the combinations. To estimate predicted means, we needed to choose fixed values for the confounders, so we selected a "hybrid" participant: we used cohort-specific percents for maternal education (INMA project 32% and Project Viva 68% college graduate), sex (50% female in both cohorts), and in INMA region (50% Sabadell) and in Viva child race/ethnicity (63% white). We used mean values for pre-pregnancy BMI (INMA project 23.8 kg/m^2 and Project Viva 24.7 kg/m^2) and child age (INMA project 7.2 years and Project Viva 8.0 years).

For each outcome, we sorted its predicted mean by number of risk factors from 0 to ≥6, we truncated the higher number of risk factors into ≥6 because of the low prevalence in our populations. We looked at the distribution of the outcomes of the 512 combinations, and we displayed for each number of factors the combinations that gave the highest and lowest predicted mean, and also the most prevalent combination for each number of risk factors.

We also ran multivariable linear models and prediction models for FMI z-score and FFMI z-score in Project Viva only, as data on these outcomes were not available in INMA project.

To account for missing data, we performed multiple imputation as described above.

-To examine the association between maternal adherence to the Mediterranean Diet during pregnancy and risk of general and abdominal obesity at pre-school age (3rd objective):

In this analysis we included data from all new INMA project cohorts. Simple and multiple linear regression models were used to estimate the β coefficients for the association between rMED in pregnancy (expressed both in tertiles and as continuous variables per two-points increment) and BMI z-scores and WC of children at 4 years of age. Three models with different levels of adjustment were used. Only covariates which influenced the association between the exposure and

outcome of interest were used for adjustment in the most adjusted model (model 3), following the backward stepwise method ($p < 0.2$). Variables that were shown to be plausible mediators of the association in mediation analyses were not included in any of the studied models (i.e. breastfeeding, for models evaluating BMI z-scores and gestational diabetes, for models evaluating waist circumference). Model 1 was a crude model for both outcomes. For the association between rMED and BMI z-score: Model 2 was a minimally adjusted model including maternal total energy intake, region, child sex and child age at anthropometric measurements. Model 3 was further adjusted for educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months. For the association between rMED and waist circumference: Model 2 was a minimally adjusted model including maternal total energy intake, region, child sex, child height and child age at anthropometric measurements. Model 3 was further adjusted for educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.

Logistic regression models were run to study the association between rMED and the odds of offspring having overweight (including obesity) (BMI z-score≥ 85^{th} percentile) and to have abdominal obesity (waist circumference >90th percentile) at 4 years of age.

Models were also stratified by child sex, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal physical activity, maternal social class, educational level and infant birth weight in order to evaluate the homogeneity of effects between these subgroups of *a priori* interest. The statistical significance of interaction terms involving the exposures and these stratification variables was assessed.

To assess heterogeneity among regions in the association between rMED ad BMI z-score and the waist circumference of offspring, regions-specific estimates were calculated by using general linear models, and random-effect meta-analyses (I^2) were used to pool the estimates.

As sensitivity analyses, we repeated all analyses using alternate Mediterranean Diet Score (aMED), another score of adherence of Mediterranean Diet developed to be applied to the US population [290], we also used the rMED in the 1^{st} and

3rd trimester of pregnancy as different exposures. We compared results defining overweight using weight-for-length age- and sex-specific z-scores [283], the IOTF criteria [291], and abdominal obesity using waist-to-height ratio. We also repeated BMI models excluding INMA-Gipuzkoa cohort from the analyses. As complementary analysis we repeated all analyses further adjusting for child diet (rMED, fiber, and fruit and vegetable intake, in different models) measured at 4 years of age. However, effects estimates did not change substantially.

Some individuals had missing values in covariables and multiple imputation was performed using chained equations, 20 completed data sets were generated and analyzed by using the standard combination rules for multiple imputations [292].

-To evaluate the association between maternal adherence to the Mediterranean Diet during pregnancy and cardiometabolic biomarkers at pre-school age (4th objective):

In this analysis, we included data from participant of all INMA project cohorts with available data of cardiometabolic biomarkers. We used simple and multiple linear regression models to estimate the β coefficients for the association between rMED in pregnancy (expressed in tertiles) and cardiometabolic risk score, lipid score and cardiometabolic biomarkers at 4 years of age. We used three models with different levels of adjustment. We selected covariates which influenced the association between the exposure and outcome of interest following the backward stepwise method ($p < 0.2$). Variables that were shown to be plausible mediators of the association in mediation analyses were included in model 3 (i.e. infant birth weight, breastfeeding and growth trajectories). Model 1 was a minimally adjusted model for all outcomes including child age, sex and region. Model 2 was a fully adjusted model. Since the models had specific adjustments for each outcome, the description of the covariables included in the models can be found in the respectively tables. For cardiometabolic risk score and lipid score in table 21; SBD, DBP, HDL and TG in table 22; leptin, c-peptide, adiponectin, Apo A-1 and Apo B in table 23 and IL-6 and CRP in table 24. For all the outcomes, model 3 included covariables included in model 2 and further adjusted for potential mediators.

As sensitivity analyses, we repeated all analyses using aMED, another score of adherence of Mediterranean Diet developed to be applied to the US population, we also used the rMED in the 1st and 3rd trimester [290].

To account for missing data, we performed multiple imputation as described in the previous section [292].

- To evaluate the association between maternal Mediterranean Diet during pregnancy and child longitudinal growth trajectories from birth to up to 4 years (5th objective):

We included data from INMA cohort in this analysis. We used multinomial logistic regression models to assess the association between maternal rMED (in tertiles) in the 3rd trimester and child early life growth trajectories from birth to up to 4 years. We used this regression model because our outcome variable had 5 categories. The reference category was Class 4, which represented the average birth size-slower growth trajectory. We exponentiated the effect estimates from the models to calculate relative risk ratios.

Three models with different levels of adjustment were used. Only covariates which influenced the association between the exposure and outcome of interest were used for adjustment, following the backward stepwise method ($p < 0.2$). Model 1 was a minimally adjusted model including child sex, age and region. Model 2 was adjusted by child sex, age, cohort, maternal age, maternal pre-pregnancy BMI, gestational weight gain, smoking during pregnancy, maternal total energy intake and parity. Model 3 was further adjusted for gestational age.
As sensitivity analyses, we repeated all analyses using rMED at 1st trimester, average rMED between the 1st and 3rd trimester and aMED [290].

To account for missing data, we performed multiple imputation as described in the previous section [292].

All statistical analyses were performed using the statistical package STATA 12.1 (Stata Corporation, College Station, TX) and SAS version 9.3 software (SAS Institute, Cary, NC).

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RESULTS

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EARLY LIFE FACTORS AND CHILDHOOD OBESITY DEVELOPMENT
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5. RESULTS

In this section, we provide description of the different results according to the specific aims of the doctoral thesis:

5.1 Identification of potential modifiable early life risk factors for offspring childhood general and abdominal obesity at mid-childhood (1st objective)

Table 10 shows the participant characteristics of Project Viva (n=1108) and INMA project (n=979). There were some differences between the participants of these cohorts. Project Viva mothers had higher pre-pregnancy BMI (mean 24.7 vs. 23.7 kg/m^2) and were more educated (67.9% vs. 31.0% college graduates) and Project Viva children were older (mean 8.0 vs. 7.2 years) and their mean BMI z-score were lower compared to Project INMA (mean 0.39 vs. 0.58 units). In both cohorts, women excluded from the analysis sample were younger, less educated, and more likely to smoke during pregnancy.

Prevalence of the modifiable risk factors of interest differed between the cohorts (**Table 10**). For example, Project Viva mothers had lower proportion of smoking during pregnancy compared with INMA project mothers (9.8% vs. 32.0%) but a higher proportion of excessive gestational weight gain (57.7% vs. 40.40%) and regular sugar sweetened beverage intake (7.7% vs. 4.0%). Project Viva children had a higher proportion of short sleep duration than the INMA project children (32.1% vs. 20.1%).

Table 10. Participant characteristics in Project Viva and INMA project

	Project Viva n=1108	INMA project n=979
Risk factors	n (%)	
Prenatal smoking		
No	1 000 (90.2)	665 (68.0)
Yes	108 (9.8)	314 (32.0)
Gestational weight gain		
Inadequate or adequate	469 (42.3)	588 (60.0)
Excessive	639 (57.7)	391 (40.0)
GDM or IGT		
No	1 068 (96.4)	922 (94.2)
Yes	40 (3.6)	57 (5.8)
Excessive sugar-sweetened beverage intake		
No	1 023 (92.3)	940 (96.0)
Yes	85 (7.7)	39 (4.0)
Cesarean section delivery		
No	862 (77.8)	791 (80.8)
Yes	246 (22.2)	188 (19.2)
Breastfeeding duration (months)		
≥12	311 (28.1)	201 (20.5)
<12	797 (71.9)	778 (79.5)
Early introduction of solid foods		
No	971 (87.7)	174 (17.8)
Yes	137 (12.3)	805 (82.2)
Short infant sleep duration		
No	752 (67.9)	782 (79.9)
Yes	356 (32.1)	197 (20.1)
Weight gain birth to 6 months		
Quartiles 1-3	831 (75.0)	733 (74.9)
Quartile 4	277 (25.0)	246 (25.1)
Children characteristics	Mean (SD)	
BMI z-score	0.39 (1.00)	0.58 (1.05)
FMI (kg/m^2)	4.4 (1.9)	-
FFMI (kg/m^2)	13.0 (1.4)	-
Waist circumference, cm	60.0 (8.3)	58.3 (7.1)
Waist-to-height ratio	46.6 (5.2)	47.0 (4.8)
Covariates		
Maternal BMI (kg/m^2)	24.7 (5.3)	23.7 (4.4)
Child Age (years)	8.0 (0.9)	7.2 (0.5)
	n (%)	
College graduate	752 (67.9)	304 (31.0)
Child race/ethnicity white	704 (63.6)	936 (95.6)
Female	553 (49.9)	474 (48.4)

GDM: Gestational diabetes mellitus; IGT: Impaired Glucose Tolerance; BMI: Body Mass Index; FMI: Fat Mass Index; FFMI: Fat-free Mass Index

In **Table 11,** we show the mutually adjusted associations of each of the 9 risk factors (prenatal smoking, gestational weight gain, gestational diabetes mellitus, sugar-sweetened beverages intake, cesarean section, breastfeeding duration, age at introduction of solid foods, short infant sleep duration and rapid infant weight gain) with child BMI z-score and WHtR in both cohorts at 7 years of age.

In Project Viva, all of 9 risk factors were individually positively associated with the outcomes when exploring the confounder-adjusted effect of each risk factor (data not shown), but some of the estimates were attenuated when including all risk factors in the model.

In contrast in INMA Project, only 4 out of the 9 were associated with BMI z-score: prenatal smoking, excessive gestational weight gain, short infant sleep duration, and rapid infant weight gain. The same risk factors were associated with WHtR except for short infant sleep duration (β: 0.52; 95%CI: -0.22, 1.27). Moreover, the presence of gestational diabetes mellitus was also associated with WHtR (β: 1.50; 95%CI: 0.01, 2.99).

The risk factor that was most strongly associated with the outcomes in both cohorts was rapid infant weight gain (BMI z-score β: 0.44; 95%CI: 0.31, 0.57 in Project Viva, and BMI z-score β: 0.33; 95%CI: 0.18, 0.48 in INMA project). In both cohorts, excessive gestational weight gain was associated with BMI z-score and gestational diabetes was associated with WHtR.

Table 11. Associations between 9 risk factors and anthropometric measures at mid-childhood in Project Viva and INMA project.

	Project Viva		INMA project	
	BMI z-score	WHtR[1]	BMI z-score	WHtR[1]
Maternal factors	Adjusted estimate (95% CI)			
Prenatal smoking	0.04 (-0.15, 0.24)	0.61 (-0.41, 1.64)	0.20 (0.06, 0.34)	0.90 (0.25, 1.56)
Excessive Gestational weight gain	0.18 (0.07, 0.29)	0.39 (-0.21, 0.98)	0.20 (0.06, 0.33)	0.68 (0.05, 1.31)
GDM or IGT	0.10 (-0.19, 0.39)	1.35 (-0.19, 2.90)	0.06 (-0.25, 0.38)	1.50 (0.01, 2.99)
Excessive sugar-sweetened beverage intake	0.19 (-0.05, 0.43)	1.03 (-0.28, 2.34)	0.00 (-0.33, 0.33)	-0.78 (-2.29, 0.73)
Cesarean section delivery	0.12 (-0.01, 0.25)	0.15 (-0.55, 0.85)	-0.06 (-0.22, 0.11)	-0.07 (-0.84, 0.70)
Infancy factors				
Breastfeeding duration (<12 months)	0.12 (-0.01, 0.25)	0.48 (-0.23, 1.19)	0.01 (-0.15, 0.18)	-0.56 (-1.32, 0.21)
Early introduction of solid foods	0.16 (-0.03, 0.35)	1.02 (-0.09, 2.12)	-0.10 (-0.27, 0.07)	0.12 (-0.69, 0.93)
Short infant sleep duration	0.20 (0.07, 0.33)	1.05 (0.32, 1.78)	0.14 (-0.02, 0.30)	0.52 (-0.22, 1.27)
Weight gain from to birth to 6 months (4^{th} Quartile)	0.44 (0.31, 0.57)	1.57 (0.88, 2.26)	0.33 (0.18, 0.48)	0.95 (0.23, 1.67)

GDM: Gestational diabetes mellitus; IGT: Impaired Glucose Tolerance; BMI: Body Mass Index; WHtR: Waist-to-height ratio
Multivariable linear model adjusted for all nine risk factors plus maternal pre-pregnancy BMI and education and child age, sex and race/ethnicity (Project Viva) and region (INMA project).
[1]Waist-to-height ratio multiplied by 100.

The mutually adjusted associations of the 9 risk factors with FMI z-score and FFMI z-score outcomes were assessed only in Project Viva. The estimates of the associations are shown in **table 12** and they were generally similar to those for BMI z-score. An exception was gestational diabetes mellitus, which showed a stronger association with FMI z-score than with BMI z-score and this association remained after adjustment for all risk factors and covariates, including maternal pre-pregnancy BMI ((BMI z-score (β: 0.10; 95%CI: -0.19, 0.39) vs. FMI z-score (β: 0.29; 95%CI: 0.00, 0.59)).

Table 12. Associations between 9 risk factors and anthropometric measures at mid-childhood in Project Viva.

	FMI z-score	FFMI z-score
	Adjusted estimate (95% CI)	
Maternal factors		
Prenatal smoking	0.15 (-0.05, 0.34)	0.02 (-0.17, 0.21)
Excessive Gestational weight gain	0.06 (-0.05, 0.17)	0.11 (0.00, 0.22)
IGT or GDM	0.29 (0.00, 0.59)	-0.06 (-0.35, 0.23)
Excessive sugar-sweetened beverage intake	0.19 (-0.06, 0.44)	0.27 (0.02, 0.52)
Cesarean-section	0.07 (-0.06, 0.20)	0.10 (-0.03, 0.23)
Infancy factors		
Breastfeeding duration (<12 months)	0.12 (-0.02, 0.26)	0.05 (-0.08, 0.18)
Early introduction of solid foods	0.20 (-0.02, 0.41)	0.20 (0.00, 0.41)
Short infant sleep duration	0.17 (0.03, 0.31)	0.11 (-0.02, 0.25)
Weight gain from birth to 6 months (4^{th} Quartile)	0.27 (0.14, 0.40)	0.40 (0.27, 0.53)

FMI: Fat Mass Index; FFMI: Fat-free Mass Index; GDM: Gestational diabetes mellitus; IGT: Impaired Glucose Tolerance; BMI: Body Mass Index; Multiple linear model adjusted for all the risk factors and maternal BMI, maternal education status, child race/ethnicity, child sex and age.

5.2 Examination of the extent to which combination of modifiable prenatal and postnatal risk factors predicts childhood general and abdominal obesity at mid-childhood (2nd objective)

We created combinations of the 9 risk factors (in total 512 combinations), and we modeled the predicted mean of the outcomes for the 9 risk factors. In **Figure 8**, we show a summary of the 512 combinations. For each number of factors, we show the combinations that predict the lowest and highest child BMI z-score, and also the most prevalent combination. In Project Viva, the range of predicted BMI z-score was -0.02 to 1.52 for 0 to 9 risk factors (**Table 13A**). In INMA project, the predicted BMI z-score was 0.41 to 1.20 for 0 to 9 risk factors (**Table 13B**). In INMA project, the highest predicted BMI-z was 1.36 for participants with the combination of 7 risk factors (all except for cesarean section and introduction of solid foods).

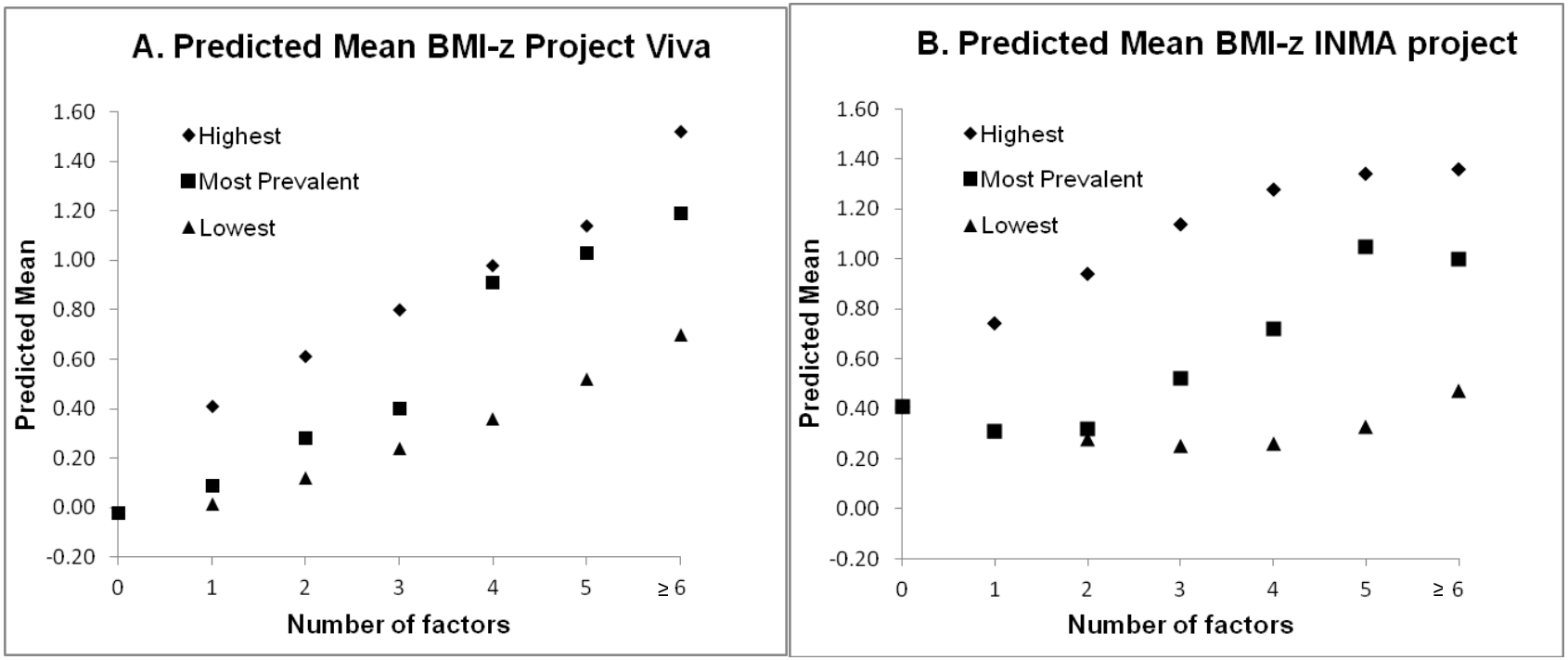

Figure 8. Predicted means of Body Mass Index (BMI) z-score at mid-childhood according to selected combinations of 9 early life risk factors

Summary of the distribution of the outcomes of the 512 combinations: for each number of factors the combinations with the highest and lowest predicted mean, and the most prevalent combination.

Results

Table 13A. Predicted means of BMI z-score at mid-childhood according to selected combinations of 9 early life risk factors in Project Viva

Number of factors	0		1			2			3			4			5			≥6	
		Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prevt	Highest Pred	Lowest Pred	Most Prevt	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred
C - section	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	+	+	+
SSB	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+
IGT or GDM	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+
Smoking	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+
Excessive GWG	-	-	-	-	-	+	-	-	+	-	-	+	+	-	+	+	+	+	+
BF<12 months	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	+
Introduced solids <4 months	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+
Infant sleep<12 h/d	-	-	-	-	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
4thQ of infant weight gain	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
Pred, BMI z-score	-0.02	0.02	0.09	0.41	0.12	0.28	0.61	0.24	0.40	0.80	0.36	0.91	0.98	0.52	1.03	1.14	0.70	1.19	1.52
Prevalence in this cohort (%)	4.65	0.10	9.16	1.39	0.01	12.22	0.92	0.02	4.43	0.06	0.00	1.35	0.01	0.00	0.78	0.00	0.00	0.26	0.00

Lowest pred: The lowest predicted mean with this number of factors; Most Prev: The most prevalent combination with this number of factors; Highest Pred: The Highest Predicted mean with this number of factor. BMI: Body Mass Index; C-section: Cesarean section; SSB: Sugar-sweetened Beverages; GWG: Gestational Weight Gain; BF: Breastfeeding duration

Table 13B. Predicted means of BMI z-score at mid-childhood according to selected combinations of 9 early life risk factors in INMA project

Number of factors	0		1			2			3			4			5			≥6	
		Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred
C - section	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-
SSB	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	+
GDM	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+
Smoking	-	-	-	-	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
Excessive GWG	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+
BF<12 months	-	-	-	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	+
Introduced solids <6 months	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
Infant sleep<11 h/d	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+
4thQ of infant weight gain	-	-	-	+	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+
Pred, BMI z-score	0.41	0.31	0.31	0.74	0.25	0.32	0.94	0.25	0.52	1.14	0.26	0.72	1.28	0.33	1.05	1.34	0.47	1.00	1.36
Prevalence in this cohort (%)	2.15	2.94	2.94	0.68	0.39	14.44	0.10	2.00	6.95	0.31	0.10	3.68	0.00	0.00	1.90	0.00	0.00	1.00	0.00

Lowest pred: The lowest predicted mean with this number of factors; Most Prev: The most prevalent combination with this number of factors; Highest Pred: The Highest Predicted mean with this number of factor. BMI: Body Mass Index; C-section: Cesarean section; SSB: Sugar-sweetened Beverages; GWG: Gestational Weight Gain; BF: Breastfeeding duration

Results

We show the predicted mean WHtR for the summary of the 512 combinations of the 9 risk factors in **Figure 9**. The predicted WHtR ranged from 45.0 to 52.6 in Project Viva and from 46.4 to 49.7 in INMA project for 0 to 9 risk factors (**Table 14A and 14B**). Combinations with the same number of risk factors predicted different WHtR means in different cohorts and within the same cohort. For example, in INMA project the combination of gestational diabetes mellitus, smoking during pregnancy, and rapid infant weight gain predicted a child mean WHtR of 49.7, while in the same cohort the presence of sugar-sweetened beverage intake, cesarean section and introduction of solid foods predicted child mean WHtR of 45.0.

In the sensitivity analyses, we explored the use of the Project Viva cut-offs for the exposures in INMA project, rather than INMA specific cut-offs and the estimates were similar.

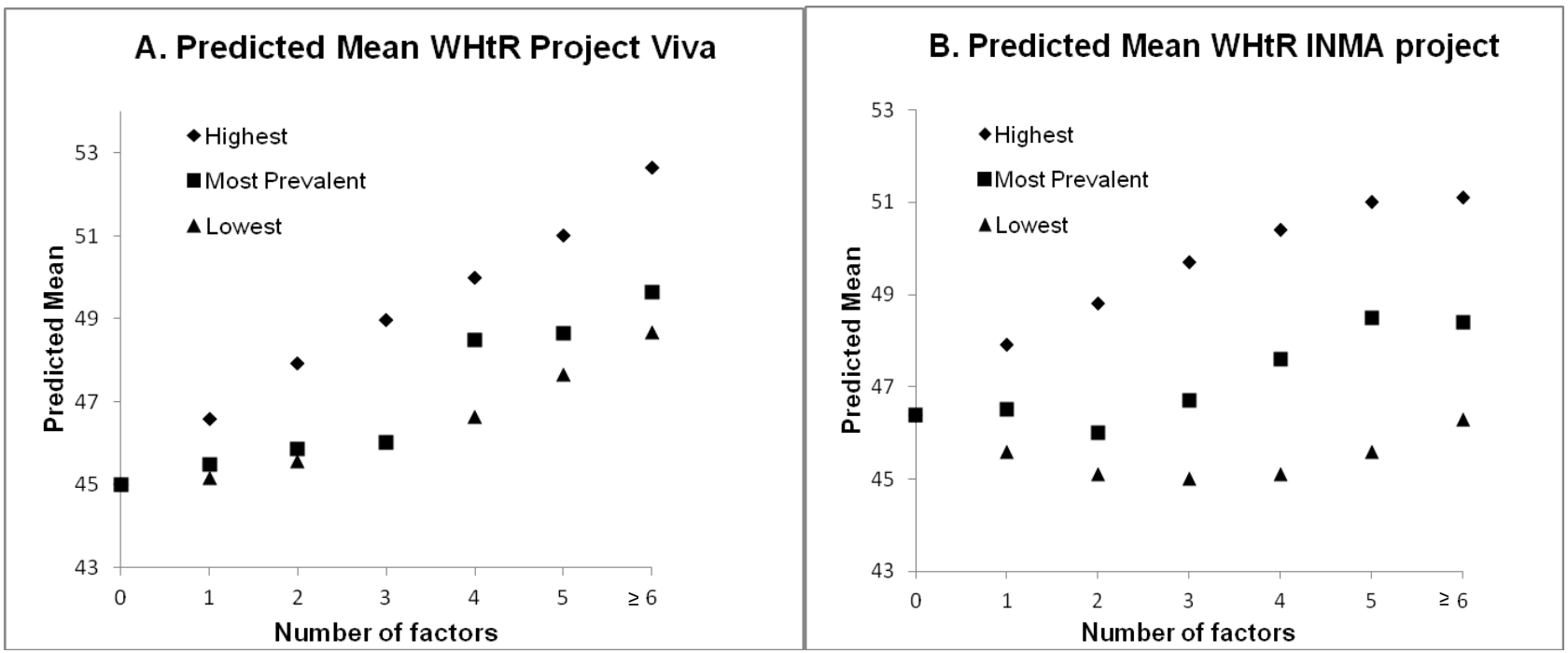

Figure 9. Predicted means of Waist-to-height ratio (WHtR) at mid-childhood according to selected combinations of 9 early life risk factors

Summary of the distribution of the outcomes of the 512 combinations: for each number of factors the combinations with the highest and lowest predicted mean, and the most prevalent combination.

Results

Table 14A. Predicted means of WHtR at mid-childhood according to selected combinations of 9 early life risk factors in Project Viva

Number of factors	0	1			2			3			4			5			≥6		
		Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred
C - section	-	+	-	-	+	-	-	+	+	-	+	-	-	+	+	-	+	+	+
SSB	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+
IGT or GDM	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Smoking	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+
Excessive GWG	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+
BF<12 months	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	+
Introduced solids <4 months	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+
Infant sleep<12 h/d	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	+	+
4thQ of infant weight gain	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
Pred, WHtR	45.0	45.2	45.5	46.6	45.5	45.9	47.9	46.0	46.0	49.0	46.6	48.5	50.0	47.6	48.6	51.0	48.7	49.6	52.6
Prevalence in this cohort (%)	4.65	0.57	9.16	1.39	1.72	12.22	0.00	4.43	4.43	0.00	0.34	1.35	0.00	0.19	0.78	0.00	0.00	0.26	0.00

Lowest pred: The lowest predicted mean with this number of factors; Most Prev: The most prevalent combination with this number of factors; Highest Pred: The Highest Predicted mean with this number of factor. WHtR: Waist-to-Height ratio WHtR; C-section: Cesarean section; SSB: Sugar-sweetened Beverages; GWG: Gestational Weight Gain; BF: Breastfeeding duration*WHtR multiplied by 100 to make the values more identifiable.

Table 14B. Predicted means of WHtR at mid-childhood according to selected combinations of 9 early life risk factors INMA project

Number of factors	0	1			2			3			4			5			≥6		
		Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred
C - section	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	+	-
SSB	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
GDM	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Smoking	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	+	+
Excessive GWG	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	+
BF<12 months	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-
Introduced solids <6 months	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	+
Infant sleep<11 h/d	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+
4thQ of infant weight gain	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+
Pred, WHtR	46.4	45.6	46.5	47.9	45.1	46.0	48.8	45.0	46.7	49.7	45.1	47.6	50.4	45.6	48.5	51.0	46.3	48.4	51.1
Prevalence in this cohort (%)	2.15	0.00	2.94	0.26	0.00	14.44	0.01	0.25	6.95	0.00	0.10	3.68	0.00	0.00	1.90	0.00	0.00	1.00	0.00

Lowest pred: The lowest predicted mean with this number of factors; Most Prev: The most prevalent combination with this number of factors; Highest Pred: The Highest Predicted mean with this number of factor. WHtR: Waist-to-Height ratio WHtR; C-section: Cesarean section; SSB: Sugar-sweetened Beverages; GWG: Gestational Weight Gain; BF: Breastfeeding duration*WHtR multiplied by 100 to make the values more identifiable.

Figure 10 show the predicted mean FMI z-score and FFMI z-score for the summary of the 512 combinations of the 9 risk factors in Project Viva. Data on FMI and FFMI was not available in INMA project.

The predicted mean FMI z-score ranged from -0.30 to 1.22 for 0 to 9 risk factors (**Table 15A**), whereas the predicted mean FFMI z-score range from -0.28 to 0.99 (**Table 15B**), for 0 to 8 risk factors (all except gestational diabetes mellitus). The most prevalent combination in this cohort was gestational weight gain and short breastfeeding duration (12.22%).

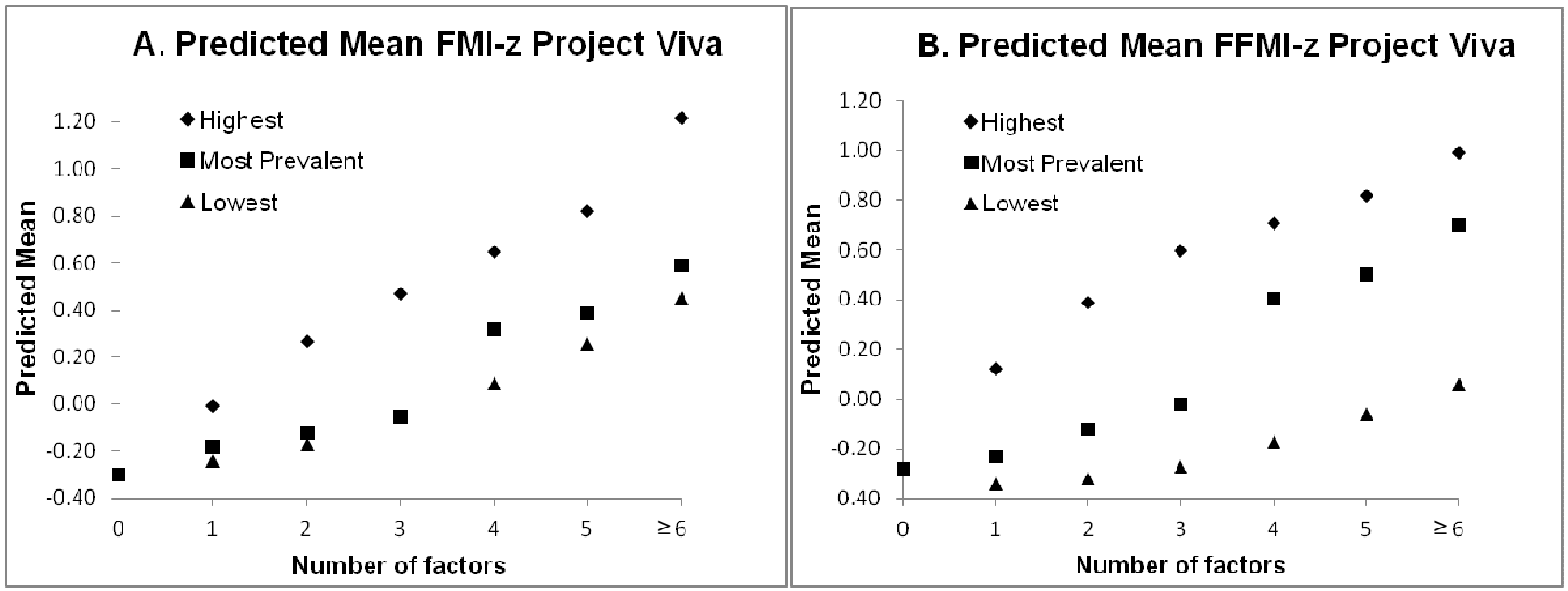

Figure 10. Predicted means of Fat Mass Index (FMI) z-score and Fat-Free Mass Index (FFMI) z-score at mid-childhood according to selected combinations of 9 early life risk factors

Summary of the distribution of the outcomes of the 512 combinations: for each number of factors the combinations with the highest and lowest predicted mean, and the most prevalent combination.

Results

Table 15A. Predicted means of FMI z-score at mid-childhood according to selected combinations of 9 early life risk factors in Project Viva

Number of factors	0		1			2			3			4			5			≥6	
		Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred
C - section	-	-	-	-	+	-	-	+	+	-	+	-	-	+	+	-	+	+	+
SSB	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+
IGT or GDM	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Smoking	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+
Excessive GWG	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+
BF<12 months	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	+
Introduced solids <4 months	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+
Infant sleep<12 h/d	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+
4thQ of infant weight gain	-	-	-	-	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
Pred, FMI z-score	-0.30	-0.24	-0.18	-0.01	-0.17	-0.12	0.27	-0.05	-0.05	0.47	0.09	0.32	0.65	0.26	0.39	0.82	0.45	0.59	1.22
Prevalence in this cohort (%)	4.65	0.10	9.16	1.39	0.01	12.22	0.92	0.02	4.43	0.06	0.00	1.35	0.01	0.00	0.78	0.00	0.00	0.26	0.00

Lowest pred: The lowest predicted mean with this number of factors; Most Prev: The most prevalent combination with this number of factors; Highest Pred: The Highest Predicted mean with this number of factor. FMI: Fat Mass Index; FFMI: Fat-free Mass Index; C-section: Cesarean section; SSB: Sugar-sweetened Beverages; GWG: Gestational Weight Gain; BF: Breastfeeding duration

Table 15B Predicted means of FFMI z-score at mid-childhood according to selected combinations of 9 early life risk factors Project Viva

Number of factors	0		1			2			3			4			5			>6	
		Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred	Lowest Pred	Most Prev	Highest Pred
C - section	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	+	+	+
SSB	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
IGT or GDM	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
Smoking	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+
Excessive GWG	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	+	+	+	+
BF<12 months	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	+
Introduced solids <4 months	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+
Infant sleep<12 h/d	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
4thQ of infant weight gain	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+
Pred, FMI z-score	-0.28	-0.34	-0.23	0.12	-0.32	-0.12	0.39	-0.27	-0.02	0.60	-0.17	0.40	0.71	-0.06	0.50	0.82	0.06	0.70	0.99
Prevalence in this cohort (%)	4.65	0.10	9.16	1.39	0.01	12.22	0.92	0.02	4.43	0.06	0.00	1.35	0.01	0.00	0.78	0.00	0.00	0.26	0.00

Lowest pred: The lowest predicted mean with this number of factors; Most Prev: The most prevalent combination with this number of factors; Highest Pred: The Highest Predicted mean with this number of factor. FMI: Fat Mass Index; FFMI: Fat-free Mass Index; C-section: Cesarean section; SSB: Sugar-sweetened Beverages; GWG: Gestational Weight Gain; BF: Breastfeeding duration

5.3 Association between maternal adherence to the Mediterranean Diet during pregnancy and risk of general and abdominal obesity at pre-school age (3rd objective)

Table 16 shows the characteristics of mothers and offspring of INMA project (n=1827), according to categories of weight status of children.
In our sample 16.3% of the children were overweighed and 12.4% were obese according the World Health Organization criteria, and 14.2% overweighed and 5.7% obese according the IOTF [291]. Mothers of obese children had higher pre-pregnancy BMI on average. Furthermore, overweight and obese children had higher birth weight on average compared to normal weight children. The prevalence of obesity was higher in the region of Asturias (24.3%) and lower in the region of Sabadell (20.8%). Prevalence of obesity was higher in boys whereas prevalence of overweight was higher in girls.

We compared characteristics between mothers included in the study and those lost to follow-up; those lost to follow-up were younger and had lower social class and education level.

Table 16. Characteristics of mothers and children, by weight status of children at 4 years of age in INMA project

	n= 1827	Normal weight (n=1303, 71.3%)	Overweight (n=298, 16.3 %)	Obese (n=226, 12.4 %)	P
Child characteristics					
Sex (%)					
Male	941	53.1	40.3	57.1	< 0.001
Female	886	46.9	59.7	42.9	
Age years*	1827	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	0.268
Region (%)					
Asturias	385	20.3	22.5	24.3	0.002
Gipuzkoa	395	19.7	29.2	23.9	
Sabadell	474	28.2	20.8	20.8	
Valencia	563	31.8	27.5	31.0	
Birth weight*	1794	3307.0	3423.4	3450.0 (362.5)	< 0.001
Breastfeeding duration (%)					
0 weeks	259	14.9	13.1	13.5	0.686
>0-16 weeks	437	25.3	21.7	22.5	
>16-24 weeks	286	15.8	16.6	15.8	
>24 weeks	812	44.0	48.6	48.2	
Maternal characteristics					
Age at delivery*	1827	31.0 (4.1)	30.7 (4.1)	31.1 (4.0)	0.567
Smoking in pregnancy (%)					
No cig/d	1493	83.8	82.9	79.5	0.233
1-4 cig/d	110	5.4	6.5	9.8	
>4-7 cig/d	93	5.0	6.2	4.9	
>7 cig/d	100	5.8	4.5	5.8	
Social class (%)					
I+II	441	25.0	23.8	19.5	0.289
III	507	28.2	26.9	26.6	
IV+V	878	46.8	49.3	54.0	
Education level (%)					
Primary or less	383	20.4	22.8	22.1	0.376
Secondary	762	41.7	39.3	46.0	
University	678	38.0	37.9	31.9	
Physical activity in pregnancy (%)					
Sedentary	131	7.3	6.1	8.4	0.213
Little active	464	25.3	24.2	29.8	
Moderately active	736	40.1	41.6	42.7	
Quite-Very active	478	27.3	28.0	19.1	
Pre-pregnancy BMI*	1827	23.1 (3.9)	24.1 (4.3)	25.9 (5.0)	< 0.001
Pregnancy weight gain, kg*	1776	13.7 (4.9)	13.6 (5.0)	14.4 (6.0)	0.119
Pregnancy EI, kcal*	1827	2074.4	2067.4	2024.8 (470.7)	0.325
Diabetes (%)					
None	1340	84.8	84.9	83.7	0.691
Impaired glucose tolerance	160	10.4	10.2	8.4	
Gestational DM	78	4.6	4.6	7.4	
DM before pregnancy	5	0.3	0.4	0.5	
rMED*	1827	8.0 (2.6)	8.2 (2.6)	8.0 (2.4)	0.504

* mean (s.d.) Variables defined as: overweight: BMI z-score >85th percentile - ≤95th percentile; obese: BMI z-score >95th percentile of WHO reference.
Abbreviations: BMI: body mass index; EI: Energy Intake; DM: diabetes mellitus. rMED: relative Mediterranean Diet Score.

Results

Table 17 presents the association between maternal adherence to the Mediterranean Diet in pregnancy, using rMED, and child BMI z-score and waist circumference at 4 years of age. The score rMED was not significantly associated with BMI z-score, in any of the studied models. There was an inverse association between the highest tertile of rMED and waist circumference (model 3: β:-0.62; 95%CI: -1.10, -0.14; *P* for trend = 0.009) and also between 2 units increase of rMED and waist circumference (model 3: β:-0.18; 95%CI: -0.33, -0.03), in models 2 and 3.

Table 17. Association between maternal relative Mediterranean Diet score in pregnancy and Body Mass Index (BMI, z-score) and Waist Circumference (WC, cm) at 4 years of age in INMA project.

rMED Range:	**Low T_1 (1-7)**	**Medium T_2 (8-9)**	**High T_3 (10-15)**		**2 units increase**
		***β* (95% CI)**	***β* (95% CI)**	***P for trend***	***β* (95% CI)**
BMI[1]	**(n=1827)**				
Model 1	Ref	0.00 (-0.11, 0.12)	-0.01 (-0.13, 0.10)	0.827	0.01 (-0.03, 0.04)
Model 2	Ref	-0.02 (-0.13, 0.10)	-0.07 (-0.20, 0.05)	0.255	-0.01 (-0.05, 0.03)
Model 3	Ref	-0.06 (-0.17, 0.05)	-0.09 (-0.20, 0.02)	0.113	-0.02 (-0.06, 0.01)
WC[2]	**(n=1398)**				
Model 1	Ref	0.13 (-0.42, 0.69)	-0.05 (-0.53, -0.64)	0.806	0.12 (-0.07, 0.30)
Model 2	Ref	-0.26(-0.73, 0.20)	-0.57 (-1.07, -0.07)	0.024	-0.15 (-0.31, 0.00)
Model 3	Ref	-0.34 (-0.78, 0.11)	-0.62(-1.10, -0.14)	0.009	-0.18 (-0.33, -0.03)

BMI: Body Mass Index z-score; WC: Waist Circumference.
rMED: relative Mediterranean Diet Score: range from 1-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet.
Model 1: Crude model. General linear regressions with no adjustments.
[1]Model 2: Model 1 further adjusted for child sex, region, child age, maternal total energy intake.
Model 3: Model 2 further adjusted for educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.
[2]Model 2: Model 1 further adjusted for child sex, region, child age, maternal total energy intake and child height.
Model 3: Model 2 further adjusted for educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.

We found no association between rMED in pregnancy and odds of overweight at 4 years of age; there was an inverse association between rMED and odds of abdominal obesity, but it was not statistically significant (**Table 18**).

Table 18. Association between maternal relative Mediterranean Diet score in pregnancy and odds of having overweight and abdominal obesity at 4 years of age in INMA project

rMED Range:	**Low T_1 (1-7)**	**Medium T_2 (8-9)**	**High T_3 (10-15)**		**2 units increase**
		***β* (95% CI)**	***β* (95% CI)**	***P for trend***	***β* (95% CI)**
Overweight[1] (n=1827)					
Model 1	Ref	1.06 (0.83, 1.35)	1.08 (0.85, 1.39)	0.506	1.04 (0.96, 1.12)
Model 2	Ref	0.99 (0.78, 1.28)	0.97 (0.75, 1.26)	0.833	1.00 (0.92, 1.09)
Model 3	Ref	0.88 (0.67, 1.15)	0.94 (0.71, 1.24)	0.596	0.98 (0.89, 1.07)
Abdominal obesity[2] (n=1398)					
Model 1	Ref	0.95 (0.63, 1.41)	0.82 (0.53, 1.27)	0.378	1.00 (0.87, 1.15)
Model 2	Ref	0.90 (0.58, 1.39)	0.62 (0.38, 1.02)	0.068	0.89 (0.48, 1.04)
Model 3	Ref	0.84 (0.53, 1.32)	0.62 (0.37, 1.03)	0.064	0.89 (0.76, 1.05)

rMED: relative Mediterranean Diet Score: range from 1-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet.
Variables defined as: general obesity: BMI >85th percentile of WHO reference; abdominal obesity: Waist Circumference> 90th percentile distribution of the sample.
Model 1: Crude model. Logistic regressions with no adjustments.
[1]Model 2: Model 1 further adjusted for child sex, region, child age, maternal total energy intake.
Model 3: Model 2 further adjusted for educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.
[2]Model 2: Model 1 further adjusted for child sex, region, child age, maternal total energy intake and child height.
Model 3: Model 2 further adjusted for educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.

Results

Table 19 shows stratified analyses run by different variables of interest. No evidence of effect modification in the associations between maternal rMED and child BMI z-score at 4 years was observed after stratification selected variables. All results were homogeneous among the four regions (I^2=0.0%) (data not shown).

Table 19. Association between relative Mediterranean Diet score (per 2 units increment) and Body Mass Index (BMI, z-score) and Waist Circumference (WC, cm) at 4 years of age by population subgroups in INMA project.

	BMI (z-score)[1]			WC[2] (cm)		
	n	**β (95% CI)**	P for interaction[3]	**n**	**β (95% CI)**	P for interaction[3]
Pre-pregnancy BMI						
<25 kg/m^2	966	-0.01 (-0.06, 0.03)	0.574	876	-0.10 (-0.29, 0.08)	0.746
>25 kg/m^2	364	-0.04 (-0.13, 0.06)		350	-0.21 (-0.54, 0.12)	
Smoking in pregnancy						
No	1094	-0.01 (-0.06, 0.03)	0.680	1001	-0.05 (-0.23, 0.12)	0.266
Yes	236	-0.03 (-0.14, 0.07)		225	-0.32 (-0.72, 0.09)	
Physical activity in pregnancy						
No	435	0.00 (-0.07, 0.08)	0.483	420	-0.20 (-0.49, 0.09)	0.482
Yes	895	-0.02 (-0.07, 0.03)		800	-0.06 (-0.26, 0.13)	
Social class						
Low	674	-0.01 (-0.06, 0.05)	0.735	595	-0.13 (-0.36, 0.10)	0.927
High	655	-0.01 (-0.07, 0.05)		630	-0.07 (-0.30, 0.15)	
Education level						
Primary or less	285	-0.05 (-0.14, 0.05)	0.187	292	-0.15 (-0.48, 0.18)	0.974
Secondary	572	0.02 (-0.04, 0.09)		538	-0.09 (-0.33, 0.14)	
University	473	-0.01 (-0.08, 0.05)		360	-0.09 (-0.39, 0.20)	
Child sex						
Male	685	-0.00 (-0.06, 0.06)	0.679	637	-0.07 (-0.30, 0.15)	0.823
Female	645	-0.02 (-0.08, 0.04)		589	-0.13 (-0.36, 0.09)	
Birth weight						
2.500 – 4.000 g	1233	-0.03 (-0.05, 0.04)	0.473	1134	-0.10 (-0.26, 0.07)	0.492
>4.000 g	80	-0.09 (-0.25, 0.07)		71	-0.30 (-1.52, 0.53)	

BMI: Body Mass Index
[1]Multiple linear regression model adjusted for child sex, region, child age, maternal total energy intake, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.
[2]Multiple linear regression model adjusted for child sex, region, child age, maternal total energy intake, child height, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.
[3]Tested by adding an interaction term in the model between the variables and rMED.

5.4 Association between maternal adherence to the Mediterranean Diet during pregnancy and cardiometabolic biomarkers at pre-school age (4th objective)

Table 20 shows the maternal and child characteristics of participants of INMA project (n=964), according to tertiles of adherence to Mediterranean Diet using the relative Mediterranean score.

Mothers who had lower adherence to Mediterranean Diet were younger (30.6 (4.2) vs. 31.7 (3.9); $p < 0.001$), had lower physical activity during pregnancy, and they had higher energy intake (2122.6 (441.9) vs. 1952.0 (403.8) kcal; $p < 0.001$) compared to women who had higher adherence to the Mediterranean Diet.

There were some differences across the four INMA cohorts; the adherence to the Mediterranean Diet was higher in Asturias and Gipuzkoa, compared to Sabadell and Valencia.

Furthermore, offspring of mothers who had lower adherence to the Mediterranean Diet were more likely to be female and received predominant breastfeeding for shorter time (11.5 (9.4) weeks vs. 14.0 (9.4); $p= 0.002$), compared to offspring of mothers who had higher adherence to the Mediterranean Diet during pregnancy.

We compared characteristics between mothers included in the study and those lost to follow-up; those lost to follow-up were younger, smoke more, and had lower social class and education level.

Table 20. Characteristics of children and mother by maternal adherence to Mediterranean Diet (rMED score) in INMA project

rMED	n=964	T_1 (n=407, 42.2 %)	T_2 (n=283, 29.4%)	T_3 (n=274, 28.4%)	P
Maternal characteristics					
Age at delivery*	963	30.6 (4.2)	31.7 (4.0)	31.7 (3.9)	< 0.001
Smoking in pregnancy					
No	799	81.3	86.4	87.3	0.063
Yes	147	18.8	13.6	12.7	
Social class (%)					
I+II	232	21.6	25.9	25.9	0.239
III	258	25.1	27.0	29.2	
IV+V	473	53.3	47.2	44.9	
Education level (%)					
Primary or less	208	25.4	19.9	17.9	0.090
Secondary	402	41.5	39.9	44.5	
University	350	33.1	40.2	37.6	
Physical activity, METS*	964	37.0 (3.1)	37.5 (2.9)	37.6 (3.2)	0.036
Pre-pregnancy BMI*	964	23.6 (4.1)	24.0 (4.4)	23.9 (4.6)	0.370
Pregnancy EI, kcal *	964	2122.6 (441.9)	2031.7 (426.5)	1952.0 (403.8)	< 0.001
Gestational weight gain*	936	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.073
Diabetes (%)					
None	925	98.0	96.8	95.6	0.185
Gestational DM (GDM)	29	2.0	3.2	4.4	
Child characteristics					
Sex (%)					
Female	483	50.4	55.8	43.8	0.018
Male	481	49.6	44.2	56.2	
Age years*	960	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	0.317
Region (%)					
Asturias	265	25.3	27.2	31.0	< 0.001
Gipuzkoa	212	14.3	21.9	33.6	
Sabadell	317	39.3	32.2	24.1	
Valencia	170	21.1	18.7	11.3	
Birth weight *	945	3339.8 (392.6)	3345.4 (412.3)	3363.7 (376.9)	0.739
Predominant breastfeeding duration*	923	11.5 (9.4)	13.6 (9.7)	14.0 (9.4)	0.002
Bmi z-score *	959	0.6 (1.0)	0.6 (1.0)	0.6 (1.0)	0.864

* mean (s.d.)
rMED: relative Mediterranean Diet Score: range from 0-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet. EI: Energy Intake; DM: diabetes mellitus. BMI: body mass index;

Table 21 presents the association between maternal adherence to the Mediterranean Diet in pregnancy, using rMED, and child cardiometabolic risk score (n=715, data not available in Gipuzkoa) and lipid score (n=958) at 4 years of age in INMA project.

We found no association between the rMED during pregnancy and the child cardiometabolic risk score in any of the studied models. Moreover, there were null associations between the rMED and the child lipid score at 4 years.

Table 21. Associations of maternal relative Mediterranean Diet score in pregnancy with metabolic risk score and lipid score at 4 years of age in INMA project

rMED Range:	**Low T_1 (1-7)**	**Medium T_2 (8-9)**	**High T_3 (10-15)**	
		***β* (95% CI)**	***β* (95% CI)**	***P for trend***
Cardiometabolic risk[1]	**(n=715)**			
Model 1	Ref	-0.01 (-0.28, 0.26)	-0.07 (-0.36, 0.23)	0.670
Model 2	Ref	0.01 (-0.26, 0.28)	-0.07 (-0.37, 0.22)	0.670
Model 3	Ref	0.11 (-0.14, 0.36)	0.03 (-0.25, 0.31)	0.719
Lipid score[2]	**(n=958)**			
Model 1	Ref	-0.01 (-0.22, 0.19)	-0.09 (-0.30, 0.13)	0.447
Model 2	Ref	-0.02 (-0.22, 0.18)	-0.12 (-0.33, 0.09)	0.286
Model 3	Ref	0.01 (-0.17, 0.19)	-0.06 (-0.25, 0.12)	0.540

rMED: relative Mediterranean Diet Score: range from 0-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet. Sex, age and cohort specific z-scores. Cariometabolic risk score: mean between inverse HDL Cholesterol z-score and Triglycerides z-score, average between height specific z-score systolic b lood pressure (SBP) and diastolic blood pressure (DBP) and waist circumference z-score. Based on IDEFICS score. Lipid score: BMI z-score and mean between z-score Triglycerides and inverse z-score HDL.
Model 1: General linear regressions adjusted for child sex, age and region.
[1]Model 2: Model 1 further adjusted for maternal educational level, maternal total energy intake, maternal pre-pregnancy BMI, gestational weight gain and maternal physical activity.
Model 3: Model 2 further adjusted for growth trajectories.
[2]Model 2: Model 1 further adjusted for maternal social class, maternal total energy intake, maternal pre-pregnancy BMI, gestational weight gain and maternal physical activity.
Model 3: Model 2 further adjusted for growth trajectories and weeks of predominant breastfeeding.

Results

Table 22 shows the association between maternal relative Mediterranean Score during pregnancy and child blood pressure and biomarkers at 4 years, all those which are components of the cardiometabolic risk score.

There were null associations between maternal rMED and child diastolic and systolic blood pressure z-score at 4 years in all the 3 models. Moreover, the rMED during pregnancy was not associated with HDL cholesterol and Triglycerides at 4 years of age.

Table 22. Associations of maternal relative Mediterranean Diet score in pregnancy with components of the offspring cardiometabolic risk score at 4 years of age in INMA project.

rMED	**Low**	**Medium**	**High**	
Range:	**T_1 (1-7)**	**T_2 (8-9)**	**T_3 (10-15)**	
		***β* (95% CI)**	***β* (95% CI)**	***P for trend***
SBP[1]	**(n=722)**			
Model 1	Ref	0.10 (-0.06, 0.25)	0.01 (-0.16, 0.18)	0.700
Model 2	Ref	0.13 (-0.13, 0.21)	0.04 (-0.13, 0.21)	0.441
Model 3	Ref	0.15 (-0.11, 0.30)	0.06 (-0.11, 0.23)	0.309
DBP[1]	**(n=722)**			
Model 1	Ref	0.05 (-0.11, 0.20)	0.00 (-0.16, 0.17)	0.881
Model 2	Ref	0.07 (-0.09, 0.22)	0.03 (-0.14, 0.20)	0.625
Model 3	Ref	0.07 (-0.08, 0.23)	0.02 (-0.15, 0.19)	0.674
HDL[2]	**(n=960)**			
Model 1	Ref	0.07 (-0.08, 0.22)	0.04 (-0.12, 0.20)	0.538
Model 2	Ref	0.07 (-0.09, 0.22)	0.05 (-0.11, 0.21)	0.514
Model 3	Ref	0.06 (-0.09, 0.22)	0.05 (-0.11, 0.21)	0.489
Triglycerides[2]	**(n=960)**			
Model 1	Ref	0.09 (-0.07, 0.24)	-0.03 (-0.19, 0.13)	0.828
Model 2	Ref	0.08 (-0.07, 0.24)	-0.04 (-0.20, 0.12)	0.725
Model 3	Ref	0.09 (-0.07, 0.24)	-0.03 (-0.19, 0.13)	0.839

rMED: relative Mediterranean Diet Score: range from 0-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet. Age, sex, height and cohort specific z-score systolic blood pressure (SBP) and diastolic blood pressure (DBP). Age, sex and cohort specific z-scores High Density Lipoprotein (HDL) and tryglicerides.
Model 1: General linear regressions adjusted for child sex, age and region.
[1]Model 2: Model 1 further adjusted for maternal social class, maternal smoking, maternal total energy intake, maternal pre-pregnancy BMI, gestational diabetes, parity and child z-score BMI at 4 years.
Model 3: Model 2 further adjusted for growth trajectories and weeks of predominant breastfeeding.
[2]Model 2: Model 1 further adjusted for maternal social class, maternal total energy intake, parity and child z-score BMI at 4 years.
Model 3: Model 2 further adjusted for weeks of predominant breastfeeding.

Table 23 shows the association between maternal relative Mediterranean Diet score during pregnancy and child biomarkers at 4 years of age in a subsample of INMA project with available data.

Although there was a modest tendency of lower leptin z score (model 2: β: -0.10; 95%CI: -0.30, 0.09) and adiponectin z-score (model 2: β: -0.52; 95%CI: -1.19, 0.14) offspring of mothers with higher rMED, these associations were not statistically significant.

We found a significant positive association between maternal rMED and child Apo A-1 at 4 years in all 3 studied models (model 2: tertile 3 β: 0.30; 95%CI: 0.08, 0.51; p for trend = 0.007). Nevertheless, there was a null association between rMED and child Apo B.

Table 23. Associations of maternal relative Mediterranean Diet score in pregnancy with offspring biomarkers at 4 years of age in a subsample of INMA project.

rMED	Low	Medium	High	
Range:	T_1 (1-7)	T_2 (8-9)	T_3 (10-15)	
		β (95% CI)	*β* (95% CI)	*P for trend*
Leptin[1]	**(n=462)**			
Model 1	Ref	0.05 (-0.18, 0.27)	-0.13 (-0.36, 0.11)	0.336
Model 2	Ref	-0.01 (-0.20, 0.17)	-0.10 (-0.30, 0.09)	0.320
Model 3	Ref	-0.02 (-0.21, 0.17)	-0.10 (-0.30, 0.10)	0.332
C-peptide[1]	**(n=462)**			
Model 1	Ref	0.03 (-0.20, 0.25)	-0.05 (-0.29, 0.18)	0.699
Model 2	Ref	-0.02 (-0.24, 0.21)	-0.07 (-0.30, 0.17)	0.579
Model 3	Ref	-0.01 (-0.24, 0.21)	-0.07 (-0.30, 0.17)	0.585
Adiponectin[1]	**(n=76)**			
Model 1	Ref	-0.11 (-0.65, 0.43)	-0.57 (-1.20, 0.05)	0.089
Model 2	Ref	-0.18 (-0.79, 0.43)	-0.52 (-1.19, 0.14)	0.122
Model 3	Ref	-0.11 (-0.77, 0.55)	-0.51 (-1.21, 0.18)	0.158
Apo A-1[2]	**(n=527)**			
Model 1	Ref	0.15 (-0.05, 0.36)	0.28 (0.07, 0.50)	0.009
Model 2	Ref	0.15 (-0.06, 0.35)	0.30 (0.08, 0.51)	0.007
Model 3	Ref	0.14 (-0.07, 0.35)	0.30 (0.08, 0.52)	0.006
Apo B[2]	**(n=527)**			
Model 1	Ref	0.13 (-0.08, 0.34)	0.03 (-0.19, 0.24)	0.723
Model 2	Ref	0.11 (-0.10, 0.33)	0.00 (-0.22, 0.22)	0.918
Model 3	Ref	0.10 (-0.11, 0.31)	-0.01 (-0.23, 0.21)	0.981

rMED: relative Mediterranean Diet Score: range from 0-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet. Age, sex and cohort specific z-scores of Leptin, C-peptide, Adiponectin, Apolipoprotein A-1 (Apo A-1) and Apolipoprotein B (Apo B).
Model 1: General linear regressions adjusted for child sex, age and region.
[1]Model 2: Model 1 further adjusted for maternal educational level, maternal smoking, maternal total energy intake, maternal pre-pregnancy BMI, gestational weight gain, parity and child z-score BMI at 4 years.
Model 3: Model 2 further adjusted for growth trajectories.
[2]Model 2: Model 1 further adjusted for maternal age, maternal smoking, maternal pre-pregnancy BMI, gestational diabetes, maternal total energy intake and parity.
Model 3: Model 2 further adjusted for growth trajectories.

We found null associations between maternal adherence to Mediterranean Diet in pregnancy and child offspring inflammatory biomarkers (Interleukin 6 and C-reactive protein) at 4 years in all the 3 studied models in a subsample of INMA project (**Table 24**).

In sensitivity analyseis, using aMED and 1st trimester and 3rd trimester rMED, the estimates of the associations between dietary patterns and cardiometabolic risk score and the biomarkers were similar (data not shown).

Table 24. Associations of maternal relative Mediterranean Diet score in pregnancy with offspring inflammatory biomarkers at 4 years of age in a subsample in INMA project.

rMED Range:	**Low T_1 (1-7)**	**Medium T_2 (8-9)**	**High T_3 (10-15)**	
		***β* (95% CI)**	***β* (95% CI)**	***P for trend***
IL-6	**(n=244)**			
Model 1	Ref	0.10 (-0.23, 0.43)	0.03 (-0.29, 0.34)	0.891
Model 2	Ref	0.12 (-0.22, 0.46)	0.02 (-0.30, 0.34)	0.926
Model 3	Ref	0.10 (-0.24, 0.44)	0.03 (-0.30, 0.35)	0.896
CRP	**(n=689)**			
Model 1	Ref	-0.04 (-0.22, 0.14)	-0.07 (-0.25, 0.12)	0.486
Model 2	Ref	-0.03 (-0.21, 0.15)	-0.06 (-0.25, 0.13)	0.546
Model 3	Ref	-0.01 (-0.20, 0.17)	-0.06 (-0.25, 0.14)	0.574

rMED: relative Mediterranean Diet Score: range from 0-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet. Age, sex and cohort specific z-scores for interleukin 6 (IL-6), and Protein C-reactive (CRP).
Model 1: General linear regressions adjusted for child sex, age and region.
Model 2: Model 1 further adjusted for maternal age, maternal educational level, maternal smoking, maternal total energy intake and maternal physical activity.
Model 3: Model 2 further adjusted for growth trajectories.

5.5 Association between maternal Mediterranean Diet during pregnancy and child growth trajectories (5th objective)

We identified 5 longitudinal growth trajectories in INMA project based on birth size and measurements of BMI z-score from birth to up to 4 years of age (**Figure 11 and Table 25**).

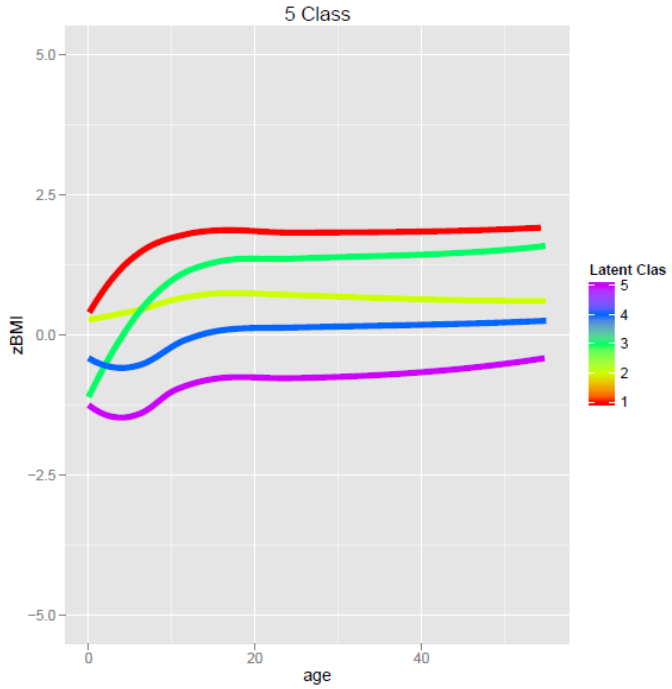

Figure 11. Child Body Mass Index z-score longitudinal growth trajectories.

Table 25. Child Body Mass Index (BMI) z-score longitudinal growth trajectories class from birth to 4 years of age in the INMA project.

Growth Trajectory	Frequency (n, %)	Description	
		Birth size	Growth
Class 1	n=268, 11.9%	Higher birth size	Accelerated growth
Class 2	n=583, 26.0%	Higher birth size	Slower growth
Class 3	n=301, 13.4%	Lower birth size	Accelerated growth
Class 4	n=806, 35.9%	Average birth size	Slower growth
Class 5	n=286, 12.8%	Lower birth size	Slower growth

Characteristics of children and mothers included in the analyses are presented in **Table 26**. Mothers of children with high birth size (Class 1 and 2) had higher gestational weight gain ($p < 0.001$), and they are more likely to be multiparous ($p < 0.001$) compared with mothers of children with low or average birth size.

Children with accelerated growth (Class 1 and 3) had mothers with wither pre-pregnancy BMI, and they had higher BMI z-score and waist circumference z-score at 4 years compared with children with slow growth. There were other statistically significant differences between the five child growth trajectories on region, gestational age and birthweight.

Mothers who were lost to follow-up differed from the mothers included in the study because they were younger, smoked more, and had lower socio economic status and educational level.

Table 26. Characteristics of children and mother by child longitudinal growth trajectories in INMA project.

	Class 1 (n=268, 11.9%)	Class 2 (n=583, 26.0%)	Class 3 (n=301, 13.4%)	Class 4 (n=806, 35.9%)	Class 5 (n=286, 12.8%)	P
Maternal characteristics						
Age at delivery*	30.5 (4.1)	30.8 (4.3)	31.0 (3.9)	30.8 (4.2)	31.0 (4.2)	0.701
Smoking in pregnancy (%)						
No	79.9	83.0	79.5	82.7	86.6	0.173
Yes	20.2	17.0	20.6	17.3	13.5	
Social class (%)						
I+II	19.4	22.2	26.6	226	22.7	0.632
III	26.5	26.6	23.6	27.9	25.2	
IV+V	54.1	51.2	49.8	49.5	52.1	
Education level (%)						
Primary or less	26.1	23.5	20.4	23.0	21.0	0.618
Secondary	40.3	42.4	39.5	41.1	44.4	
University	33.6	34.0	40.1	35.9	34.6	
Physical activity, METS*	37.2 (3.2)	37.5 (3.0)	37.6 (2.9)	37.2 (3.1)	37.3 (3.3)	0.234
Pre-pregnancy BMI*	24.4 (4.4)	23.6 (4.1)	24.2 (4.5)	23.3 (4.3)	22.5 (3.6)	<0.001
3rd trim EI*	2062.8 (537.1)	2068.8	2020.5 (507.8)	2048.5 (535.0)	2101.2 (530.2)	0.434
rMED 3rd trim*	7.8 (2.5)	8.1 (2.6)	8.1 (2.5)	8.0 (2.7)	7.8 (2.7)	0.319
Gestational weight gain*	0.4 (0.1)	0.4 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	<0.001
Parity (%)						
Primiparous	53.6	49.4	66.5	56.6	66.0	<0.001
Multiparous	46.4	50.6	33.5	43.4	34.0	
Child characteristics						
Sex (%)						
Female	41.8	48.0	48.8	52.5	43.0	0.009
Male	58.2	52.0	51.2	47.5	57.0	
Age years*	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	0.421
Region (%)						
Asturias	23.5	17.2	28.6	17.5	14.7	<0.001
Gipuzkoa	24.6	25.2	25.6	24.9	22.0	
Sabadell	23.9	25.2	21.6	25.4	29.4	
Valencia	28.0	32.4	24.3	32.1	33.9	
Gestational age*	39.7 (1.3)	39.8 (1.3)	39.3 (1.7)	39.7 (1.5)	39.3 (2.1)	<0.001
Birth weight*	3561.1 (397.2)	3484.8 (373.3)	3252.0 (345.9)	3296.6 (367.6)	3069.2 (372.0)	<0.001
Bmi z-score*	1.8 (1.0)	0.7 (0.7)	1.3 (1.0)	0.3 (0.8)	-0.5 (0.8)	<0.001
WC z-score *	0.9 (1.1)	0.0 (0.8)	0.6 (1.1)	-0.2 (0.8)	-0.8 (0.7)	<0.001

* mean (s.d.) rMED: relative Mediterranean Diet Score; MET: Metabolic Equivalents; EI: Energy intake; BMI: Body Mass Index; WC: waist circumference

Table 27 shows the association between maternal relative Mediterranean Diet score in the 3rd trimester of pregnancy and offspring growth trajectories from birth to up to 4 years of age.

Higher adherence to the Mediterranean Diet was associated with lower Relative Risk of having a baby with high birth size and accelerated growth (RR 0.64; 95%CI: 0.45, 0.92; p for trend 0.021) when compared to the reference trajectory (Class 4: average birth size-slower growth). This association remained after controlling for potential confounders such as maternal pre-pregnancy BMI and gestational weight gain (RR: 0.68; 95%CI: 0.47, 0.99; p for trend 0.056) and potential mediators such as gestational age.

In sensitivity analyses, aMED during pregnancy, rMED at 1st trimester or the average between the 1st and the 3rd trimester were not associated with any of the longitudinal growth patterns (data not shown).

Table 27. Associations of maternal alternate Mediterranean Diet score at 3rd trimester of pregnancy with offspring growth trajectories from birth to up to 4 years of age in INMA project.

rMED	Range:	Low T_1 (1-7)	Medium T_2 (8-9)	High T_3 (10-15)	
			β (95% CI)	*β* (95% CI)	*P for trend*
Class 1	(n=268)				
higher birth size-	Model 1	Ref	0.96 (0.69, 1.34)	0.64 (0.45, 0.92)	0.021
accelerated growth	Model 2	Ref	0.99 (0.71, 1.40)	0.68 (0.47, 0.99)	0.056
	Model 3	Ref	0.99 (0.71, 1.40)	0.68 (0.47, 0.99)	0.056
Class 2	(n=583)				
higher birth size-	Model 1	Ref	0.98 (0.76, 1.28)	0.93 (0.71, 1.22)	0.610
slower growth	Model 2	Ref	1.02 (0.78, 1.33)	0.98 (0.74, 1.29)	0.900
	Model 3	Ref	1.02 (0.78, 1.34)	0.99 (0.75, 1.30)	0.942
Class 3	(n=301)				
lower birth size-	Model 1	Ref	1.01 (0.72, 1.40)	0.97 (0.70, 1.36)	0.884
accelerated growth	Model 2	Ref	0.97 (0.69, 1.37)	0.92 (0.65, 1.30)	0.643
	Model 3	Ref	0.98 (0.70, 1.37)	0.92 (0.65, 1.30)	0.651
Class 5	(n=286)				
lower birth size-	Model 1	Ref	0.84 (0.60, 1.19)	0.84 (0.60, 1.19)	0.294
slower growth	Model 2	Ref	0.80 (0.57, 1.14)	0.78 (0.55, 1.12)	0.158
	Model 3	Ref	0.82 (0.58, 1.16)	0.79 (0.55, 1.12)	0.168

rMED: relative Mediterranean Diet Score: range from 0-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean Diet. Reference group for the outcome is Class 4 (average birth size-slower growth) trajectory (n=806).
Model 1: Multinomial logistic regressions adjusted for child sex, age and region.
Model 2: Model 1 further adjusted for maternal age, maternal pre pregnancy body mass index, smoking during pregnancy, maternal total energy intake, gestational weight gain, physical activity (3rd Trim) and parity.
Model 3: Model 2 further adjusted for gestational age.

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DISCUSSION

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6. DISCUSSION

This section includes a brief summary of the main findings, an overall discussion of methodological issues and interpretation of the results of this thesis.

6.1 Main findings

This longitudinal follow-up study aimed to investigate the prospective association between potentially modifiable early life factors and offspring childhood obesity development.

In the first part of the thesis, we assessed the association of 9 early life risk factors with offspring BMI and WHtR in two different cohort studies (the INMA project in the regions of Sabadell and Valencia (Spain) and the Project Viva in Eastern Massachusetts (Boston)). We found that excessive gestational weight gain, short infant sleep duration and rapid infant weight gain were associated with offspring BMI in both cohorts with β estimates ranging from 0.14 to 0.44. Gestational diabetes and rapid infant weight gain were associated with offspring WC at 7 years in both cohorts.
The modifiable risk factor that was more strongly associated with the adiposity outcomes was rapid infant weight gain.
When we assessed to what extent the combinations of these risk factors predict higher offspring adiposity, we found that there was a graded increase of prediction when increasing the number of risk factors. However, the prevalence and the prediction of the combinations of early risk factors differed across settings and populations.

In the second part of the thesis, we hypothesized that maternal diet could play an important role in offspring obesity development, and we found that adherence to Mediterranean Diet was inversely associated with waist circumference (a proxy of abdominal obesity) at 4 years of age (β: -0.57; 95%CI: -1.07, -0.07; p for trend = 0.024), but not with offspring BMI among children in the INMA Project.

Furthermore, the results from the multivariable analyses suggested a lack of association between maternal adherence to Mediterranean Diet with offspring metabolic risk and related biomarkers at age 4. Except for Apo A-1, which showed

a positive association with maternal dietary intake (β: 0.30; 95%CI: 0.08, 0.51; p for trend = 0.007).

The adherence to the Mediterranean Diet during the 3rd trimester of pregnancy was associated in adjusted multinomial analyses with lower risk of a detrimental child longitudinal growth pattern (RR: 0.68; 95%CI: 0.47, 0.99; p for trend = 0.056), characterized by higher birth size and accelerated growth compared with the reference growth pattern (average birth size and slower growth).

6.2 Study design and participants

The prospective design of the studies included in this thesis enables us to assess the longitudinal influence of prenatal and postnatal factors on the offspring obesity development during childhood.

We were able to collect data on exposures, outcomes and covariates at pregnancy, infancy and childhood, since there were several follow-up assessments in different time-points. Due to these continuous assessments, we had data on modifiable risk factors at least in one time-point of pregnancy and in infancy. Furthermore, we were able to collect anthropometric data from birth, infancy, at 4 and 7 years and derived growth trajectories. Finally, we could control our analyses for several potential confounders.

There are other benefits of using a prospective design, as the reduction of the risk of reverse causation, compared to cross-sectional studies or recall bias, present in retrospective studies (case-control studies)

Although, the design of the study is one of the main strength of this thesis, this design is not exempt of limitations, and those are described in section 6.5.1.

Other strengths of the study are the sample size and the long follow-up. In INMA project there was data available for 1827 children at 4 year assessment, and 960 in the 7 year assessment (including only 2 of the new 4 cohorts). In Project Viva, we included in our analysis 1108 children who were assessed at 7 years.

These studies have a long follow-up, in contrast with other studies that included dietary data during pregnancy and they were focused on birth outcomes, and they did not include data during childhood.

Furthermore, we included population of different countries (USA and Spain) and from 4 different regions of Spain (Sabadell, Valencia, Asturias and Gipuzkoa).

Moreover, this thesis used data from population-based cohort studies, that means that data come from general population, and their external validity is higher than studies conducted in clinical population.

6.3 Methods

6.3.1 Exposure assessment

We included several exposure variables in our analyses during pregnancy and infancy. In order to obtain them, trained staff from INMA project and Project Viva performed interviews, questionnaires and obtain data from clinical records.
The dietary data was obtained by using 2 validated food frequency questionnaires (one in INMA project and one in Project Viva), adapted from the same original food frequency questionnaire for pregnant population. The semi quantitative nature of the questionnaire permits us to estimate energy and nutrient intake.

We decided to use in our analyses a dietary pattern, since dietary patterns provide an overall description of peoples' eating habits and preference, take into account the synergies between foods and nutrients, and enables the study the association between diet and disease and facilitate the generation of public health recommendations [186]. We used the relative Mediterranean Diet Score because it was created for Spanish population and it is energy adjusted. In sensitivity analyses, we used the alternate Mediterranean Diet score with similar results.

6.3.2 Outcome assessment

Study trained staff measured anthropometric data with the same standardized protocol. The direct determination of weight, height and waist circumference represents an advantage compared to self reported studies, since mothers tend to misreport the children weight [293].

We included data on cardiometabolic risk based on a large number of related biomarkers. Our cardiometabolic risk score was based on the IDEFIC definition, because this score was derived in a population of children similar to ours (i.e. European origin and similar age)[287].

The inclusion of longitudinal growth trajectories based on BMI enables us to see longitudinal growth patterns, not only BMI at just one time-point. Some studies indicated that these trajectories may be better predictor of later obesity than BMI at one time-point [294].

6.3.3 Statistical analyses

We used multiple imputation to deal with missing values in covariables in order to include participants with partial information. We performed multiple imputation to maintain the sample size and also to reduce the potential selection bias and inaccurate inference [295]. In sensitivity analyses, we run the models with and without imputed data, and the estimates were similar.

6.4 Interpretation of results

6.4.1 Combination of modifiable early life risk factors and childhood general and abdominal obesity

In these analyses, we used data from Project Viva (in USA) and INMA project (Spain), and we assessed the associations of combination of 9 modifiable pre- and postnatal risk factors with BMI z-score and WHtR in mid-childhood. We found only 6-7 of the 9 pre- and postnatal risk factors of childhood adiposity in the US cohort also predicted adiposity in the Spanish setting. Nevertheless, combinations of these risk factors predicted a similarly wide range of BMI z-score and WHtR in both cohorts.

In both cohorts, there was a graded increase of risk of adiposity with a higher number of modifiable risk factors, in line with previous studies [296, 297]. Furthermore, in our analyses we found that some of the risk factors of BMI z-score and WHtR in mid-childhood were similar for both cohorts. According to our findings, the interventions aiming to reduce childhood adiposity should focus on the prevention of gestational diabetes mellitus, excessive gestational weight gain, rapid infant weight gain during the first six months of life and short infant sleep duration, since these are the predictors of adiposity in both cohorts. Rapid infant weight gain was the strongest single risk factor, consistent with previous studies [34, 121, 145, 146, 256, 298, 299].

Although risk of general and central adiposity shares most of the same risk factors, the presence of altered glucose had a stronger association with central adiposity than general adiposity in both cohorts. This specific association with abdominal adiposity is consistent with other studies, especially after adjustment for pre-pregnancy BMI [123, 300]. One potential explanation could be that an altered glucose supply *in utero* may predispose to an altered metabolic pattern during childhood, with an increment of fat mass in detrimental of lean mass [301]. Another explanation may be that the effect of gestational diabetes mellitus on general adiposity may appear later in life than the effect on abdominal adiposity [123, 300, 301]. Furthermore, the association of gestational diabetes mellitus is stronger with FMI than in BMI z-score, consistent with previous studies that suggest that gestational diabetes mellitus may increase childhood adiposity rather than size alone; FMI is a better indicator of overall adiposity than is BMI, which incorporates both fat and lean mass [122, 301].

There were also differences in the associations between some risk factors and the outcomes in the 2 cohorts, mainly in sugar-sweetened beverage intake, cesarean section, breastfeeding duration and early introduction of solids. These differences may be due to different characteristics in the populations. Women in Project Viva (USA) had higher intake of sugar-sweetened beverage intake, higher rate of cesarean deliveries, and were more likely to breastfeed for at least 12 months, compared with the women in INMA (Spain).

Different populations may have different associations, as Brion et al. found in their study between breastfeeding duration and childhood BMI; in their study, they argued that different confounding structures exist in different populations [302]. In our study, we tested different confounding structures for the two cohorts, and we retained the confounders that had an effect on the estimates. Even though the difference in the associations between certain risk factors remained after cohort specific adjustments, they could be affected by residual or uncontrolled confounding. These discrepancies found between the two cohorts regarding these risk factors are consistent with other studies. For example, some but not all observational studies show that breastfeeding duration is inversely associated with childhood obesity. A recent review conducted by Yan et al. suggested that these discrepancies may be due to the different populations [241, 303].

Timing of introduction of solids is another risk factor with inconclusive evidence, as previous reviews did not find a clear association with childhood obesity [250, 251]. Pearce J et al. reported that 16 studies (out of 21) found no association with childhood obesity, and only one study found association with body composition [250]. A recent study conducted in the Netherlands found higher risk of obesity with early introduction of solids but this association was substantially attenuated after adjustments for confounders, such as sociodemographics and pre-pregnancy BMI [304].

The association between cesarean delivery and childhood obesity is more consistent across studies. A recent systematic review and meta-analyses found that the difference in childhood BMI between cesarean delivery and vaginal delivery was 0.44 kg/m^2 (0.17, 0.72), but in our results in INMA, the presence of cesarean delivery predicted a lower BMI z-scores and WHtR in mid-childhood [202]. According to Li et al. the lack of association in some of the studies could be due to an insufficient statistical power or an inadequate adjustment for

confounding [203]. However, in the INMA study with a large sample size and after accounting for several possible confounders, we found a negative association. The wide variations in clinician and individual preferences of cesarean section over vaginal delivery noted across different regions could be another reason partially explaining the inconsistency in associations.

In these analyses, we included all the risk factors in the models, and there may have been an overadjusment. Some of the associations were not "statistically significant" after adjustments; however, we focused more on the magnitude and the direction of the estimates. For some risk factors we used different cut-offs for Project Viva and INMA, based on guidelines and recommendations and also in empirical reasons (mostly in Viva, based in our previous publications).

In this study, we found that two different populations, in USA and Spain, have different pre- and postnatal modifiable risk factors of adiposity. The strongest modifiable risk factor in both cohorts was rapid infant weight gain, followed by gestational diabetes mellitus, excessive gestational weight gain and short sleep duration. Taken together these results suggest that testing multifactorial interventions to prevent excess childhood adiposity are warranted, although foci of interventions might differ by context. Specific public health policies and interventions that focus on modifiable multi risk factors may be useful to prevent obesity and abdominal adiposity risk in childhood.

6.4.2 Mediterranean Diet during pregnancy and risk of general and abdominal obesity at pre-school age

In the present analyses, higher adherence to the Mediterranean Diet in pregnancy was not clearly associated with BMI in 4-year-old offspring, but it was modestly associated with lower waist circumference: children born of mothers with high adherence to the Mediterranean Diet had a waist circumference on average 0.6 cm smaller than children born to mothers with low adherence among children in INMA Project. While this difference in waist circumference may seem small, it is similar to that reported in previous studies evaluating adherence to the Mediterranean Diet and waist circumference in other populations [305-307]. At this early age, we do not know if this difference is clinically relevant and will have an impact on future cardiometabolic outcomes; however these results add to the evidence that early life nutritional factors might have an influence on body composition in early childhood.

To the best of our knowledge, no previous cohort study has evaluated the association between (Mediterranean) dietary patterns in pregnancy and childhood overweight and abdominal obesity risk. Previous human studies have focused on evaluating specific nutrients or foods, and results were mostly inconsistent. Maternal sugar and saturated fatty acid intakes were associated with offspring adiposity at 5 years of age [161]. Another study found no association between maternal macronutrient and energy intakes in pregnancy and offspring adiposity at 10 years of age [162]. Both maternal meat intake and protein intake were associated with adiposity in studies conducted in adolescents [163]. A few studies have evaluated the association between dietary patterns in pregnancy and birth outcomes, including birth weight and fetal birth restriction, potential risk factors for the obesity development later in life; however, results are also inconsistent. In a previous INMA study, diet quality measured with the Alternative Healthy Eating Index adapted for pregnancy was associated with a lower risk of delivering an infant with fetal growth restriction [192]. In another study that included INMA and a Greek birth cohort, higher adherence to the Mediterranean Diet was associated with a lower risk of delivering a fetal-growth-restricted infant, but only in the Spanish Mediterranean INMA regions (Sabadell and Valencia); no association was observed in the Spanish Atlantic regions (Asturias and Gipuzkoa) or the Greek cohort[190]. It was hypothesized that the existence of different Mediterranean Diet patterns in the different geographic areas could explain the

diverse associations observed. Degree of adherence to a Mediterranean dietary pattern in the first semester of pregnancy was associated with several features of intra-uterine growth in the Generation R birth cohort: women with low adherence had a lower placental weight and a lower birth weight [191]. Other studies conducted in the US, such as the Project Viva [193], did not find significant associations between diet quality measures and birth weight characteristics.

In theses analyses, we observed no association between maternal diet in pregnancy and offspring overweight, but some evidence of an inverse association between diet quality in pregnancy and offspring waist circumference, a marker of abdominal obesity. As pointed out in different studies, maternal weight status seems to be the strongest predictor of obesity later in life [308]. Some animal experiments have argued that maternal obesity – as a consequence of a high-fat diet – but not a high-fat diet *per se*, is necessary to program obesity predisposition in the offspring [218, 309]. Also, studies in animals have observed that post-natal offspring diet seems to combine with prenatal maternal diet in exacerbating obesity risk [218]. In rodents the consumption of a western diet during lactation is particularly critical for the ability of a maternal western diet to cause obesity and associated metabolic consequences in childhood [218]. Also, it has been speculated that the effects of intrauterine over-nutrition are hard to detect early in childhood and may predominantly appear later in life. This hypothesis is supported by studies of offspring exposed to gestational diabetes, where it seems that this effect on childhood obesity becomes apparent from the age of 9-10 years but not earlier [310]. Finally, it may be possible that Mediterranean Diet in pregnancy has a specific effect on programming body fat distribution leading to a lower abdominal obesity risk without influencing general obesity. As observed in this cohort, children born of mothers with higher adherence to Mediterranean Diet in pregnancy tend to show a lower waist circumference. This is consistent with previous studies that shown that Mediterranean Diet influence abdominal obesity independently of total body weight in adults and children [305].

In conclusion, our data suggests that Mediterranean Diet during pregnancy was not associated with measures of overweight in 4-year-old offspring, but was inversely associated with offspring waist circumference, a marker of abdominal obesity. Long term studies with a larger sample size, better measurements of body fat distribution, and biomarkers of cardiometabolic risk are needed to

disentangle the plausible effect of Mediterranean Diet in pregnancy on visceral fat accumulation in childhood.

6.4.3 Mediterranean Diet during pregnancy and offspring cardiometabolic risk

We found that higher adherence to the Mediterranean Diet in pregnancy was not associated with cardiometabolic risk factor in 4-year-old offspring, nor with blood pressure, HDL-cholesterol and triglycerides in INMA project. Moreover, the maternal Mediterranean Diet was not associated with offspring leptin, C-peptide, Apo B, adiponectines, IL-6 and CRP. However, we found a positive association with Apo A-1.

To the best of our knowledge, no previous cohort study has evaluated the association between adherence to Mediterranean Diet in pregnancy and offspring cardiometabolic risk during childhood, including blood pressure and lipid biomarkers. Previous studies have focused their interest in the association between maternal Mediterranean Diet and outcomes at birth. For example, one study conducted in Spain found that offspring of women with low adherence to Mediterranean Diet had higher LDL-cholesterol, Apo B, insulin resistance markers but lower Apo A-1 / Apo B ratio at birth, in comparison with those with higher adherence [195, 311]. Disparities in study findings may be due to differences in sample size and age of outcome assessment.

Other studies have explored the influence of dietary intake on offspring lipid biomarkers. Our findings are in agreement with two previous studies that assessed the association between glycemic index and load and protein intake during pregnancy and offspring biomarkers at age 20, that found no association with HDL cholesterol and triglycerides [163, 200].

Although, in adult population higher adherence to Mediterranean Diet was associated with lower systolic and diastolic blood pressure as reported in a recent meta-analysis [312]; we could not find an association between this dietary pattern in pregnancy and offspring blood pressure. The few studies that have evaluated the association between dietary intake and offspring blood pressure show different results and most of them have reported null associations [111],

[200, 313]. Except for an Australian study, that found that higher intake of maternal PUFA was associated with higher offspring systolic blood pressure [164].

We assessed the association between Mediterranean Diet and other biomarkers, such as leptin and adiponectin and there was a lack of association. In line with a previous study conducted in Project Viva that showed no association between this dietary pattern in pregnancy and adiponectin and leptin levels in cord blood [314]. However, in the Danish cohort study maternal glycemic index (not glycemic load), was positive associated with leptin concentrations in the offspring at 20 years of age [200].

There may be several explanations for these null results. Firs, the Mediterranean Diet may not play an important role on the risk of metabolic syndrome development in the offspring.

Second, the assessment of cardiometabolic risk and other biomarkers at 4 years of age may have been too early to detect any cardiometabolic alteration. Furthermore, the effect of prenatal exposures on metabolic syndrome development may appeared later in childhood, as observed in previous studies which explored the influence of being born large for gestational age (from diabetic mothers) or early body mass index trajectories on the development of metabolic syndrome [224, 315]. This may respond to the "amplification" hypothesis, that describe how early life exposures (e.g. low birth weight) influence offspring outcomes (e.g. blood pressure), and the influence increase with age, maybe due to interaction with child lifestyle patterns [316].
There may be a time-delayed effect between the development of abdominal adiposity and cardiometabolic comorbidities, this could explain the association found between rMED during pregnancy and waist circumference in children in our previous study, but not with cardiometabolic risk or other biomarkers.

Furthermore, according to the International Diabetes Federation, children under 10 years old should not be diagnosed of metabolic syndrome [72]. In this line, recent reviews conducted by the US Preventive Services Task Force conclude that the current evidence is insufficient to assess the potential benefits and harms of lipid screening in children [317, 318]. It is unclear if elevated lipid levels in children are associated with future disease risk, however, some of these altered biomarkers (e.g. blood pressure and triglycerides) may track into adulthood and

be predictors of later metabolic syndrome [317, 319, 320]. Further analyses with cardiometabolic data in older ages are needed.

In the present analysis, maternal Mediterranean dietary pattern was related to offspring Apo A-1 at 4 years of age. This finding is consistent with a results from the PREDIMED study, that found that an intervention with Mediterranean Diet supplemented with extra virgin olive oil increased Apo A-1 and decreased Apo B/ Apo A-1 ratio [321].

The potential mechanism may be related to the polyphenols content of the olive oil, as observed in studies where dietary supplementation with polyphenols increased Apo A-1 [322, 323]. Besides polyphenols, other components of the Mediterranean Diet may play a role increasing Apo A-1, like high content of fiber, and mono and polyunsaturated fatty acids. Recent studies have suggested that Apo A-1 and the ratio Apo B/Apo A-1 are good indicators of cardiovascular disease in adults, and some argued that may be more useful than LDL, TG and HDL measurements, but others observed that do not provided additional advantage [61, 62, 244].

We expected that an increase of Apo A-1 would mean an increase of HDL, since Apo A-1 is the major apolipoprotein component of HDL-cholesterol. Nevertheless, in our analyses there was no an association between Mediterranean Diet and HDL. One potential reason could be that Apo A-1 is a prospective marker of HDL, and our study was conducted in too early age to detect also an increase of HDL [69, 324, 325]. In addition, we cannot rule out the possibility that this is a chance finding due to multiple comparison applied.

In conclusion, higher adherence to Mediterranean Diet in pregnancy was not associated with a continuous offspring cardiometabolic risk score, nor was associated with blood pressure or biomarkers (i.e. HDL-cholesterol, triglycerides, leptin, adiponectin, CRP, IL6, C-peptide, Apo B) at 4 years of age, except for Apo A-1.

6.4.4 Mediterranean Diet during pregnancy and early life growth trajectories

Our analyses suggested that higher adherence to a Mediterranean dietary pattern during the 3rd trimester of pregnancy was associated with lower risk of having an offspring with higher birth size and accelerated growth from birth to 4 years of age in INMA project.

Recent interest has raised on the study of causes and consequences of early life growth trajectories. Recent studies have reported relations between early life longitudinal growth patterns and risk of disease (e.g. asthma) in later life including cardiovascular disease (e.g. blood pressure and obesity) [326-328]. However, few studies have explored the potential risk factors of certain growth trajectories, and evidence has been found for: maternal sociodemographic factors, smoking, paternal overweight / obesity, gestational weight gain, and breastfeeding duration [327, 329].
We are not aware of any study exploring the association between maternal dietary pattern (Mediterranean pattern) during pregnancy and longitudinal growth trajectories in childhood.

In our analyses, we detected five child longitudinal BMI growth trajectories from birth to 4 years in INMA project. They are based on birth size (lower, average or higher birth size) and growth (accelerated or slower BMI gain). These growth patterns are consistent with previous growth trajectories described in other populations [327, 330-332].

Some longitudinal growth trajectories have been associated with increased risk of later obesity, mainly the two that included accelerated growth [332]. The "accelerated postnatal growth hypothesis" links rapid weight gain in infancy and early childhood with increased risk of later obesity and other chronic diseases [333].
Several studies conducted in different populations have identified this "accelerating" growth pattern. In one Australian study, maternal obesity was associated with a fourfold risk for the offspring of being in the "accelerating" group from birth to up to 3 years and half, and these children were at higher risk of being overweight at age 9 [332]. Another study conducted in UK, found that a

similar longitudinal growth pattern was associated with higher systolic and diastolic blood pressure at 18 years old [327].

In our analyses, higher adherence to the Mediterranean Diet was associated with offspring lower risk of developing this adverse longitudinal growth pattern. This association remained even after controlling by maternal pre-pregnancy BMI and gestational weight gain, two previously identified determinants of this growth pattern in other studies [331].

There is another growth trajectory that may increase later disease risk and this is the "mismatch" that links low birth weight combined with high postnatal weight gain (Class 3) [333]. However, in our analyses there was not a clear association between this growth pattern and maternal Mediterranean Diet.

In a previous INMA project study, the adherence to the Mediterranean Diet was associated with lower risk of having a fetal-growth-restricted infant in the Spanish Mediterranean INMA cohorts (Sabadell and Valencia), but not in the Spanish Atlantic regions (Asturias and Gipuzkoa) or the Greek RHEA cohort [190]. However, in our previous study also in INMA project we found that the Mediterranean Diet was not associated with child BMI at 4 years [334]. In the current analyses, we used several measures of BMI in different time points, taking into account the dynamics of BMI over time. We found that offspring higher birth size and accelerated growth were associated with maternal adherence to Mediterranean Diet during late pregnancy but not with the dietary pattern during the 1st trimester. It is biologically plausible that fetal nutrition supply could vary from early to late pregnancy. The 2nd and 3rd trimesters of pregnancy could be more critical periods for the offspring susceptibility to obesity, since fetal fat accumulation is accelerating [335, 336].

The potential mechanism behind the association between maternal diet and longitudinal growth trajectory may be explained by the impact of the Mediterranean Diet on gestational weight gain and this may influence birth weight [337, 338]. However, the association remains significant after controlling for these factors. Other potential explanations may be due to epigenetic modifications but also to share lifestyles within the family, the potential mechanism should be disentangled with further studies.

In conclusion, higher adherence to the Mediterranean Diet in the 3rd trimester of pregnancy is associated with lower risk of having an offspring with higher birth size and accelerated growth from 0 up to 4 years of age. Since longitudinal growth patterns that involved accelerated growth seems to be detrimental for development of chronic disease, detecting potential early life determinants of longitudinal growth trajectories is of special interest. Therefore, further research is needed.

6.5 Limitations

The present thesis is not exempt of limitations, and these are described as follows:

6.5.1 Study design and population

The observational design of the study limits us to talk about causality. Moreover, for the nature of the study it is difficult to distinguish the effect of early life exposures and the effect of shared lifestyles between the parents and offspring. To address these questions, it may necessary to conduct randomized controlled trials or to use different statistical methods (such as negative control approaches, sibling comparison, cross-cohort comparison or Mendelian randomization analyses) to test for causality [339, 340].

Overall, the population size was adequate to assess the association between the exposures and the outcomes. However, in the different analyses the sample size depended on availability of exposure and outcome variables. Therefore, in stratified analyses or analyses including certain biomarkers (i.e. adiponectin), the sample size of the subsamples used in these analyses may have been too small to detect an effect.

One common limitation of prospective cohort studies is the potential selection bias due to losses to follow-up. In our analyses, we included 35% - 66% participants (who had data on exposure and outcome variables) of the total recruitment sample.

We compared participants who were included and excluded from the analyses, and we found that excluded women were younger, less educated and more likely to smoke during pregnancy. This fact may mean that not all our findings are generalizable to all populations (i.e. populations with low socioeconomic status).

6.5.2 .Methods

Some of the data collected (exposures and covariates) were self-reported (e.g. Dietary intake, physical activity and socio economic status) and thus subjected to misreporting or misclassification.

Diet was evaluated using a food frequency questionnaire, subjected to measurement error that may lead to an attenuation of effect estimates. This attenuation could explain the modest magnitude of the estimates in most of the associations found (e.g. between rMED in pregnancy and waist circumference at 4 years). We used dietary data adjusted by total energy intake in order to try to mitigate this measurement error [341].

In the second part of this thesis, we used the relative Mediterranean Score to assess the overall quality of maternal diet during pregnancy, although we used another score (i.e. aMED), this may represented a limitation. Firstly, because there are several scores to assess the adherence to the Mediterranean Diet, and each of them is related to a specific region and / or specific recommendations (for instance, the fat item differed between scores, or some included dairy products and others not). Another limitation is that all the items included have the same importance, independently of the component's proportion in the overall diet, and not all of them may have the same association with health/disease outcome [342]. Finally, all the dietary scores, Mediterranean and also others such as alternative Healthy Eating Index, presented a limitation to represent the overall diet of an individual with accuracy. Furthermore, other food habits, like cravings, TV viewing during meals, etc may have influenced the overall quality dietary pattern.

There are also some limitations in the outcome assessment. Regarding the anthropometric outcomes, we used measures of height and weight, BMI and waist circumference. However, we had available data on Fat Mass Index and Fat-Free Mass Index only in Project Viva. Although a recent review showed high correlations between BMI and waist circumference with total body fat (measured by skinfold, DXA or bioimpedance), these techniques allow us to know the body fat and fat distribution [343]. This may be a key factor for its relation with the metabolic risk.

We had no-fasted blood samples due to logistical issues and the age of the children. We believe that the lack of fasting blood did not affect our results and their interpretation [344]. Difference on the biomarkers methods may also mean a limitation. For further INMA follow-up, a common protocol between the INMA-cohorts may be useful for collection of blood samples and measurement of blood lipids at the same time-point.

We made a huge effort to harmonize the variables across the cohorts. However, in the first part of the thesis due to the differences between the cohorts (INMA project and Project Viva), in participant characteristics and exposure measurement and variables, we could not perform a joint analyses.

6.5.3 Confounders

The thesis contains information on many potential confounders, collected during pregnancy, infancy and childhood. We included these potential confounders based on previous literature and their association with both the exposure and the outcome, and we used the forward stepwise method to decide the inclusion of the covariates.

Furthermore, we performed mediation analyses, in order to assess covariables that may mediate our associations of interest. However, residual confounding is always potentially present. Despite the huge number of covariates included, we fail to include potential confounders such as: Vitamin D intake during pregnancy, TV viewing during meals or other lifestyle practices that could affect dietary intake, maternal diet during lactation, infant diet quality during first year, weaning practices, exclusive breastfeeding, TV viewing during meals.

6.6 Implications and future research

This thesis provides the results of a large longitudinal study of potentially modifiable early risk factors of obesity development.

In our prediction models, we showed that potential interventions including combination of modifiable risk factors could prevent a wide range of general obesity and adiposity. However, randomized controlled studies (mostly focused in one risk factor) had modest effect on obesity development prevention [345]. Future early life interventions including these modifiable early risk factors are needed to confirm our observational findings.

Since the evidence on the role of early life risk factors of obesity development remains unclear and based on our findings and the limitations detected, we have other objectives to assess within INMA project and also in collaboration with other cohort studies (e.g. with the Greek RHEA cohort).

First, for the next follow-up assessment direct measures of body fat distribution will be taken, and also biomarkers related to cardiometabolic risk with similar protocols.

We will further investigate the potential role of a maternal pro-inflammatory diet (by using the Dietary Inflammatory Index) during pregnancy on the birth outcomes and obesity development, since inflammation has been related to obesity in previous cross-sectional studies.

In order to try to disentangle the effect of intrauterine environment and the shared lifestyles between parents and children, we will assess the association between maternal dietary patterns, infancy quality of the diet and offspring dietary patterns.

As we hypothesized that child own lifestyle patterns may have an effect on his obesity risk, we will assess also the effect of mid-childhood lifestyle patterns (dietary patterns and physical activity at 4 years) on obesity and cardiometabolic risk, in a cross-sectional design study and also longitudinal (at 7 years assessment).

Finally, we will evaluate gene-environment interactions influencing the obesity development in childhood.

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Conclusions

- Excessive gestational weight gain, short infant sleep duration and rapid infant weight gain were associated with a higher offspring body mass index at 7 years, whereas gestational diabetes mellitus and rapid infant weight gain were associated with offspring waist-to-height ratio in two birth cohort studies in USA and Spain.

- Rapid weight gain from birth to six months is one of the strongest early life risk factors for general and abdominal adiposity during childhood. Our results were consistent across cohorts.

- Although the prenatal and postnatal risk factors for childhood adiposity differed across cohorts, the combinations of these risk factors predicted a similar wide range of body mass index z-score and waist-to-height ratio in both cohorts.

- Maternal adherence to a Mediterranean Diet during pregnancy was not associated with measures of overweight in 4-year-old offspring, but was inversely associated with offspring waist circumference, a marker of abdominal obesity.

- There were no clear associations between the Mediterranean dietary pattern in pregnancy and cardiometabolic risk in 4-year-old offspring, nor between this diet and blood pressure, lipids (HDL, Triglycerides and Apo B), leptin, C-peptide, adiponectin or inflammatory biomarkers (CRP and IL-6). However, we observed a positive association with Apo A-1, indicating a potential beneficial effect of the diet.

- Children exposed to a maternal Mediterranean Diet during the 3rd trimester of pregnancy had a lower risk of a detrimental longitudinal growth trajectory, characterized by higher birth size and accelerated growth from birth to 4 years of age.

Global conclusion

During the prenatal and postnatal periods, modifiable early life risk factors play an important role in the development of childhood obesity. According to our findings, the combination of modifiable risk factors that predicted higher obesity differed across settings and populations. However, rapid infant weight gain was a common risk factor for general and abdominal obesity in childhood. Another of the risk factors studied, the Mediterranean Diet during pregnancy, may have a protective effect on childhood health because it decreased the risk of higher birth size and accelerated growth, and waist circumference. It also increased the circulating Apo A-1 in 4-year-old children. However, this dietary pattern did not show an association with BMI, cardiometabolic risk, or other biomarkers.

Early life interventions focused on pregnant women, including modifiable risk factors, may be effective to prevent the development of childhood obesity. Further research in this field is needed with longer follow-up and direct measures of adiposity.

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ANNEXES

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1- SCIENTIFIC CONTRIBUTIONS

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SCIENTIFIC CONTRIBUTIONS

Publications related to the thesis:

1st and 2nd objectives

Fernández-Barrés S, Rifas-Shiman S, Romaguera D, Valvi D, Arija V, Íñiguez C, Kleinman K, Oken E, Taveras EM, Vioque J, Sunyer J, Vrijheid M, Gillman MW. Combining Modifiable Prenatal and Postnatal Risk Factors of Childhood Adiposity. *International Journal of Obesity*

Submitted

3rd objective

Fernández-Barrés S, Romaguera D, Valvi D, Martínez D, Vioque J, Navarrete-Muñoz EM, Amiano P, Gonzalez-Palacios S, Guxens M, Iñiguez C, Pereda E, Riaño I, Tardón A, Arija V, Sunyer J, Vrijheid M. Mediterranean dietary pattern in pregnant women and offspring risk of overweight and abdominal obesity in early childhood: the INMA birth cohort study. *Pediatric Obesity* 2016. Published

4th objective

Fernández-Barrés S, Manzano C, Valvi D, Martínez D, Arija V, Sunyer J, Vrijheid M, Romaguera D. Maternal Mediterranean Diet during pregnancy and offspring cardiometabolic risk in early childhood.

In preparation.

5th objective

Fernández-Barrés S, Valvi D, Martínez D, Arija V, Sunyer J, Vrijheid M Romaguera D. Mediterranean Diet in prengnat women and child longitudinal growth trajectories from birth to up to 4 years in the INMA birth cohort study.

In preparation.

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Combining Modifiable Prenatal and Postnatal Risk Factors of Childhood Adiposity

Fernández-Barrés S, Rifas-Shiman S, Romaguera D, Valvi D, Arija V, Íñiguez C, Oken E, Taveras EM, Vioque J, Sunyer J, Vrijheid M, Gillman MW

Submitted to **International Journal of Obesity**
(IF: 5.337; Q1 Nutrition & Dietetics / Endocrinology & Metabolism)

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Manuscript #	2016IJO01398
Current Revision #	0
Submission Date	23rd Aug 16
Current Stage	Initial QC Started
Title	Combining Modifiable Prenatal and Postnatal Risk Factors of Childhood Adiposity
Running Title	Combining Risk Factors of Childhood Adiposity
Manuscript Type	Original Article
Special Section	N/A
Category	*Pediatric (This option must be selected for ALL Pediatric articles)
Word Count	4070
Corresponding Author	Ms. Sílvia fernández-barrés (silvia.fernandezb@urv.cat) (Universitat Rovira i Virgili)
Contributing Authors	Ms. Sheryl Rifas-Shiman , Dr. Dora Romaguera , Dr. Damaskini Valvi , Prof. Victoria Arija , Dr. Carmen Iñiguez , Emily Oken , Dr. Elsie Taveras , Prof. Jesus vioque , Dr. Jordi Sunyer , Dr. Martine Vrijheid , Matthew Gillman
Abstract	299
Associate Editor	Not Assigned
Techniques	Not Applicable;
Subject Terms	Health sciences/Medical research/Epidemiology Health sciences/Risk factors
Conflict of Interest Statement	There is **NO** conflict of interest to disclose
Clinical Trial	No

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EARLY LIFE FACTORS AND CHILDHOOD OBESITY DEVELOPMENT
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Abstract:

Background: Prenatal and postnatal factors affect the risk of childhood adiposity, but few studies have examined them in combination.

Aim: We examined the extent to which combination of 9 modifiable pre- and postnatal risk factors predict childhood body mass index (BMI) and waist-to-height ratio (WHtR).

Methods: We included 1 108 mother-child pairs from Project Viva (USA) and 979 from INMA project (Spain). The 9 risk factors were: prenatal maternal smoking, sugar-sweetened beverage intake, gestational weight gain, and gestational diabetes; cesarean section; and infant breastfeeding duration, timing of introduction of solids, sleep duration, and rapid weight gain. The outcomes were age- and sex- specific BMI z-score and WHtR at mean 8.0 years in Viva and 7.2 years in INMA. We ran multivariable linear regressions with all 9 risk factors. We obtained predicted means of BMI-z and WHtR for the 512 combinations of the 9 risk factors.

Results: Mean (SD) BMI-z was 0.39 (1.00) in Viva and 0.58 (1.05) in INMA; respective values for WHtR*100 were 46.6 (5.2) and 47.0 (4.8). In multivariable models, all risk factors were associated with BMI-z and WHtR in Viva, with regression coefficients from 0.04 to 0.44 for BMI-z and 0.15 to 1.57 for WHtR. In INMA, 4 of the 9 risk factors were associated with BMI-z and WHtR. Predicted BMI-z scores ranged from -0.02 to 1.52 in Viva and from 0.41 to 1.20 in INMA for children with 0 to 9 risk factors. Predicted child WHtR ranged from 45.0 to 52.6 in Viva and from 46.4 to 49.7 in INMA for 0 to 9 risk factors.

Conclusion: The pre- and postnatal predictors of childhood adiposity differed across the 2 cohorts. While combinations of these risk factors predicted a wide range of BMI and WHtR in both cohorts, suggesting that multifactorial interventions are warranted, foci of interventions might differ by context.

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Published in **Pediatric Obesity**
(IF: 3.689; Q1 Pediatrics)

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PEDIATRICOBESITY
ORIGINALRESEARCH
doi:10.1111/ijpo.12092

Mediterranean dietary pattern in pregnant women and offspring risk of overweight and abdominal obesity in early childhood: the INMA birth cohort study

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Received 5 August 2015; revised 19 October 2015; accepted 10 November 2015

Summary

Background: Animal models have suggested that maternal diet quality may reduce offspring obesity risk regardless of maternal body weight; however, evidence from human studies is scarce.

Objective: The aim of this study was to evaluate associations between adherence to the Mediterranean diet (MD) during pregnancy and childhood overweight and abdominal obesity risk at 4 years of age.

Methods: We analysed 1827 mother–child pairs from the Spanish 'Infancia y Medio Ambiente' cohort study, recruited between 2003 and 2008. Diet was assessed during pregnancy using a food frequency questionnaire and MD adherence by the relative Mediterranean diet score (rMED). Overweight (including obesity) was defined as an age-specific and sex-specific body mass index ≥85th percentile (World Health Organization referent), and abdominal obesity as a waist circumference (WC) >90th percentile. Multivariate adjusted linear and logistic regression models were used to evaluate associations between pregnancy rMED and offspring overweight and abdominal obesity.

Result: There was no association between rMED and body mass index *z*-score, whereas there was a significant association between higher adherence to MD and lower WC (β of high vs. low rMED: −0.62 cm; 95% confidence interval: −1.10, −0.14 cm, *P* for trend = 0.009).

Conclusion: Pregnancy adherence to the MD was not associated with childhood overweight risk, but it was associated with lower WC, a marker of abdominal obesity.

Keywords: Abdominal obesity, childhood obesity, Mediterranean diet, pregnancy.

Introduction

The current childhood obesity epidemic is a major problem of public health. Overweight and obese children are more likely to have obesity and other metabolic diseases later in life; thus, early prevention is critical (1).

Several early life environmental factors have been associated with increased childhood obesity risk, such as pregnancy weight gain, birth weight, rapid postnatal growth and gestational diabetes (2,3). In contrast, preventive factors are breastfeeding and late solids introduction. Maternal overweight is an

important predictor of childhood obesity (4). What it is not yet clear is whether maternal diet in pregnancy has an effect on childhood obesity risk beyond maternal weight status. Some animal models have suggested that high-quality maternal diet may reduce childhood obesity risk regardless of maternal body weight (5); however, other studies have concluded that maternal diet itself, in the absence of obesity, is insufficient to predispose offspring to obesity; maternal obesity is required for this programming effect (6). The mechanisms of action are uncertain; suggested mechanisms include the glycemic effect of certain diets with a high glycemic index, which lead to hyperglycaemia *in utero* and foetal hyper-insulinemia, which may influence individual susceptibly to weight gain later in life (7). On the other hand, maternal obesity and high-fat intake in pregnancy may induce placental inflammation, and thus increase nutrient transport and foetal growth. Finally, western diet in pregnancy and lactation has been associated with leptin insensitivity in the offspring, leading to hyperphagia and increased weight gain (5). Only few studies have evaluated the role of maternal diet on childhood obesity risk in humans. Most studies have focussed on the macronutrient composition of the diet, and results have been mostly inconsistent (7–10). The study of dietary patterns represents a broader picture of dietary intake and may be more useful to assess the diet quality and to predict disease risk (11).

The Mediterranean diet (MD) is considered a healthy dietary pattern and has been associated with lower obesity development risk in both children (12) and adults (13). Recent studies have suggested that a lower maternal adherence to an MD in pregnancy is associated with early life risk factors of childhood obesity, such as foetal growth-restricted infants (14) and neonatal insulin resistance (15), all are potential risk factors for obesity later in life. Therefore, the aim of the study was to examine the association between maternal adherence to the MD in pregnancy and the risk of overweight and abdominal obesity in 4 years old children.

Methods

Subjects

The population-based 'Infancia y Medio Ambiente' (INMA – environment and childhood) birth cohort study recruited 2765 pregnant women between 2003 and 2008 in the Spanish regions of Asturias, Gipuzkoa, Sabadell and Valencia (16). Inclusion criteria were as follows: ≥16 years of age, intention to deliver at the reference hospital, ability to communicate in Spanish or regional languages, singleton pregnancy and no assisted conception. Pregnant women were recruited during prenatal visits in the first trimester of pregnancy at public healthcare centres or hospitals. All participants provided written informed consent, and the study was approved by hospital and institutional ethics committees in each region.

Mother–child pairs were afterwards followed at third pregnancy trimester, birth and at child ages 6 months, 1 and 4 years. Flowchart is shown in Figure S1. The final analytic population included 1827 pairs (66% of initially enrolled) of mothers and children.

Dietary assessment and MD

Maternal diet was assessed in the first and third trimesters of pregnancy using a 101-item food frequency questionnaire (FFQ). The FFQ was an adapted version of Willett's questionnaire developed and validated for use among adults and pregnant women living in Spain with satisfactory coefficients for validity and reproducibility (17). In the first trimester, we asked mothers about their diet during the first trimester of pregnancy and in the third trimester about their diet during second and third trimesters. Standard units and serving sizes were specified for each food item. Nutrient values and total energy intake were obtained from the US Department of Agriculture food composition tables and other published sources (18,19).

Adherence to the MD was assessed with the relative Mediterranean diet score (rMED) (13). The rMED was constructed with the average dietary data from the first and third trimesters of pregnancy. The consumption of vegetables, fruits and nuts, cereals, legumes, fish, olive oil, total meat and dairy products (except alcohol because most of the pregnant women did not consumed it in our study) were measured as grammes per 1000 kcal d^{-1} and were divided into tertiles. A value of 0, 1 and 2 was assigned to the intake tertiles, positively scoring higher intakes for the six components presumed to fit the MD. The scoring was reversed for two components presumed not to fit the MD (meat and dairy), positively scoring lower intakes. After summing up scores of each component, the final potential score range was 0–16; it was further divided into tertiles to identify those with low, medium and high adherence to the MD.

Child anthropometry

Repeated weight and length measures from birth to 6 months of age were extracted from medical records. For children without weight measurement available within ±14 d of their exact 6-month anniversary (10.2% of children), we used preexisting sex-specific growth models developed to predict weight

at 6 months of age (20). Age-specific and sex-specific *z*-scores for weight at birth and at 6 months of age were calculated using the World Health Organisation (WHO) referent (21). Rapid growth from birth to 6 months of age was defined as a *z*-score weight gain greater than 0.67 standard deviation (22).

Child weight (nearest gramme) and height (nearest 0.1 cm) at 4 years of age were measured by trained staff using standard protocols (without shoes and in light clothing). Child body mass index (BMI, weight/length^2) was used to estimate age-specific and sex-specific *z*-scores based on the WHO referent (21). Overweight was defined as a BMI equal to or above the 85th percentile and obesity as BMI *z*-score equal or above the 95th percentile.

At 4 years of age, waist circumference (WC) was measured at the nearest 0.1 cm using an inelastic tape (SECA model 201; SECA, Hamburg, Germany), at the midpoint between the lowest rib margin and the iliac crest, in a standing position and after a gentle expiration, by trained staff using standard protocols. This measurement was not obtained in the region of Gipuzkoa, and hence, Gipuzkoa was excluded from analyses using WC. WC >90th sex-specific percentile (of the sample distribution) was considered increased risk for abdominal obesity (23). In sensitivity analysis, we used waist-to-height ratio (i.e. WC in cm/height in cm) and the cut-off waist-to-height ratio >0.5 as increased risk for abdominal obesity (24).

Covariates

Based on previous knowledge, the following variables were considered *a priori* as potential confounding factors, mediators or effect modificators of the association between maternal diet in pregnancy and childhood obesity: study region, maternal social class and educational level, maternal pre-pregnancy BMI (based on measured height at recruitment and pre-pregnancy self-reported weight (kg m^{-2})), total energy intake in pregnancy, weight gain during pregnancy (extracted from prenatal visit records), maternal physical activity during pregnancy, gestational diabetes, smoking during pregnancy, maternal age at delivery, breastfeeding duration, child birth weight, child sex, rapid growth from birth to 6 months and child age at anthropometry measurements.

Some individuals had missing values in covariables, and multiple imputations were performed using chained equations; 20 completed data sets were generated and analysed by using the standard combination rules for multiple imputations (25).

Statistical analysis

Descriptive statistics were used to compare socio-demographic characteristics of mother and children included in our study by BMI category of children at age 4 years. Difference in categorical variables was assessed using the chi-squared test; difference in continuous variables was analysed by ANOVA.

Simple and multiple linear regression models were used to estimate the β-coefficients for the association between rMED in pregnancy (expressed both in tertiles and as continuous variables per two-points increment) and BMI *z*-scores and WC of children at 4 years of age. Three models with different levels of adjustment were used. Only covariates that influenced the association between the exposure and outcome of interest were used for adjustment in the most adjusted model (Model 3), following the backward stepwise method ($p < 0.2$). Variables that were shown to be plausible mediators of the association in mediation analyses were not included in any of the studied models (i.e. breastfeeding for models evaluating BMI *z*-scores and gestational diabetes for models evaluating WC). Model 1 was a crude model for both outcomes, Model 2 was a minimally adjusted model and Model 3 was the fully adjusted model (the covariates included for each outcome in each model can be found in Tables 2 and 3).

Logistic regression models were run to study the association between rMED and the odds of offspring having overweight (including obesity) (BMI *z*-score ≥ 85th percentile) and to have abdominal obesity (WC > 90th percentile) at 4 years of age.

To assess heterogeneity among regions in the association between rMED and BMI *z*-score and the WC of offspring at 4 years of age, region-specific estimates were calculated by using general linear models, and random-effect meta-analyses (I^2) were used to pool the estimates.

Models were also stratified by child sex, maternal pre-pregnancy BMI, smoking status, maternal physical activity, social class, educational level and infant birth weight in order to evaluate the homogeneity of effects between these subgroups of *a priori* interest. The statistical significance of interaction terms involving the exposures and these stratification variables was assessed.

As a sensitivity analysis, we repeated all analyses using the alternate Mediterranean Diet score (aMED), another score of adherence of MD (26) developed to be applied to the US population. We compared results defining overweight using weight-for-length age-specific and sex-specific *z*-scores (21), the IOTF criteria (27) and abdominal obesity using waist-to-height ratio. We also repeated BMI models excluding

Gipuzkoa region from the analyses. As complementary analysis, we repeated all analyses further adjusting for child diet (rMED, fibre, and fruit and vegetable intake, in different models) measured at 4 years of age. However, effects estimates did not change substantially, and therefore, these are not shown in the paper.

All statistical analyses were performed using the statistical package STATA 12.1 (Stata Corporation, College Station, TX, USA).

Results

We compared characteristics between mothers included in the study and those lost to follow-up; those lost to follow-up were younger and had lower social class and education level.

The characteristics of mothers and offspring according to categories of weight status of children are shown in Table 1. In our sample, 16.3% of the children were overweighed, and 12.4% were obese according the WHO criteria, and 14.2% overweighed and 5.7% obese according the IOTF (data not shown in tables) (27). Mothers of obese children had higher pre-pregnancy BMI on average. Further, overweight and obese children had higher birth weight on average compared with normal weight children. The prevalence of obesity was higher in the region of Asturias (14.3%) and lower in the region of Sabadell (9.9%). Prevalence of obesity was higher in boys, whereas prevalence of overweight was higher in girls.

Table 2 presents the association between maternal adherence to the MD in pregnancy, using rMED, and BMI *z*-score and WC at 4 years of age. The score rMED was not significantly associated with BMI *z*-score, in any of the studied models. There was an inverse association between the highest tertile of rMED and WC [$\beta = -0.62$; 95% confidence interval (CI): −1.10, −0.14; *P* for trend = 0.009] and also between two units increase of rMED and WC ($\beta = -0.18$; 95% CI: −0.33, −0.03), in Models 2 and 3. We found no association between score rMED in pregnancy and odds of overweight; the observed inverse association between rMED and odds of abdominal obesity was not statistically significant (Table 3).

Stratified analyses were run by different variables of interest. No evidence of effect modification in the associations between rMED and BMI *z*-score, or between rMED and WC were observed after stratification selected variables (Table S1).

All results were homogeneous among the four regions ($I^2 = 0.0\%$) (Figures S2 and S3).

Discussion

In the present study, higher adherence to the MD in pregnancy was not clearly associated with BMI in 4 years old offspring, but it was modestly associated with lower WC: children born of mothers with high adherence to the MD had a WC on average 0.6 cm smaller than children born to mothers with low adherence. While this difference in WC may seem small, it is similar to that reported in previous studies evaluating adherence to the MD and WC in other populations (28–30). At this early age, we do not know if this difference is clinically relevant and will have an impact on future cardiometabolic outcomes; however, these results add to the evidence that early life nutritional factors might have an influence on body composition in early childhood.

To the best of our knowledge, no previous cohort study has evaluated the association between (Mediterranean) dietary patterns in pregnancy and childhood overweight and abdominal obesity risk. Previous human studies have focused on evaluating specific nutrients or foods, and results were mostly inconsistent. Maternal sugar and saturated fatty acid intakes were associated with offspring adiposity at 5 years of age (9). Another study found no association between maternal macronutrient and energy intakes in pregnancy and offspring adiposity at 10 years of age (7). Both maternal meat intake and protein intake were associated with adiposity in studies conducted in adolescents (10). A few studies have evaluated the association between dietary patterns in pregnancy and birth outcomes, including birth weight and foetal birth restriction, potential risk factors for the obesity development later in life; however, results are also inconsistent. In a previous INMA study, diet quality measured with the alternative healthy eating index adapted for pregnancy was associated with a lower risk of delivering an infant with foetal growth restriction (31). In another study that included INMA and a Greek birth cohort, higher adherence to the MD was associated with a lower risk of delivering a foetal-growth-restricted infant, but only in the Spanish Mediterranean INMA regions (Sabadell and Valencia); no association was observed in the Spanish Atlantic regions (Asturias and Gipuzkoa) or the Greek cohort (14). It was hypothesized that the existence of different MD patterns in the different geographic areas could explain the diverse associations observed. Degree of adherence to a Mediterranean dietary pattern in the first semester of pregnancy was associated with several features of intra-uterine growth in the Generation R birth cohort: women with low adherence had a lower placental weight and a lower birth weight (32). Other studies conducted in the USA, such as the Project Viva (33), did not find significant associations between diet quality measures and birth weight characteristics.

Table 1 Characteristics of mothers and children, by weight status of children at 4 years of age

	n = 1827	Normal weight (*n* = 1303, 71.3%)	Overweight (*n* = 298, 16.3%)	Obese (*n* = 226, 12.4%)	*P*
Child characteristics					
Sex (%)					
Male	941	73.5	12.8	13.7	0.000
Female	886	69.0	20.1	10.9	
Age years [mean (SD)]	1827	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	0.268
Region (%)					
Asturias	385	68.5	17.3	14.2	0.002
Gipuzkoa	395	64.6	21.8	13.6	
Sabadell	474	77.1	13.0	9.9	
Valencia	563	73.1	14.5	12.4	
Birth weight [mean (SD)]	1794	3307.0 (403.2)	3423.4 (392.2)	3450.0 (362.5)	0.000
Breastfeeding duration (%)					
0 weeks	259	73.7	14.7	11.6	0.686
>0–16 weeks	437	74.1	14.4	11.5	
>16–24 weeks	286	71.0	16.8	12.2	
>24 weeks	812	69.4	17.4	13.2	
Maternal characteristics					
Age at delivery [mean (SD)]	1827	31.0 (4.1)	30.7 (4.1)	31.1 (4.0)	0.567
Smoking in pregnancy (%)					
No cig/d	1493	71.9	16.2	11.9	0.233
1–4 cig/d	110	62.7	17.3	20.0	
>4–7 cig/d	93	68.8	19.3	11.8	
>7 cig/d	100	74.0	13.0	13.0	
Social class (%)					
I + II	441	73.9	16.1	10.0	0.289
III	507	72.4	15.8	11.8	
IV + V	878	69.4	16.7	13.9	
Education level (%)					
Primary or less	383	69.2	17.8	13.0	0.376
Secondary	762	71.0	15.4	13.6	
University	678	72.7	16.7	10.6	
Physical activity in pregnancy (%)					
Sedentary	131	71.8	13.7	14.5	0.213
Little active	464	70.3	15.3	14.4	
Moderately active	736	70.4	16.6	13.0	
Quite-very active	478	73.8	17.2	9.0	
Pre-pregnancy BMI [mean (SD)]	1827	23.1 (3.9)	24.1 (4.3)	25.9 (5.0)	0.000
Pregnancy weight gain, kg [mean (SD)]	1776	13.7 (4.9)	13.6 (5.0)	14.4 (6.0)	0.119
Pregnancy EI, kcal [mean (SD)]	1827	2074.4 (463.4)	2067.4 (431.3)	2024.8 (470.7)	0.325
Diabetes (%)					
None	1340	70.6	16.7	12.7	0.691
Impaired glucose tolerance	160	72.5	16.9	10.6	
Gestational DM	78	65.4	15.4	19.2	
DM before pregnancy	5	60.0	20.0	20.0	
rMED [mean (SD)]	1827	8.0 (2.6)	8.2 (2.6)	8.0 (2.4)	0.504

Variables defined as follows: overweight: BMI >85th percentile to ≤95th percentile; obese: BMI >95th percentile of World Health Organization reference. BMI, body mass index; DM, diabetes mellitus; EI, energy intake; rMED, relative Mediterranean diet score; SD, standard deviation.

Table 2 Association between maternal rMED in pregnancy and BMI (*z*-score) and WC (cm) at 4 years of age

		T_1	T_2	T_3		2 units increase
	Range:	(1–7)	(8–9)	(10–15)		
rMED			β (95% CI)	β (95% CI)	*P* for trend	β (95% CI)
BMI[1] ($n = 1827$)						
	Model 1	Ref	0.00 (−0.11, 0.12)	−0.01 (−0.13, 0.10)	0.827	0.01 (−0.03, 0.04)
	Model 2	Ref	−0.02 (−0.13, 0.10)	−0.07 (−0.20, 0.05)	0.255	−0.01 (−0.05, 0.03)
	Model 3	Ref	−0.06 (−0.17, 0.05)	−0.09 (−0.20, 0.02)	0.113	−0.02 (−0.06, 0.01)
WC[2] ($n = 1398$)						
	Model 1	Ref	0.13 (−0.42, 0.69)	−0.05 (−0.53,–0.64)	0.806	0.12 (−0.07, 0.30)
	Model 2	Ref	−0.26 (−0.73, 0.20)	−0.57 (−1.07,–0.07)	0.024	−0.15 (−0.31, 0.00)
	Model 3	Ref	−0.34 (−0.78, 0.11)	−0.62 (−1.10,–0.14)	0.009	−0.18 (−0.33,–0.03)

rMED score: range 1–15, in 3 tertiles, to define: low (T_1), medium (T_2) and high (T_3) adherence to the Mediterranean diet.
[1]Model 1: Crude model. General linear regressions with no adjustments.
Model 2: General linear regressions adjusted for child sex, region, child age and maternal total energy intake.
Model 3: General linear regressions adjusted for child sex, region, child age, maternal total energy intake, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.
[2]Model 1: Crude model. General linear regressions with no adjustments.
Model 2: General linear regressions adjusted for child sex, region, child age, maternal total energy intake and child height.
Model 3: General linear regressions adjusted for child sex, region, child age, maternal total energy intake, child height, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight and rapid growth from birth to 6 months and breastfeeding duration.
BMI, body mass index; CI, confidence interval; rMED, relative Mediterranean diet score; WC, waist circumference.

In this study, we observed no association between maternal diet in pregnancy and offspring overweight, but some evidence of an inverse association between diet quality in pregnancy and offspring WC, a marker of abdominal obesity. As pointed out in different studies, maternal weight status seems to be the strongest predictor of obesity later in life (4). Some animal experiments have argued that maternal obesity – as a consequence of a high-fat diet – but not a high-fat diet *per se*, is necessary to programme obesity predisposition in the offspring (5,6). Also, studies in animals have observed that post-natal offspring diet seems to combine with

Table 3 Association between maternal rMED in pregnancy and odds of having overweight and abdominal obesity at 4 years of age

		T_1	T_2	T_3		2 units increase
	Range	(1–7)	(8–9)	(10–15)		
rMED			OR (95% CI)	OR (95% CI)	*P* for trend	OR (95% CI)
Overweight[1] ($n = 1827$)						
	Model 1	Ref	1.06 (0.83, 1.35)	1.08 (0.85, 1.39)	0.506	1.04 (0.96, 1.12)
	Model 2	Ref	0.99 (0.78, 1.28)	0.97 (0.75, 1.26)	0.833	1.00 (0.92, 1.09)
	Model 3	Ref	0.88 (0.67, 1.15)	0.94 (0.71, 1.24)	0.596	0.98 (0.89, 1.07)
Abdominal obesity[2] ($n = 1398$)						
	Model 1	Ref	0.95 (0.63, 1.41)	0.82 (0.53, 1.27)	0.378	1.00 (0.87, 1.15)
	Model 2	Ref	0.90 (0.58, 1.39)	0.62 (0.38, 1.02)	0.068	0.89 (0.48, 1.04)
	Model 3	Ref	0.84 (0.53, 1.32)	0.62 (0.37, 1.03)	0.064	0.89 (0.76, 1.05)

rMED score: range 1–15, in 3 tertiles, to define low (T_1), medium (T_2) and high (T_3) adherence to the Mediterranean diet.
Variables defined as: overweight (including obesity) BMI >85th percentile of World Health Organization reference; abdominal obesity: waist circumference >90th percentile distribution of the sample.
[1]Model 1: Crude model. Logistic regressions with no adjustments.
Model 2: Logistic regressions adjusted for child sex, region, child age and maternal total energy intake.
Model 3: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.
[2]Model 1: Crude model. Logistic regressions with no adjustments.
Model 2: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake and child height.
Model 3: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake, child height, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.
CI, confidence interval; OR, odds ratio; rMED, relative Mediterranean diet score.

prenatal maternal diet in exacerbating obesity risk (5). In rodents, the consumption of a western diet during lactation is particularly critical for the ability of a maternal western diet to cause obesity and associated metabolic consequences in childhood (5). Also, it has been speculated that the effects of intrauterine over-nutrition are hard to detect early in childhood and may predominantly appear later in life. This hypothesis is supported by studies of offspring exposed to gestational diabetes, where it seems that this effect on childhood obesity becomes apparent from the age of 9–10 years but not earlier (34). Finally, it may be possible that MD in pregnancy has a specific effect on programming body fat distribution leading to a lower abdominal obesity risk without influencing general obesity. As observed in this cohort, children born of mothers with higher adherence to MD in pregnancy tend to show a lower WC. This is consistent with previous studies that show that MD influence abdominal obesity independently of total body weight in adults and children (28).

This study has several limitations: diet was evaluated in pregnancy (both first and third trimester), but not during lactation, which might be a critical window of exposure to programme later obesity risk. Nevertheless, results were similar using both first and third trimester dietary data, and it is unlikely that dietary patterns change drastically in lactation compared with third trimester (35). Diet was evaluated using a food frequency questionnaire, subjected to measurement errors that may led to an attenuation of effect estimates. Energy-adjusted dietary data was used in order to try to decrease measurement error. Overweight in children was evaluated using anthropometrical measurements at 4 years of age. It may be too early to detect any potential effect of diet on childhood obesity risk, and hence, further studies are needed at older ages. Also, other indicators of visceral fat accumulation, such as precise measurements of body composition, would be necessary to confirm these findings. Finally, as in any observational study, residual confounding and selection bias cannot totally be ruled out; in this sense, there may be other postpartum unmeasured variables associated with the adherence to MD that may contribute to abdominal adiposity.

The strengths of the present study include the use of a large population-based birth cohort study set-up in several geographical areas of Spain and the prospective design. We used a validated FFQ (17), and anthropometric indicators were measured and not self-reported. We assessed several potential confounders, and we also evaluated the potential mediating effect of these mentioned variables. We also conducted several sensibility and effect modification analyses to test the robustness of our findings.

In conclusion, our data suggest that MD during pregnancy is not associated with measures of overweight in 4 years old offspring, but is inversely associated with offspring WC, a marker of abdominal obesity. Long-term studies with a larger sample size, better measurements of body fat distribution and biomarkers of cardio-metabolic risk are needed to disentangle the plausible effect of MD in pregnancy on visceral fat accumulation in childhood.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to acknowledge all the study participants for their generous collaboration and the interviewers for their assistance in contacting the families and administering the questionnaires. The authors are grateful to Lourdes Cirugeda for her support and assistance in preparing the data set.

A full roster of the INMA Project Investigators can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html.

The study was supported by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041; FIS-FEDER 03/1615, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/1213, 07/0314, 09/02647, 11/0178, 11/01007, 11/02591, 11/02038, 13/1944, 13/2032, 14/00891 and 14/01687; FIS-PI041436; FIS-PI081151; FIS-PI042018 FIS-PI09/02311; and ISPI13/02429), UE (FP7-ENV-2011 cod 282957 and HEALTH.2010.2.4.5-1), Conselleria de Sanitat, GeneralitatValenciana, Generalitat de Catalunya – CIRIT 1999SGR 00241, Obra Social CAJASTUR, Universidad de Oviedo, Department of Health of the Basque Government (2005111093 and 2009111069) and the Provincial Government of Gipuzkoa (DFG06/004 and DFG08/001). Dora Romaguera holds a Ramon y Cajal contract (Ministerio de Economía y Competitividad, Spain and European Regional Development Fund; RYC-2011-08796).

The author's responsibilities were as follows: D. R. designed the research; S. F. B. and D. M. analysed the data; S. F. B. and D. R. wrote the manuscript, with close assistance from D. V., J. V., E. M. N. M. and M. V., taking into account comments and suggestions of other co-authors; J. S. is the overall coordinator of the INMA project, which was conceptualized, designed and implemented in collaboration with the principal investigators in the collaborating centres. S. F. B. and D. R. had

primary responsibility for the final content; all authors read and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1: Flow chart describing the selection process among participants of the INMA project to be included in the present analyses.

Table S1: Association between relative Mediterranean diet score (per 2 units increment) and Body Mass Index (BMI, z-score) and Waist Circumference (WC, cm) at 4 years of age by population subgroups

Figure S2: Region specific association between 2-points increase in the maternal relative Mediterranean Diet Score (rMED) and BMI z-score of offspring at 4 years of age.

Figure S3: Region specific association between 2-points increase in the maternal relative Mediterranean Diet Score (rMED) and Waist Circumference of offspring at 4 years of age.

UNIVERSITAT ROVIRA I VIRGILI
EARLY LIFE FACTORS AND CHILDHOOD OBESITY DEVELOPMENT
Sílvia Fernández Barrés

Manuscript in preparation

Maternal Mediterranean Diet during pregnancy and offspring cardiometabolic risk in early childhood

Abstract

Background: Several observational and intervention studies suggested that the Mediterranean diet has protective effects for cardiometabolic risk in adults.

Objective: To evaluate the association between maternal adherence to the Mediterranean Diet during pregnancy and cardiometabolic biomarkers at pre-school age.

Methods: We included 964 mother-child pairs from the Spanish INMA cohort study. We assessed the dietary intake using a food frequency questionnaire and we used the relative Mediterranean Diet score (rMED). We measured systolic and diastolic blood pressure, high-density cholesterol (HDL), triglycerides and waist circumference at 4 years of age. We calculated a cardiometabolic risk score as the sum of age, sex and cohort specific z-scores of blood pressure, mean between triglycerides and inverse HDL, and waist circumference. We also assessed age, sex and cohort specific z-score biomarkers: leptin, c-peptide, adiponectin, Apo A-1, Apo B, Interleukin 6 and protein C-reactive. We run multivariable adjusted linear models.

Results: rMED during pregnancy was not associated with cardiometabolic risk, blood pressure, lipids or other biomarkers. Higher rMED was associated with higher Apo A-1 levels at 4 years (β: 0.30; 95%CI: 0.08, 0.51: p for trend = 0.007).

Conclusions: Higher adherence to Mediterranean Diet in pregnancy was neither associated with a continuous offspring cardiometabolic risk score, nor was associated with blood pressure or biomarkers at 4 years of age, except for Apo A-1. Further research is needed at older ages.

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EARLY LIFE FACTORS AND CHILDHOOD OBESITY DEVELOPMENT
Sílvia Fernández Barrés

Manuscript in preparation

Mediterranean diet in pregnant women and child longitudinal growth trajectories from birth to up to 4 years in the INMA birth cohort study

Abstract

Background: Few studies have assessed the early life risk factors of child longitudinal growth trajectories, and no study has explored the association between maternal dietary pattern during pregnancy and longitudinal growth trajectories in childhood.

Objective: To evaluate the association between dietary patterns in pregnancy and child longitudinal growth trajectories from birth to 4 years

Methods: We included 2244 mother-child pairs from the longitudinal cohort study INMA project in Spain. We measured dietary intake at 3rd trimester of pregnancy using a food frequency questionnaire. We assessed adherence to Mediterranean Diet by using the relative Mediterranean diet score (rMED) at 3rd trimester of pregnancy. We used body mass index z-scores to developed child longitudinal trajectories from birth to up to 4 years of age using latent class growth analyses. Multinomial adjusted models were used to assess the association between maternal rMED and the child longitudinal growth trajectories.

Results: We identified 5 child longitudinal growth trajectories based on birth size (lower, average and highier) and growth (slower and accelerated). Maternal rMED at 3rd trimester was associated with lower risk of high birth size and accelerated growth (RR: 0.68; 95%CI: 0.47, 0.99; p for trend = 0.056). There were no associations between rMED and the other child longitudinal trajectories.

Conclusions: Higher adherence to the Mediterranean Diet was associated with lower risk of having an offspring with an accelerated growth pattern. Since accelerated growth seems to be detrimental for development of chronic diseases, detecting potential early-life determinants of longitudinal growth trajectories is of special interest. Further research is needed to confirm these results.

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OTHER SCIENTIFIC CONTRIBUTIONS

Fernández-Barrés S, García-Barco M, Basora J, Martínez T, Pedret R, Arija V. Project ATDOM-NUT group. The efficacy of a nutrition education intervention to prevent risk of malnutrition for dependent elderly patients receiving Home Care: a randomized controlled trial. *Int Journal of Nursing Studies*.

Submitted

Fernández-Barrés S, Martín N, Canela T, García-Barco M, Basora J, Arija V; Project ATDOM-NUT group. Dietary intake in the dependent elderly: evaluation of the risk of nutritional deficit. *Journal of Human Nutrition and Dietetics*. 2016;29(2):174-84.

Published

Arija V, Martín N, Canela T, Anguera C, Castelao AI, García-Barco M, García-Campo A, González-Bravo AI, Lucena C, Martínez T, Fernández-Barrés S, Pedret R, Badia W, Basora J. Nutrition education intervention for dependent patients: protocol of a randomized controlled trial. *BMC Public Health*. 2012;12:373.

Published

Annexes

Gillman MW, Rifas-Shiman SL, Fernández-Barrés S, Kleinman K, Taveras EM, Oken E. Beverage Intake during Pregnancy and Childhood Obesity. *Pediatrics.*

Submitted

Julvez J, Méndez M, Fernández-Barrés S, Romaguera D, Vioque J, Llop S, Ibarluzea J, Guxens M, Avella-Garcia C, Tardón A, Riaño I, Andiarena A, Robinson O, Arija V, Esnaola M, Ballester F, Sunyer J. Maternal Consumption of Seafood in Pregnancy and Child Neuropsychological Development: A Longitudinal Study Based on a Population With High Consumption Levels. *Am J Epidemiol* 2016;183(3):169-82. Published.

Poblet-Calaf C, Fernández-Barrés S, Hernández-Gauchia N, Hamudi-Kadhin A, Arija V. Effect of an Educational Intervention Adapted for Obese Immigrant Moroccan Women. *Public Health Nursing.*

Submitted.

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2- OTHER ACHIEVEMENTS

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Sílvia Fernández Barrés

COMMUNICATIONS IN CONGRESSES

Fernández Barrés S, Romaguera D, Valvi D, Martínez D, Arija V, Sunyer J, Vrijheid M. Mediterranean dietary pattern in pregnant women and offspring risk of overweight and abdominal obesity in early childhood: INMA cohort study. Advances and controversies in clinical nutrition conference. American Society of Nutrition. Long Beach (USA), 2015
Participation: oral communication and poster
Publication:

Fernández Barrés S, Arija V, Sunyer J, Vrijheid, M, Valvi D, Martínez D, Riaño I, Santa Marina L, Iñiguez C, Romaguera D. Effect of the Mediterranean Diet during pregnancy and childhood overweight. INMA cohort study. 3r FESNAD Congress. Federación Española de Sociedades de Nutricion, Alimentación y Dietética (FESNAD). Sevilla (Spain), 2015
Participation: oral communication

Fernandez-Barres S, Romaguera D, Cirugeda L, Valvi D, Martínez D, Riaño I, Santa Marina L, Iñiguez C, Arija V, Sunyer J, Vrijheid M. Maternal diet in pregnancy and offspring obesity based on the INMA cohort project. 12th INMA Scientific Conference. CREAL. Barcelona (Spain), 2015
Participation: oral communication

Julvez J, Méndez M, **Fernandez-Barres S**, Sunyer J. Maternal consumption of seafood in pregnancy and child neuropsychological development: A longitudinal study based on a population with high consumption levels. 12th INMA Scientific Conference. CREAL. Barcelona (Spain), 2015
Participation: oral communication

Poblet C, **Fernández-Barrés S**, Escudier E, Hernández N, Hamudy Amal, Domingo L. Intervención intercultural e interdisciplinaria en mujeres con sobrepeso u obesidad y bajo nivel socioeconómico. PACAP 2014. XVI Encuentro del Programa de Actividades Comunitarias en Atención Primaria. Sociedad Española de Medicina Familiar y Comunitaria (SemFYC). Madrid (Spain), 2014
Participation: Poster

Fernández-Barrés S, Romaguera D, Valvi D, Martínez D, Arija V, Sunyer J, Vrijheid M. Mediterranean diet and offspring obesity based on the INMA cohort project. 1st PhD Symposium. ISGlobal. Barcelona (Spain), 2014
Participation: Poster

Julvez J, Méndez M, **Fernández-Barrés S**, Romaguera D, Guxens M, Avella C, Sunyer J. Maternal intakes of seafood types and child neurodevelopment: A longitudinal study based on a population with high consumption levels. Prenatal Programming and Toxicity PPTOX IV Conference. Endocrinology Society. Boston (USA), 2014.
Participation: Poster

Poblet C, Escudier E, **Fernández-Barrés S**, Hernàndez N, Al Mhdawi A, Arija J. Interdisciplinary and intercultural intervention on low-income women with overweight and obesity. 5th EUPHA European Conference on migrant and ethnic minority health. European Public Health Society (EUPHA). Granada (Spain), 2014
Participation: Oral communication

Fernández-Barrés S, Martí N, Canela T, Anguera C, Castelao-Alvarez A, Martínez-Blesa T, Pedret R, Basora J, Arija V. Adequacy of food consumption and nutritional intake of dependent patients in a home care program. 1st World Forum for Nutrition Research. International Nut and Dried Fruit Foundation (INC), Mediterranean Diet Foundation (FDM), International Union of Nutritional Sciences (IUNS) / Fundación iberoamericana de Nutrición (FINUT) y Federación Española de Sociedades de Nutricion, Alimentación y Dietética (FESNAD). Reus (Spain), 2013
Participation: Poster

Arija V, **Fernández Barrés S**, Canela T, Badia W, Anguera C, Castelao AI, Martínez T, Pedret R, Basora J, Martín N. Effect of a Nutrition Education Intervention for Dependent Patients at Risk of Malnutrition of a Home Care Program. 1st World Forum for Nutrition Research. International Nut and Dried Fruit Foundation (INC), Mediterranean Diet Foundation (FDM), International Union of Nutritional Sciences (IUNS) / Fundación iberoamericana de Nutrición (FINUT) y Federación Española de Sociedades de Nutricion, Alimentación y Dietética (FESNAD). Reus (Spain), 2013
Participation: Poster

Canela T, **Fernández Barrés S**, Badia W, García M, García A, González AI, Lucena C, Rovira S, Arija V, Basora J. Relationship Between Dependency and Malnutrition in Home Care Patients with Caregivers. 1st World Forum for Nutrition Research. International Nut and Dried Fruit Foundation (INC), Mediterranean Diet Foundation (FDM), International Union of Nutritional Sciences (IUNS) / Fundación iberoamericana de Nutrición (FINUT) y Federación Española de Sociedades de Nutricion, Alimentación y Dietética (FESNAD). Reus (Spain), 2013
Participation: Poster

Martín N, **Fernández Barrés S**, Arija V, Canela T, Martínez T, García M, Pedret R, Vázquez O, Anguera C
Martínez T, Arija V. Adecuación nutricional en sujetos con riesgo nutricional. 17th International Nursing Research Conference. Sociedad Española de Enfermería Geriátrica y Gerontológica. Lleida (Spain), 2013
Participation: Oral communication

Martín N, **Fernández Barrés S**, Canela T, García M, Vázquez O, Arija V. Ingesta de macronutrientes en una muestra de sujetos en riesgo de desnutrición. XI Congreso de la Asociación de enfermería Familiar y Comunitaria de Cataluña. Sitges (Spain), 2012.
Participation: Oral communication

Fernández Barrés S, González AI, Castelao AI, Anguera C, Martínez T, Arija V. Valoración de la ingesta nutricional de personas mayores en programa de atención domiciliaria. XIX Congreso Nacional de la Sociedad Española de Enfermería Geriátrica y Gerontológica. IV Jornada para Auxiliares, Gerocultores y Cuidadores. Sociedad Española de Enfermería Geriátrica y Gerontológica. Tarragona (Spain), 2012
Participation: Oral communication

Fernández-Barrés S, González Bravo A, Castelao A, Martínez T, Anguera C, Arija V. Factors associated with degree of malnutrition in dependent patients. XXXII Congress of Spanish Society of Family and Community Medicine. Bilbao (Spain), 2012.
Participation: Oral communication

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January 31, 2016

Victoria Arija Val
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Spain

Dear Professor Arija Val:

I am pleased to certify that Sílvia Fernandez Barres' research internship with us at the Department of Population Medicine has been a great success. She completed her research internship under my supervision; the internship ran from September 28, 2015 to January 31, 2016.

During her time here with us at the Harvard Pilgrim Health Care Institute, she examined the extent to which combination of 9 modifiable prenatal and infant risk factors predict childhood obesity and adiposity at mid-childhood, employing dietary intake assessment methodologies to data collected in the Project Viva and INMA cohorts, and examined similarities and divergences in results from the two cohort studies.

She performed this research under my direct supervision, and I met with her formally every week to review her progress, assign readings, discuss methodologies and techniques, and work with her to produce a high-quality scientific manuscript suitable for publication.

Yours sincerely,

Matthew W. Gillman, MD, SM

GRANTS, AWARDS AND TEACHING

Grants

Travel award

Travel award to attend to the Advances and Controversies in Clinical Nutrition by the American Society of Nutrition 2015

Mobility Grant

Mobility Grant by the Biomedicine Doctoral program of Universitat Rovira I Virgili 2015

PhD Grant

Doctoral Program Martí Franqués by the Universitat Rovira I Virgili 2013

Teaching

- Public Health and research related courses of BSc Human Nutrition and Dietetics (Universitat Rovira I Virgili).
- Research seminars of MSc Nutrition and Metabolism (Universitat Rovira I Virgili and Universidad de Barcelona)
- Codirector of 2 Final projects of bachelor studies (Nutrition in Universitat Rovira I Virgili, and Biology in Universitat Pompeu Fabra).
- Codirector of a Final Master project (Nutrition and Metabolism in Universitat Rovira I Virgili).