UNIVERSITÀ DI PAVIA

SCUOLA DI ALTA FORMAZIONE DOTTORALE

MACRO-AREA SCIENZE DELLA VITA

PhD in Psychology, Neuroscience and Data Science
Department of Brain and Behavioral Sciences

Early-Life Origins of Child Growth: Maternal Pre-Pregnancy BMI, Gestational Exposures, and Neonatal Microbiome Signatures

Academic year 2023-2026
Cycle XXXVIII

Coordinatore
Prof.ssa Elena Cavallini

Doctoral candidate
Dr. Dana El Masri

Tutor
Prof.ssa Hellas Cena

Co-Tutor
Prof.ssa Rachele De Giuseppe

Abstract

Childhood obesity represents a major global public health challenge, with increasing evidence identifying the prenatal period as a critical window for metabolic programming. This PhD thesis investigates early-life determinants relevant to childhood obesity risk, focusing on maternal pre-pregnancy body mass index (BMI), gestational weight gain (GWG), lifestyle factors during pregnancy, and neonatal gut microbiota signatures at birth.

This thesis is structured as a publication-based doctoral work and is embedded within the LIMIT (LIfestyle and Microbiome InTeraction Early Adiposity Rebound in Children) prospective cohort study. It primarily addresses baseline (T0) assessments conducted at delivery. Framed within the Developmental Origins of Health and Disease (DOHaD) paradigm and the first 1,000 days framework, the thesis integrates evidence synthesis with original empirical analyses to examine how prenatal exposures may shape early metabolic and microbial profiles.

The publication-based structure comprises a series of peer-reviewed and under-submission studies. First, a literature synthesis includes narrative and systematic reviews examining maternal lifestyle and nutritional exposures, early microbial environments, and the offspring exposome as determinants of obesity risk. Second, an original empirical study evaluates associations between maternal lifestyle behaviors during pregnancy and the adequacy of gestational weight gain. Third, a cross-sectional analysis investigates relationships between maternal pre-pregnancy BMI, GWG, lifestyle factors, and neonatal meconium microbiota composition at birth using 16S rRNA gene sequencing.

Overall, this thesis characterizes key maternal and neonatal factors present at delivery that are biologically and behaviorally relevant to later obesity susceptibility. By focusing on early-life exposures rather than downstream obesity outcomes, this publication-based work contributes

to advancing understanding of early metabolic programming and informs future preventive strategies beginning before and during pregnancy.

Table of Contents

Chapter 1: General Introduction

1.1. Global Burden of Overweight and Obesity

Over the last decades, obesity has become one of the most serious public-health issues worldwide, affecting individuals from all age groups, including women of reproductive age as well as children[1,2]. As stated by the World Health Organization (WHO), overweight and obesity are the major risk factors for several non-communicable diseases (NCDs), including type II diabetes[3], cardiovascular diseases[3], metabolic syndrome, various types of cancer[3], musculoskeletal disorders[3], and overall impaired health status[3].

The WHO has defined obesity as an abnormal or excessive accumulation of body fat that can negatively affect the human health[3]. In adults, it is usually assessed through calculating the body mass index (BMI) and having a value more than or equal to 30 Kg/m^2[3]. However, in children and adolescents, age- and sex-specific BMI z-scores are used, based on international growth references[4]. While BMI does not directly measure body fat distribution at the individual level, it remains the most widely used indicator in population-based research and public health surveillance due to its feasibility and strong association with morbidity and mortality outcomes[5].

Latest updates reveal that by 2050, overweight and obesity rates are expected to reach approximately 3.8 billion adults, corresponding to nearly 60% of the adult population (10.2% to 20.8% are women), and 746 million children and adolescents, representing 31% of the young population[2].

Trends in global obesity prevalence and future projections are summarized in ***Figure 1***[2].

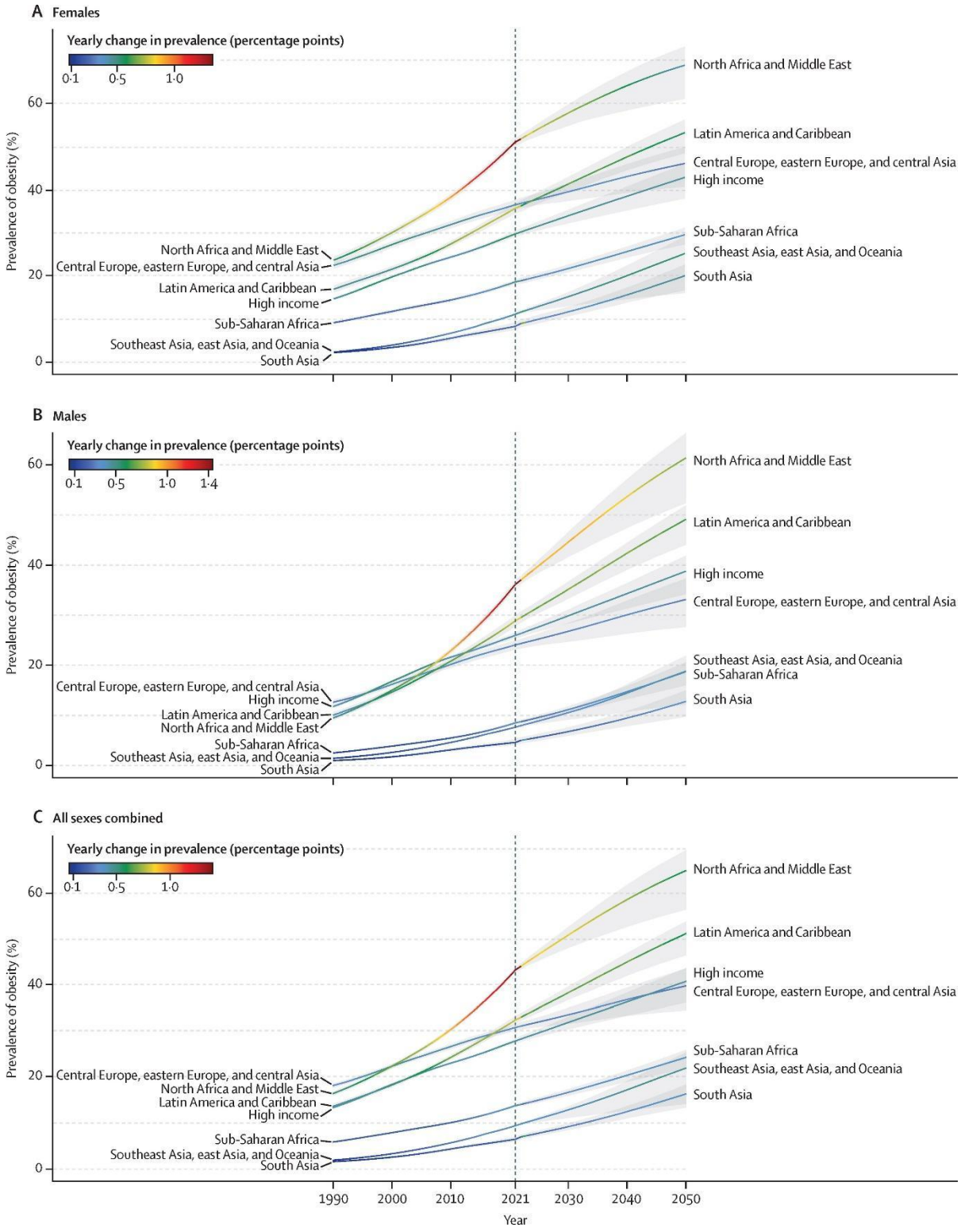


Figure 1. *Estimated age-standardised prevalence of obesity in adults aged 25 years and older by sex, globally and by super-region, 1990–2050. (A) Females. (B) Males. (C) All sexes combined. Shaded regions are 95% uncertainty intervals. Reprinted from GBD 2021 Adult BMI Collaborators (2025)*[2].

This rapid expansion has been driven by societal and environmental changes, including urbanization, reduced physical activity (PA), and increased availability of energy-dense ultra-processed foods (UPF)[6,7]. Further concern is that nowadays obesity coexists with undernutrition in many regions, generating what we now call the "Double Burden of Malnutrition" that has significant effects on women and children[8,9].

In Europe, the number of individuals affected by obesity has tripled over the last few decades[10], reflecting substantial changes in lifestyle, dietary patterns, and environmental exposures[10]. As shown in the WHO European Childhood Obesity Surveillance Initiative (COSI)[11], a high and persistent prevalence of overweight and obesity is detected among primary school–aged children (6–9 years), with limited evidence of meaningful declines over time[11]. Approximately 25% of children in this age range in the WHO European Region are affected by being overweight (including obesity), with obesity alone accounting for 11% of them ***(Figure 2)***. This emphasises the magnitude of this public health challenge and highlights the limited effectiveness of interventions that could be applied once the individual has reached the class of obesity[11,12].

Marked socioeconomic and geographic inequalities characterize childhood obesity prevalence across Europe. Data from the WHO European COSI also indicate higher prevalences among children from families with lower parental educational attainment ***(Figure 3)***, and substantial variation across countries, with higher rates observed in Southern and Eastern Europe compared with Northern regions. Through this, we can observe the influence of social and environmental determinants beyond individual behaviours[11].

According to data from the World Obesity Federation, Italy currently shows a prevalence of obesity affecting approximately 17.8% of adults and 9.8% of children, highlighting the growing public health relevance of this condition at the national level[13]. Despite being a Mediterranean country, Italy nowadays is facing a declining adherence to the Mediterranean dietary pattern coupled with increased consumption of energy-dense and processed foods, especially in southern areas[14,15].

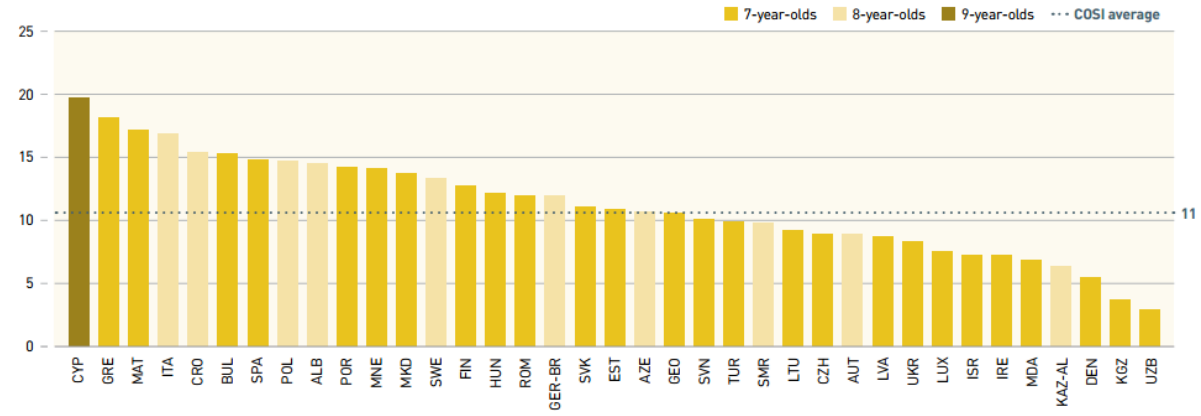


Figure 2 *Prevalence of obesity (WHO definitions) in children aged 7–9 years (%).Copied from WHO COSI data*[11].

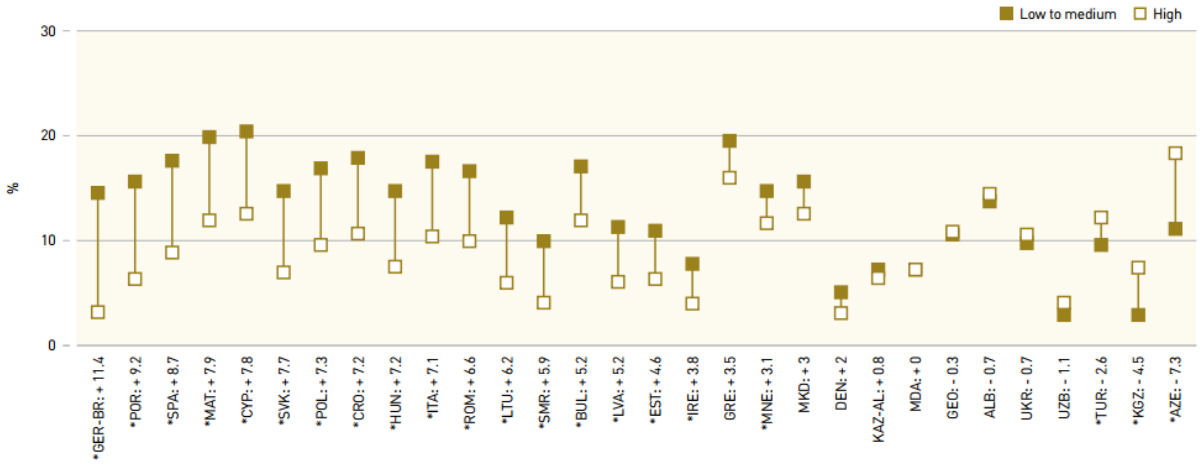


Figure 3. *Prevalence of obesity (WHO definitions) in children aged 6–9 years, by level of parental education (low to medium versus high) (%). a Variations, measured in percentage points, were calculated as the difference between the estimate for children with low to medium parental education and the estimate for children with high parental education*
Data relate to all children aged 6–9 years for whom data about parental education and weight status were available. The asterisk indicates a significant difference (P < 0.05). Copied from WHO COSI data[11].

From a public health perspective, the persistence of obesity across the life course reflects strong tracking of adiposity from childhood into adulthood[16,17]. Children with overweight or obesity are more likely to remain with obesity through adulthood, with limited effectiveness of interventions implemented after obesity is already established[18]. These observations highlight the need to shift preventive efforts toward earlier stages of life, before excess adiposity and metabolic dysfunction become hard to reverse[11,19].

In recent years, this need has become particularly urgent due to the rising prevalence of overweight and obesity among women of reproductive age, a trend that directly affects the intrauterine environment and early developmental trajectories of the offspring. Traditional interventions implemented after birth or during childhood have shown limited long-term

effectiveness, highlighting the importance of shifting prevention strategies toward earlier life stages. Increasing attention has therefore focused on the combined role of maternal lifestyle behaviours, metabolic status, and early microbial exposures as modifiable determinants acting before obesity becomes established. Within this framework, pregnancy and the periconceptional period represent a critical window for early prevention, with the potential to interrupt intergenerational cycles of obesity risk.

Rising global rates of overweight and obesity are particularly alarming among women of childbearing age[10]. Evidence suggests that excessive maternal adiposity, before and during pregnancy, is linked to adverse pregnancy outcomes, including gestational diabetes mellitus (GDM)[20], pre-eclampsia[20], caesarean delivery[20], and excessive gestational weight gain (GWG), as well as a higher risk of giving birth to large-for-gestational age infants[21]. Furthermore, it may predispose offspring to develop obesity and experience other metabolic disorders in the future[10,22,23], creating a cycle of intergenerational health risk.

Children born to mothers with overweight or obesity are more likely to experience accelerated postnatal weight gain, higher adiposity rates, and altered metabolic profiles during childhood, which may continue into adolescence and adulthood[17,24]. These observations highlight the importance of targeting maternal health before and during pregnancy, as a critical phase for implementing strategies to prevent childhood obesity[18,19].

Strategies for prevention have increasingly prioritised early life, particularly within the “first 1,000 days” window, the critical period of life that starts from preconception and ends at the second year of age[25,26]. This window is considered a critical stage of plasticity during children's growth, metabolic health and disease susceptibility could be influenced by environmental, nutritional, and other lifestyle exposures[26–28]. Within this period, early-life interventions can be very effective in preventing the risk of childhood obesity and its related consequences, through targeting biological and behavioural determinants before the unhealthy trajectories take the lead[27].

In this context, ensuring optimal maternal weight and metabolic health as well as healthy lifestyle behaviours may serve as a defensive opportunity against the intergenerational transmission of obesity and consequently will reduce the risk of obesity-related diseases**.**

1.2 Maternal Lifestyle Factors

Maternal lifestyle factors before and during pregnancy, including dietary patterns, PA, smoking, alcohol consumption, sleep quality, and psychosocial stress, play an important role in shaping maternal metabolic health[29]. They also contribute to shaping the intrauterine environment[30,31]. Evidence from life-course and developmental origins research[32] highlights that these exposures can influence maternal GWG, glucose metabolism, inflammatory pathways, and hormonal regulation, thereby affecting both maternal and fetal health[23]. Unhealthy lifestyle behaviours are common among women of reproductive age and have been associated with high risks of GDM, hypertensive disorders, and adverse cardiometabolic outcomes in the offspring[32].

Maternal nutrition is not limited to what a mother consumes during her pregnancy [33]. Rather, it represents a continuum of nutritional status and behaviours all over the periods of preconception, pregnancy, and postpartum[32]. Adequate preconception nutrition is fundamental for the mother to establish sufficient nutrient stores, such as iron and folic acid, aiming at supporting early fetal development[10,29,34], particularly during the first weeks of gestation when many women are not yet aware they are pregnant[32]. Adequate nutrition before and during pregnancy has been associated with optimal GWG, adequate fetal growth, and reduced rates of pregnancy complications[20,35].

During pregnancy, diet quality has emerged as a critical determinant of maternal and neonatal metabolic health[36]. Dietary patterns rich in fruits, vegetables, whole grains, legumes, nuts, and unsaturated fats, such as the Mediterranean diet (MD)[35], are associated with improved insulin sensitivity, reduced systemic inflammation, and better regulation of GWG[35,37]. In contrast, diets high in saturated fats, refined carbohydrates, ultra-processed foods and added sugars have been linked to excessive GWG, maternal and fetal metabolic dysregulation[38] and higher rates of preterm birth and low birth weight[37,38].

The MD pattern features a high consumption of fruits, vegetables, whole grains, legumes, nuts, olive oil, and moderate fish consumption, and adhering to this pattern offers a well-balanced macronutrient profile with anti-inflammatory and antioxidant properties[35,39]. In a systematic review conducted by Sharma et al. 202[39] on a large-scale of observational studies and randomized controlled trials to evaluate the protective effect of MD adherence on metabolic and obstetric complications, it was observed that higher adherence was associated with a lower risk of developing GDM for up to 28%.

By providing abundant dietary fiber, polyphenols, and unsaturated fatty acids, the MD may influence maternal gut microbiota and early microbial transmission at birth, providing a mechanistic pathway linking maternal diet quality with GWG, and the composition of neonatal microbiota[40].

In addition to nutrition, regular PA before and during pregnancy contributes to improved metabolic health[41] and supports appropriate GWG[42], although adherence remains low due to multiple factors[43].

In a recent systematic review and meta-analysis conducted by Xie W et al (2024)[44], the authors demonstrated an important role of PA in improving insulin sensitivity and reducing GDM risk by up to 36%. The study also highlights a clear dose-response relationship, showing that increasing PA levels is significantly linked to lower risks of developing GDM. The findings emphasize that pregnant women are a primary target for interventions aimed at maintaining moderate-intensity activity to ensure optimal pregnancy outcomes[44].

Smoking[46,47], alcohol consumption[47], inadequate sleep[48], and high stress levels[49] further contribute to adverse pregnancy outcomes and may interact with maternal overweight or obesity to exacerbate metabolic risk[50].

In a cohort study conducted by Schnurr M. T et al. (2022)[51], a sample of 700 mother-infant dyads taken from the German GINIplus and LISA birth cohorts was examined to assess the effects of maternal smoking during pregnancy on longer-term health outcomes in offspring. Children exposed to smoking in utero were found to have a significantly higher risk of being overweight by the age of 10 years, even after adjusting for maternal pre-pregnancy BMI (pre-BMI) and the child's genetic predisposition to adiposity (measured via polygenic risk scores). These findings suggest an association between smoking during pregnancy, as a direct intrauterine stressor, and children's metabolic risk[51].

A literature review explored the relationship between maternal alcohol consumption during pregnancy and long-term health outcomes in children. The analysis highlighted that alcohol exposure in utero can induce oxidative stress, impair cellular function and neurodevelopment, and increase the risk of obesity later in life, as well as insulin resistance, dyslipidaemia, and neurocognitive deficits[52].

Poor maternal sleep and elevated stress levels were also found in the literature to have an impact on the offspring outcomes, including risk of GDM, higher values of BMI and waist circumference in children, as well as increasing adiposity[48,53].

Collectively, these maternal lifestyle factors may also influence early biological pathways relevant to offspring health, including the establishment of maternal and neonatal microbiota, which have been proposed as potential mediators linking prenatal exposures to later obesity risk[54].

1.3 Gestational Weight Gain (GWG)

GWG is an important indicator of maternal nutritional and metabolic status during the period of pregnancy[55], and reflects the combined influence of pre-BMI, maternal lifestyle behaviours, and fetal growth[56]. Both low and excessive GWG have been consistently associated with adverse maternal and neonatal outcomes[56–58], making GWG a central focus in maternal and child health research.

To standardize the assessment of GWG adequacy, the Institute of Medicine (IOM) has created evidence-based recommendations defining optimal ranges of GWG based on pre-BMI categories[55].

Studies have demonstrated an association between excessive GWG with higher likelihood of GDM, hypertensive disorders of pregnancy, caesarean delivery, postpartum weight retention, and delivery of large-for-gestational-age infants, whereas low GWG is linked to preterm birth and small-for-gestational age infants[57,59]. Moreover, excessive GWG may contribute to long-term maternal obesity[58] and increase offspring susceptibility to metabolic disorders, thereby reinforcing intergenerational patterns of obesity risk.

Although pre-pregnancy is a major determinant of GWG[59,60]; growing evidence indicates that modifiable lifestyle factors during pregnancy, particularly PA[42] and nutrition[60], can independently provide a positive influence on GWG trajectories.

Regular PA, before and during pregnancy, can reduce the risk of excessive GWG by approximately 32% and decrease total weight gain by an average of 0.9 Kg[42]. It is also associated with reduced risk of developing GDM by 38%, gestational hypertension by 39%, and pre-eclampsia by 41%[41]. Moreover, women who exercise during pregnancy, are less likely to experience postpartum water retention, which is a risk factor for developing obesity, and more likely to have vaginal deliveries[42].

Pre-pregnancy BMI	BMI Category (Kg/m^2)	Total Weight Gain (lbs)	Total Weight Gain (Kg)
Underweight	< 18.5	28 – 40	12.5 – 18
Normal Weight	18.5 – 24.9	25 – 35	11.5 – 16
Overweight	25.0 – 29.9	15 – 25	7 – 11.5
Obese (all classes)	≥ 30.0	11 – 20	5 – 9

Table 1. *IOM 2009 Recommendations for Total Gestational Weight Gain*

1.4 Pregnancy as a Critical Period for Early-Life Programming

Pregnancy is a foundational phase of the first 1000 days of life, during which environmental exposures can alter fetal development and health trajectories throughout the life course[27]. This concept is central to the Developmental Origins of Health and Disease (DOHaD) framework[61,62], which proposes that nutritional, metabolic, and lifestyle-related exposures during prenatal and early postnatal life may influence susceptibility to chronic diseases, including obesity and metabolic disorders, later in life[61].

Maternal metabolic status, GWG, and lifestyle behaviours during pregnancy act as critical signals that shape fetal growth and development through multiple biological pathways, including altered nutrient supply, hormonal signalling, inflammatory processes, and epigenetic modifications[27,63,64]. These biological mechanisms play a role in developmental programming, creating metabolic patterns during infancy that could continue into adulthood and affect future health outcomes[27].

Adiposity rebound (AR) is the primary indicator of early-life metabolic programming and represents the secondary rise in BMI that typically occurs between ages 4 and 7 years. However, an accelerated onset of this phase could happen earlier, and it is widely known as early adiposity rebound (EAR). This early transition signals an imbalance in developmental programming, which is a risk factor for the future development of metabolic syndrome and a higher likelihood of having insulin resistance and chronic cardiometabolic complications[65]. Research suggests that maternal weight status and the intrauterine environment are the main drivers for this early transition[65]. For this reason, EAR is used as a clinical indicator for developmental programming that can detect early metabolic exposures influencing children's health and growth trends[65].

While AR and EAR are not evaluated as outcomes within my PhD thesis, they are targeted as outcomes within the LIMIT (LIfestyle and Microbiome InTeraction early adiposity rebound in children) prospective cohort study[66].

At the offspring level, maternal PA has a central role in managing the intrauterine environment through boosting placental function and nutrient transport to the fetus. This optimization helps ensure healthy fetal growth, preventing excess fat accumulation (macrosomia) while preserving lean muscle mass[68,69]. Maternal PA during and after pregnancy positively influences weight trajectories in offspring[68].

Other maternal lifestyle factors, such as the quality of dietary consumption, smoking status, alcohol consumption, quality of sleeping, and psychosocial stress levels, may also influence developmental programming through different metabolic and inflammatory systems[27]. These lifestyle exposures likely interact with maternal weight status and shape the infant's risk of lifelong metabolic disorders.

1.5 Early-Life Microbiota and Metabolic Programming

The human gut microbiome is a fundamental moderator for metabolic function, energy balance, and the immune system. A growing body of evidence identifies gut microbial dysbiosis (alterations in composition and diversity) as key influencers for weight gain and metabolic disorders. These alterations can adversely influence the caloric extraction from the diet, create systemic inflammation, and reduce insulin sensitivity[69,70].

Maternal microbiota during pregnancy and at delivery is fundamental for shaping the infant's immune and microbial systems through multiple biological pathways. These include the systemic transfer of metabolites across the placenta and the exposure to maternal microbes during delivery through vaginal, skin and mucosal contacts[69]. Moreover, the presence of maternal short-chain fatty acids (SCFAs) and secondary bile acids of maternal origin helps in regulating fetal immunity and preparing infants' gut for postnatal colonization[71].

Beyond these mechanisms, the early development of neonatal gut microbiota within the first 1,000 days of life is affected by multiple interactions between maternal, perinatal, and environmental exposures[69] ***(Figure 4***[72]***)***. These include pre-BMI[54], GWG[54], maternal dietary pattern[73], delivery mode[74], infant feeding choices[74], antibiotic use[74], and broader environmental exposures[74]. Among these determinants, maternal lifestyle factors

and weight status have been linked to the specific diversity and density of the newborn's gut microbiota[54,73,75].

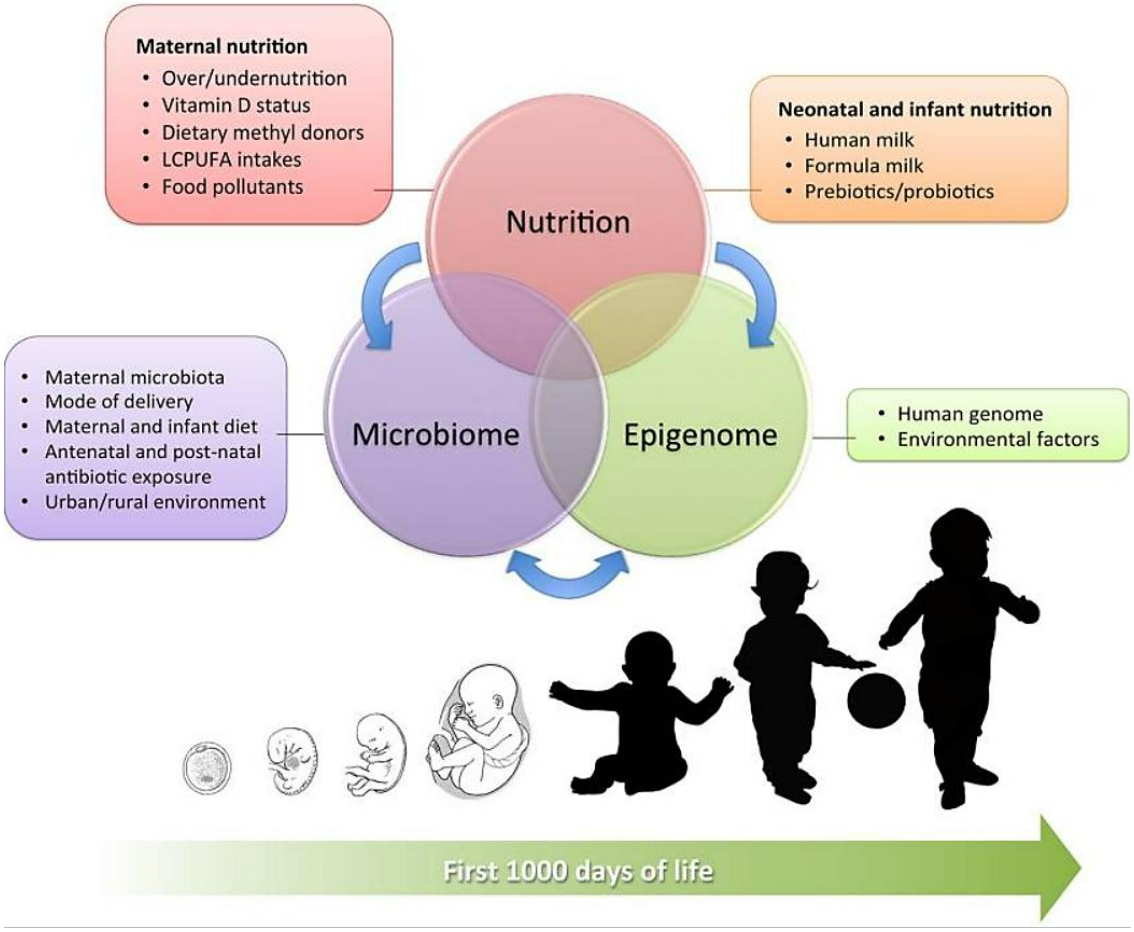


***Figure 4**. Interrelation between maternal and neonatal nutrition, gut microbiota, and epigenetics during the first 1,000 days of life. The main influencing factors are detailed in the boxes. Copyright © 2017 Indrio, Martini, Francavilla, Corvaglia, Cristofori, Mastrolia, Neu, Rautava, Russo Spena, Raimondi and Loverro*[72].

Growing evidence suggests that these maternal influences on early microbial composition may represent a biological pathway linking prenatal exposures to early-life metabolic programming. Maternal pre-BMI, GWG, and dietary quality have been associated with differences in neonatal gut microbiota, including reduced microbial diversity and altered abundance of taxa involved in metabolic regulation[54,73]. Such microbial signatures may contribute to offspring metabolic susceptibility by modulating the maturation of the immune system, the integrity of the intestinal barrier, and the regulation of systemic inflammatory pathways[69].

Despite the need for further investigations to confirm a causal mechanism for early-life microbiota, the consistency of findings in the literature highlights the relevance of the infant microbiome. The investigation of microbial profiles at delivery provides a unique opportunity for identifying early markers of developmental programming[76][69,76]. By understanding the

pathways by which these exposures can influence neonatal microbiota, targeted early prevention strategies must be implemented.

1.6 Meconium Microbiota at Delivery (T0)

Meconium, the first neonatal stool, provides a unique biological image for the intrauterine environment that holds the history of fetal exposures, including maternal influences. It helps in understanding the microbial exchange occurred during late pregnancy between the mother and her infant[77]. Traditionally considered sterile, advanced molecular techniques have demonstrated that meconium contains a wide array of microbial-derived metabolites. These chemical signatures reflect a complex exchange of microbial and metabolic signals from the mother to the fetus during late gestation and the perinatal period[77]. As reported by Bekhti et al. (2022) [77], the metabolic profile of meconium has matured rapidly between delivery and almost the third day of birth, influenced by several exposomes including the initiation of feeding[77]. This rapid transition confirms the importance of analysing meconium to identify early signs of fetal programming before the effect of postnatal exposures.

The microbial composition of meconium is mainly influenced by multiple interactions between maternal, placental, and perinatal factors. As demonstrated by Liu et al. (2025)[78], infants born to mothers with GDM had a lower microbial diversity and composition. Maternal pre-BMI, age, and GWG were also shown as potential moderators for these microbial profiles[78].

Based on the framework of the first 1,000 days and the DOHaD paradigm[61,62], meconium microbiota can be conceptualized as an early biological “print” of prenatal and perinatal influences.

In the LIMIT[66] prospective cohort study, baseline (T0: at delivery) assessments include the systematic collection and analysis of meconium samples as a baseline biological assessment upon which all subsequent longitudinal analyses will be performed.

Within the DOHaD framework, these early microbial and metabolic signatures at T0 may contribute to developmental programming processes that influence postnatal growth trajectories and the timing of adiposity rebound, thereby providing biological context for later outcomes such as early adiposity rebound, even when these outcomes are not directly assessed at baseline.

1.7 The LIMIT Prospective Cohort Study and Scope of the Present PhD Thesis

The present PhD thesis is embedded within the LIMIT (; www.clinicaltrials.gov NCT04960670)[66] study, an ongoing observational study designed to investigate early-life determinants of childhood obesity risk. The LIMIT[66] study follows 272 mother–child dyads from delivery until early childhood, with follow-up extending up to 36 months of age. It aims at examining the link between infant gut microbiota during the first 6 months of life, and EAR by the age of 36 months, as well as exploring how maternal and infant lifestyle factors, and environmental exposures shape infant gut microbiome at 6 time points spanning pregnancy, delivery, and postnatal follow-up (T0, at delivery; T1, 1 month; T2, 6 months; T3, 12 months; T4, 24 months; T5, 36 months after birth). ***Figure 5***: Graphical abstract of LIMIT[66] study

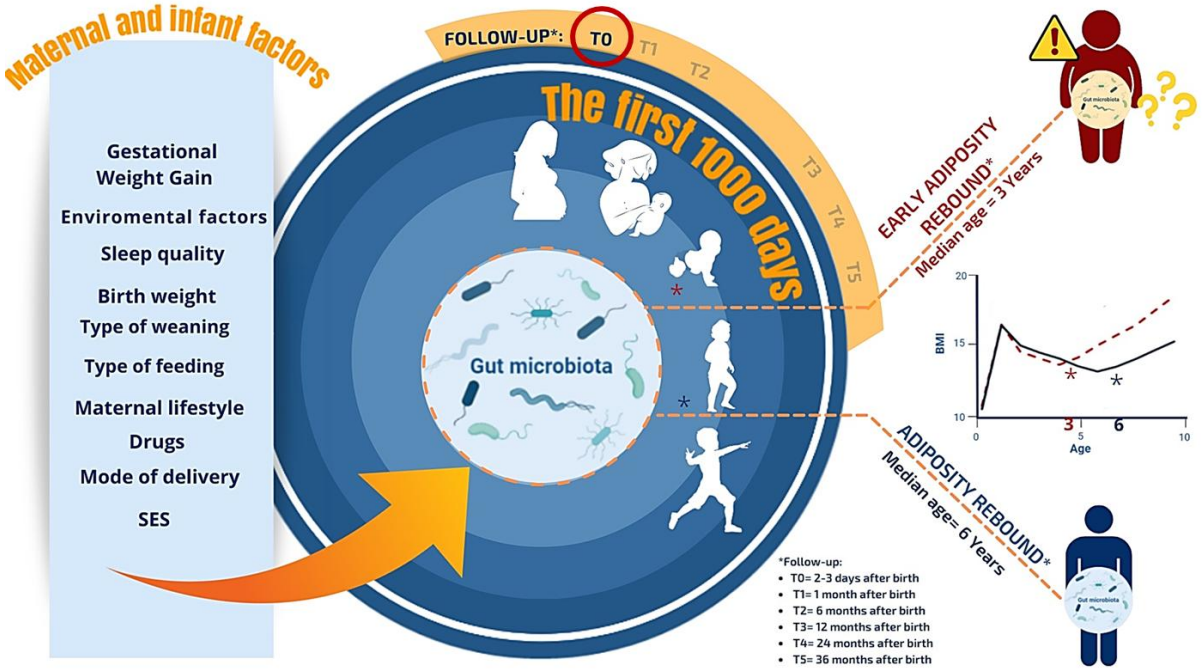


Figure 5. *Graphical abstract of LIMIT study representing all time points of measurement. T0 measurements are the scope of the present thesis*[66]

The LIMIT study was funded by:

1. *The National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.4-Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP F13C22000720007, Project title "National Biodiversity Future Center-NBFC"*

2. *The National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.3-Call for proposal No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU. Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by*

the Italian Ministry of University and Research, CUP F13C22001210007, Project title "ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security-Working ON Foods.

3. *The University of Pavia's "Fondo Ricerca e Giovani, years 2020–2021 and 2021-2022.*

Subsequent follow-up assessments (T1–T5) will capture dynamic changes in infant gut microbiota, growth patterns, lifestyle exposures, and metabolic indicators across early childhood, ultimately allowing evaluation of early adiposity rebound and related obesity risk within a longitudinal developmental framework.

Despite the longitudinal nature of the LIMIT[66] study, the scope of this PhD thesis is mainly restricted to baseline assessments (T0) conducted at delivery. Specifically focusing on maternal pre- BMI, GWG, lifestyle factors and environmental exposures, and their associations with neonatal meconium microbiota composition at birth.

The comprehensive framework of the LIMIT study is reported in the published study protocol[66]. In accordance with the objectives of this PhD thesis, only methodological aspects directly relevant to baseline data collection and T0 microbiota analyses are addressed.

From a conceptual perspective, T0 measurements could be considered as the starting conditions of a long developmental trajectory. While the LIMIT[66] study follows children across early life, enabling the evaluation of growth patterns and obesity-related outcomes, the present thesis focuses on identifying early biological and lifestyle-related determinants present at delivery. Therefore, this work refines current hypotheses regarding early microbial shaping and informs the interpretation of metabolic programming processes relevant to childhood obesity risk.

1.8 Aims of the PhD Thesis

The overall aim is to cross-sectionally characterise, within the LIMIT[66] study, key early-life exposures that have been linked to later childhood obesity risk, focusing on maternal pre-pregnancy weight status (e.g. pre-BMI), GWG, and pregnancy lifestyle factors (including nutrition and other behavioural determinants), together with the neonatal meconium microbiota profile at delivery. Specifically, the thesis will describe these maternal and neonatal features and explore their interrelationships at birth, without evaluating childhood obesity as an outcome. Therefore, specific objectives are

1. To synthesize current evidence on maternal lifestyle, nutrition, and early microbial exposures relevant to offspring obesity risk.

2. To assess the relationship between maternal lifestyle factors during pregnancy and GWG adequacy.

3. To investigate associations between maternal weight status (pre-BMI and GWG) and lifestyle factors, with infant microbiota composition of the meconium at the delivery (Bacteria 16S rRNA gene variable regions 1-8, bV18-A)

1.9 Structure of the Thesis

This PhD thesis is structured around three complementary objectives based on the published and under-submission studies conducted during my doctoral program (2023-2026).

Briefly, first (**Chapter 2**), it aims to synthesise the current evidence on maternal lifestyle and nutrition during pregnancy, as well as early microbial exposures, as determinants relevant to offspring obesity risk. To address this objective, I conducted a structured body of literature work, comprising two narrative reviews[27,79] and one systematic review[80], which together provide a critical and updated framework on the biological plausibility, consistency of findings, and methodological gaps in this research area.
Building on this evidence base, the thesis then moves to an empirical component embedded within the ongoing LIMIT study[66] (**Chapter 3**). Indeed, the second objective focuses on the relationship between maternal lifestyle factors during pregnancy (particularly nutrition and related behavioural determinants) and the adequacy of GWG, which was examined through a cross-sectional analysis at baseline/time of delivery and resulted in one original research paper[81]. The third objective further expands this investigation by exploring, within the same cohort and at T0, the associations between maternal weight status (pre-BMI and GWG) and maternal lifestyle factors with the neonatal meconium microbiota profile at birth, also using a cross-sectional design and resulting in a second original research paper.

Overall, the thesis combines a robust synthesis of existing evidence with original analyses of maternal and neonatal characteristics within LIMIT[66] study, providing an integrated view of pregnancy-related exposures and early-life microbial patterns that are considered relevant to later obesity susceptibility, while not assessing childhood obesity outcomes within the scope of this doctoral work.

Last, **Chapter 4** integrates findings and concludes with implications and future directions both for the LIMIT[66] study as well as for clinical practice. For each included publication[27,79,80], I made substantial contributions to the study and methodology design,

data collection and analysis and scientific writing process, including manuscript drafting and iterative revision across multiple rounds. Beyond producing original text, I actively shaped the overall structure and narrative coherence of each paper, refined the rationale and framing in line with the study objectives, and strengthened the clarity, consistency, and scientific tone of the Methods, Results, and Discussion sections.

I also contributed to the interpretation of findings and their contextualisation within the existing literature, ensuring that conclusions were appropriately supported by the data and aligned with current evidence.

In publications where I was not the first author[81], I nonetheless played an important role in critically revising key sections, integrating co-authors' feedback, addressing methodological and editorial comments, and supporting the finalisation of the submission-ready version.

My contributions are documented in the "Author Contribution" statements of the respective publications.

CHAPTER 2: LITERATURE SYNTHESIS

This chapter integrates three literature reviews carried out during my PhD journey (2023-2026) at Laboratory of Dietetics and Clinical Nutrition (LDNC) - Department of Public Health, Experimental and Forensic Medicine - University of Pavia.

The three reviews presented in this chapter form a coherent conceptual point by jointly addressing maternal lifestyle, nutritional exposures, and early biological pathways that emphasize developmental programming and childhood obesity risk. Together, these reviews focus on early-life determinants of childhood obesity, including maternal lifestyle factors, human breast milk microbiota, and folic acid supplementation in pregnant women affected by overweight and obesity.

The full published versions of the three reviews are provided in Appendices A,B and C.

Paper 1: The Influence of Maternal Lifestyle Factors on Human Breast Milk Microbial Composition: A Narrative Review

Published online at: https://doi.org/10.3390/biomedicines12112423

This narrative review investigates the relationship between maternal lifestyle behaviours and the microbial composition of human breast milk, highlighting the important role of maternal consumption for a plant based dietary pattern.

Brief Introduction

In this narrative review[79], our aim was to investigate the association of maternal lifestyle behaviours and environmental exposures during pregnancy and lactation, involving dietary habits, PA/exercise, sleep quality, smoking status, alcohol consumption, psychological stress/distress, with the human breast milk (HBM) microbiota. We also framed the topic within Lifestyle Medicine by focusing on modifiable variables, during pregnancy and breastfeeding phases, and their potential influence on infant gut development and long-term health outcomes[82–87].

We approached the topic from the view that HBM is more than just a rich biological source of nutrition, but also as a highly complex system characterized by immune factors and microbial

communities that help in shaping early microbial colonisations and metabolic and immune programming[82–86]. We described breastfeeding as a major early-life exposure that impacts the establishment of neonatal gut microbiota and is linked to reduced risks of infectious diseases and various chronic conditions later in life, including allergic and metabolic disorders. Furthermore, we suggested that these health benefits are maybe, at least partially, influenced by milk-derived microbes and their functional activities such as the antimicrobial activity, competitive exclusion of pathogens, and interactions with the intestinal epithelium and mucosal immune responses[82,86,87].

Methodology:

From a methodological standpoint, we realized that the use of culture-independent technologies, specifically the 16SrRNA gene sequencing and related techniques, has considerably advanced our understanding of the HBM microbiota while also demonstrating marked inter- and intra-individual variability[87,88]. We documented that HBM contains hundreds of microbial taxa, with streptococcus and staphylococcus being the most abundant. We further noted that the "core" set of genera represent a substantial proportion of the community, although relative abundances varied markedly across samples[82]. We also highlighted the biological feasibility of milk-to-infant microbial transfer by estimating that a substantial microbial daily dose is consumed by the infants through breastfeeding[82].

We presented the following two main overlapping pathways that could drive the microbial communities in HBM:

1- **Reverse flow:** we outlined that during infant suckling; maternal cutaneous infant oral microbes can migrate to the milk ducts potentially influencing milk and infant gut microbial communities[89–91].

2- **Enteromammary pathways or gut-breast axis:** we cited evidence suggesting that maternal intestinal microbes may translocate across barriers to the mammary gland, possibly via cellular immune transport, enabling a vertical microbial transfer through breast milk[92–94]. We used this pathway as a mechanistic framework, to support the idea of how maternal lifestyle factors may influence HBM composition, through their effect on the intestinal gut microbiota.

In this narrative, we also emphasized on the relationship between breastfeeding practices as, at-the-breast feeding, and as expressed milk feeding, and the distinct patterns of microbial co-occurence, between milk and infant stool. In this way, we strengthened the concept that the HBM microbiota is part of a bidirectional mother-infant microbial ecosystem[95–98].

We then summarized human evidence on direct relationships between certain determinants, such as diet, alcohol, smoking, and stress, with the microbial composition of HBM. In contrast, we addressed PA and sleeping patterns primarily as factors that altered intestinal microbiota of the mother, with a proposed indirect influence on HBM through the enteromammary pathways. Importantly, at the time of writing this narrative, there were no other studies that directly tested the effect of maternal PA and sleep quality on HBM microbial composition. The reason why our discussion was partly speculated and supported by biological mechanisms and reasonings[99–103].

Results:

Across lifestyle domains, our main findings involved maternal diet, PA, quality of sleep, exposure to smoking and alcohol, psychological stress/distress.

1- Maternal Diet (the most robust evidence base we found):

Maternal diet was the most investigated among all lifestyle variables as having an influence on HBM microbiota through dual mechanisms as direct or gut-mediated pathways. Among the

observational studies, we reported that overall dietary patterns (vegan, vegetarian, omnivorous diets) were found to be associated with variations in microbial environments in some populations, with effects in some cases becoming more detectable when examined at finer taxonomic resolutions, including species-level markers[104]. Broader dietary clusters and macronutrients profiles appeared to be associated with distinct microbial configurations in the HBM. Dietary patterns based mainly on protein plant sources, fibers, and complex carbohydrates were linked to the HBM profiles in a different way than those based on protein animal sources, fats, and simple sugars, with a distinct observation at the phylum and genus levels and through discriminant analysis in specific datasets[105].

The quality of fat (saturated, unsaturated, trans fatty acids) emerged as an important differentiating variable. Various studies classified milk according to its fatty acid composition and demonstrated differences in microbial community structure, with particular taxa linked to specific fat profiles; trans fatty acids were often shown as informative markers[104]. In some studies, individual nutrients and micronutrients, demonstrated associations with specific genera, with effects that differ based on whether dietary intake was assessed during gestation or during breastfeeding period[106,107]. Evidence highlighted that the dietary exposures during pregnancy may have a stronger effect on overall microbial profile, while during lactation, they were more associated with shifts in particular genera[106,107].

Fibers and other non-digestible carbohydrates were reported not only as markers for the quality of diet, but also as functional substrates that can affect microbial metabolism. We then highlighted the connection between non-digestible carbohydrates, butyrate levels, and the microbial composition in HBM[105]. Multi-omics studies have shown minimal differences in overall diversity in specific dietary restrictions, such as the gluten-free diet for celiac disease, while indicating also potential changes in selected taxa[107].

We concluded these dietary associations through the gut-breast axis, proposing that diet-induced changes in the gut microbiota of the mother and its metabolic products, could have affected which microbes or microbial metabolites entered to the mammary glands or persisted in breast milk[92–94].

Overall, our investigations, we concluded that diet was the most consistently reported modifiable variable for the HBM microbiota, while highlighting that the differences in the studied populations, timing of the sampling, and the used analytical approaches restricted the direct comparison across all the included studies.

2- Physical activity/exercise:

We examined PA and exercise as biologically plausible modifiers for the HBM microbiota, due to their well-known effects on the maternal intestinal microbiota and systemic physiology. However, we did not find any studies that directly examined PA or exercise in relation to the microbial composition of the HBM[99]. As a result, this variable was widely discussed as a hypothesis informed by indirect evidence, to negatively affect the maternal gut microbiota when it is in low levels, and to influence microbial composition of breast milk through endogenous transfer.

3- Sleep quality:

Similarly to PA, sleep disruptions were found to be associated with alteration in the gut microbiota in other settings, which could plausibly influence HBM through the enteromammary mechanism. We highlighted that paucity of evidence directly measuring the relationship between sleeping and HBM microbiota[99].

4- Smoking and alcohol:

Limited evidence was also detected in terms of smoking and alcohol exposures. For the alcohol we found at least one study that reported no significant association with overall microbial

profile of the HBM, while describing a core milk microbiome dominated by a particular genera[108]. Available evidence for smoking was sparse, and the link with microbial diversity was inconsistently observed.

However, even with the absence of a direct effect, we noted that both variables, alcohol and smoking, were associated with alterations in the gut microbiota, which are relevant within a gut-breast axis framework.

5- Psychological stress/distress:

This variable has emerged as potentially important but not well-explored. Longitudinal studies identified in some populations an association between higher distress levels and lower microbial diversity and /or temporal shifts in particular phyla or genera during early stages of lactation, even where large cross-sectional differences were not evident[109,110].

In the BINGO cohort, no significant differences were detected over the genus composition between groups of high- and low- distress levels. However, mothers experiencing a lower distress level, showed higher alpha diversity and progressive taxonomic changes over time[109]. In mothers of very preterm infants, higher stress was linked to limited early milk production and with temporal phylum-level differences, including reduction in Firmicutes and increase in Proteobacteria over the first two weeks after delivery[111].

Overall, we identified that psychological stressors may affect the microbiota of HBM through direct physiological effects and gut-regulated pathways, however, further investigations are still needed.

6- Translational considerations in terms of probiotics in infant formulas:

In our narrative, we decided to include probiotics (and synbiotic, including HMOs) exposure, even though they are obviously not maternal lifestyle exposures, but to understand if they could

influence the gut microbiota of formula-fed infants toward profiles that are closely similar to those in breastfed infants. We found evidence indicating that certain probiotic strains, namely Bifidobacterium and Lactobacillus, are associated with higher rates of beneficial taxa, and in some contexts, with immune-related benefits. We also emphasized that these effects varied depending on several factors, including strain, dose, and duration, and were often transient[91,98,112].

We discussed that probiotics alone were unlikely to reproduce the full bioactive and immunomodulatory complexity of HBM, particularly without HMOs, and the synbiotic approaches may be more promising but still require stronger long-term evidence.

Finally in this narrative review, we devoted substantial attention to methodological limitations affecting comparability and causal interpretation. These limitations include sampling variability, and contamination risks in low-biomass milk samples, and carryover from skin or nipple sources. Technical restrictions of 16S rRNA sequencing, which often limits the resolution and functional insights at the genus level. Whole-genome metagenomics provided a deeper taxonomic and functional characterization; however, it required higher financial and computational resources[88]. We also underlined the limitations of the study design, as cross-sectional studies predominated and could not capture dynamic changes across lactation, while longitudinal studies were fewer and more resource intensive.

Most of the findings remained correlational rather than causal. We called for greater protocol standardization, more longitudinal and interventional designs, and wider multi-omics approaches to improve functional interpretation and reduce heterogeneity.

In conclusion, we can say that maternal lifestyle exposures were relevant to HBM microbiota, with diet being the most consistently supported influence, while PA, sleep, and stress remained key evidence gaps requiring targeted investigations.

These areas required dedicated research. We encouraged integrating these insights into prenatal and postnatal care. We supported balanced dietary patterns, regular PA, and stress management. The goal was to promote favourable HBM composition. This could also support infant gut microbiota maturation and immune development. Finally, we reiterated the need for stronger longitudinal and intervention studies. This would help move from association to actionable recommendations. We also underscored the importance of addressing methodological barriers in milk microbiome research.

Paper 2: Folic Acid Supplementation in European Women of Reproductive Age and During Pregnancy with Excessive Weight

This systematic review examines folic acid supplementation practices among European women with overweight and obesity, addressing a potential nutritional gap within the critical period for preventing NTDs.

published online at: https://pmc.ncbi.nlm.nih.gov/articles/PMC11786555/

Brief Introduction

In this systematic review, we aimed at synthesizing the evidence on folic acid (FA) supplementation practices among European women of reproductive age, and pregnant women with overweight or obesity, with a specific focus on whether these women are supplemented according to current clinical recommendations and whether recommended doses are applied in practice.

We also discussed the biological and clinical implications of FA status in this population, highlighting how contextual factors and circumstances, including socioeconomic and behavioural determinants, influence the effectiveness and uptake of supplementation protocols.

We started by observing that neural tube defects (NTDs), severe congenital conditions including spina bifida and anencephaly, are primarily caused by maternal folate deficiency in the periconceptional period and represent a major public health burden in Europe, occurring in approximately 1 per 1,000 pregnancies[113,114]. International guidelines recommend daily FA supplementation of 0.4 mg before conception and through the first trimester to prevent NTDs, with a higher dose (5 mg) advised for women with obesity due to their altered metabolic demands and lower circulating folate levels[115–117]. These biological risks are thought to be mediated by chronic low-grade inflammation and increased nutrient requirements associated with higher BMI.

Methodology:

To address our aim, we followed PRISMA[118] guidelines to identify relevant studies, searching databases including PubMed, Web of Science, and Medline without time restrictions. Eligibility criteria focused on human studies conducted in European settings that assessed FA supplementation patterns in women with overweight/obesity and reported dosage, timing, and compliance outcomes. Eight studies met inclusion criteria and were included in the synthesis.

Results:

Across the included studies, adherence to FA supplementation guidelines among women with overweight or obesity was consistently low. Only 4–9.5% of pregnant women with obesity took the recommended 5 mg FA dose for enhanced neural tube defect prevention[119]. While most of the participants, including those with obesity, received standard 0.4 mg doses or did not take FA at all before conception. Moreover, we reported that up to 61% of women began supplementation after conception, thus missing the critical periconceptional window when FA has maximal preventive effect against NTDs[120].

Despite consistent clinical guidance, this finding revealed that real-world supplementation practices frequently fall short of recommendations, leaving women with excessive weight inadequately protected.

Several studies within the review reported that women with obesity exhibited significantly lower serum plasma folate levels in early and late pregnancy compared with normal-weight counterparts, even when supplementation was reported. For example, in one cohort, mean serum folate at 16 and 28 weeks was 7.9 ng/mL and 3.9 ng/mL respectively in women with severe obesity compared with 15.0 ng/mL and 10.6 ng/mL in control women without obesity[119,121]. These biochemical disparities occurred even though almost all women reported taking some form of FA supplementation, underlining that standard doses may be insufficient in the context of high BMI and altered folate metabolism. In other reports, serum folate differences between BMI categories remained significant, although red cell folate measures did not differ significantly.

Our synthesis for these data provides direct evidence of altered folate status in women with excessive weight and support the rationale for heightened dosing and early supplementation protocols in this group.

Concerning the timing of supplementation, we reported it as a critical determinant of periconceptional folate status. Preconceptional initiation was uncommon in most studies, with many women first taking FA after pregnancy recognition. Suboptimal timing is particularly relevant given that the neural tube closes by approximately day 28 post-conception, often before pregnancy awareness, which underscores why preconception supplementation is crucial. Only a minority of women began FA supplementation more than 12 weeks prior to conception in some cohorts.

As the timing of starting with FA supplementation significantly influences tissue folate levels during early embryogenesis, these findings suggest that behavioural and contextual barriers to preconception care are an important part of the exposome around FA supplementation practices.

Within our systematic review, we summarized evidence suggesting that diet quality and related lifestyle factors influence both biologic folate status and supplementation patterns. Pregnant women with severe obesity generally reported lower dietary folate intake, reinforcing the joint role of diet and supplementation in determining folate status[122].

While in terms of behavioural and sociodemographic factors, including age, education, smoking, alcohol use, and pregnancy planning status, these emerged to be associated with compliance to FA supplementation recommendations[120,122,123].

We reported that these factors are more likely to interact with broader socioeconomic conditions such that women with lower socioeconomic resources or less health literacy may be at greater risk for both folate insufficiency and poor supplementation adherence, consistent with evidence from social exposome research showing that environmental exposures and health behaviours do not act uniformly across populations. Accordingly, the effects of FA supplementation practices cannot be interpreted solely through a biological lens, because education, planning, and access to healthcare resources shape preconception and prenatal behaviours.

Through our investigation, we highlighted on the biological need of adequate folate status in the periconceptional period for proper neural tube closure and embryonic development. We reported how obesity-related metabolic alterations, such as chronic inflammation and increased nutrient turnover, compromise folate homeostasis, thereby increasing the risk of NTDs even when FA supplementation is reported. Moreover, the lower preconception folate status in women with obesity may reflect a combination of reduced dietary folate intake, suboptimal adherence to supplementation protocols, and physiologic metabolic demands that attenuate plasma folate levels.

This set of mechanisms highlights the internal exposome mechanisms (e.g., metabolic adaptation, inflammation) by which external behaviors and contexts manifest biologically relevant deficiencies.

We noted some limitations within our included studies[119,120,122–124]. Most of them were observational cohort or case–control designs, which limits the causal inference. The sample sizes varied between studies, and measurement of adherence often relied on self-reported supplementation practices, which may introduce recall bias. Reporting on sociodemographic correlates and comprehensive diet patterns was inconsistent across studies, limiting the ability to fully model contextual modifiers of supplementation practices.

These limitations underscore the need for prospective, longitudinal studies with validated biomarkers to better ascertain the effects of both supplementation behaviours and underlying metabolic variation.

In conclusion we can say that European women with overweight or obesity are frequently non-adherent to current FA supplementation recommendations, particularly the enhanced 5 mg dose recommended for high-BMI women. We call for standardization of FA supplementation guidance across European countries, stronger emphasis on preconception care, and tailored public health programming to ensure vulnerable populations fully benefit from evidence-based recommendations.

Paper 3: Offspring's Exposome: Early-Life Factors and Childhood Obesity Risk

This review situates maternal and early-life exposures within the exposome framework, providing a comprehensive overview of pathways influencing childhood obesity risk.

published online at: https://pmc.ncbi.nlm.nih.gov/articles/PMC12535905/

Brief Introduction

In this narrative review, our main aim was to create a comprehensive overview of how environmental exposures early in life, usually referred to as the "offspring's exposome", can increase the risk of developing childhood obesity, while focusing on all variables within the first 1,000 days window of life.

We outlined our discussion within the Developmental Origins of Health and Disease (DOHaD) framework[61,62], highlighting that during critical window of development, lifestyle and environmental exposures may create long-term metabolic trajectories. This can happen through alterations in the biological systems associated with adiposity, energy balance, immunity, and endocrine signalling.

We started with the rising prevalences of childhood obesity which become a global health challenge, known to be associated with long-term risks of non-communicable diseases (NCDs). These chronic diseases include type 2 diabetes, cardiovascular diseases, and other metabolic disorders[125–127].

In this context, we introduced the exposome framework as a method to detect all possible environmental determinants, involving external environmental variables, lifestyle factors, and internal biological reactions[128–130].

Methodology:

To address our aim, we categorized possible exposures into two external exposomes (general and specific), and one internal exposome **(Figure 1)**. The category of the general external exposome involved air pollutants, metals, chemicals (eg. endocrine-disrupting chemicals or EDCs), urbanization, and climate change. The category of specific external exposome involved modifiable lifestyle factors such as diet, PA, sleeping pattern, socioeconomic status (SES) and infant feeding practices. The internal exposome included endogenic biological interactions

such as metabolic regulations, inflammation, oxidative stress, hormonal milieu, and the microbiome.

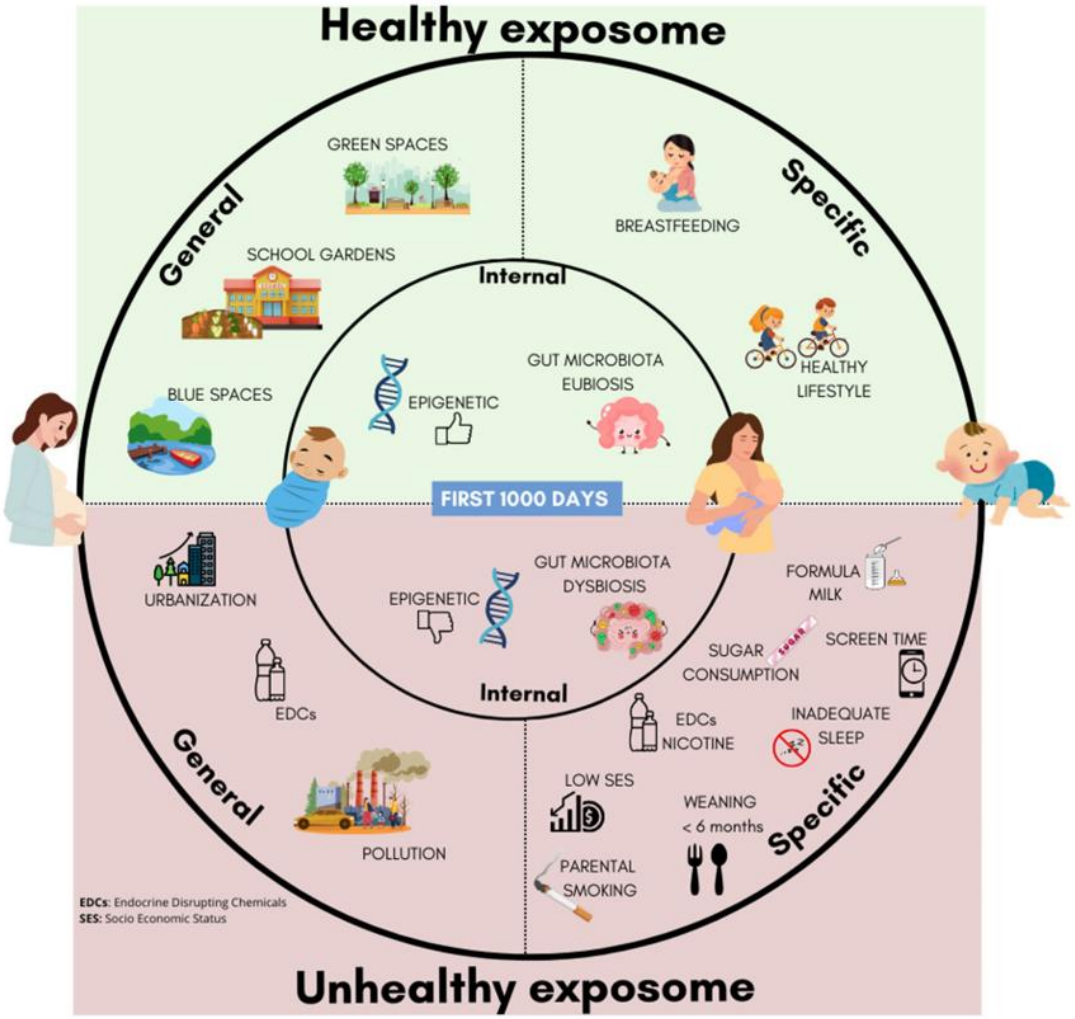


Figure 1. *Healthy and unhealthy exposome factors in early life: general, specific, and internal determinants influencing childhood obesity risk.*

Results:

We then investigated how the components of these three domains interact dynamically across early development and influence the risk of obesity. Our findings revealed that:

1- **General External Exposome and Obesity**

1.1 Endocrine-Disrupting Chemicals (EDCs)

One the most studied exposures was the effect of EDCs, specifically bisphenol A (BPA), phthalates, and perfluoroalkyl substances (PFAS), since they are widely available in all industrial products that are of daily use[131–137]. These EDCs are present in packages,

plastics, and food items, and they can alter the functions of the endocrine system through different mechanisms. Through our investigation, we noted that:

- Prenatal BPA exposure was associated with higher BMI z-scores, increased waist circumference, and greater fat mass in childhood, with several studies reporting sex-specific effects, often stronger in girls.
- Phthalate exposure during pregnancy was linked to accelerated postnatal weight gain and increased adiposity, although associations varied by phthalate metabolite and timing of exposure.
- PFAS exposure showed mixed but concerning associations, with several studies reporting higher BMI or overweight risk in childhood following prenatal exposure, particularly at higher exposure quartiles.

Our findings from original empirical studies and systematic reviews, revealed that the exposure to the chemicals within the critical period of life interact with fat cells production, and consequently affect the metabolic development of the infant. However, these findings were varied depending on the type of EDCs as well as the timing of exposure.

1.2 Air Pollution

Evidence suggested that prenatal exposure to particulate matter ($PM_{2.5}$ and PM_{10}) was associated in some cohorts with higher BMI trajectories, increased fat mass, or rapid postnatal weight gain, while other studies reported null associations. Moreover, traffic-related pollutants (e.g., NO_2) were associated with metabolic dysregulation markers, including insulin resistance, which may precede overt obesity[138,139].

We highlighted that inconsistencies, likely reflect differences in exposure assessment, timing, and confounding by socioeconomic and lifestyle factors, but overall findings suggest that air pollution may act as an indirect obesogenic stressor through inflammation and oxidative stress pathways.

1.3 Urbanization and Climate Change:

We noted that the access to green spaces during early childhood was associated with lower BMI trajectories and reduced obesity risk, mediated partly by increased PA and reduced psychosocial stress. Conversely, highly urbanized environments were associated with greater sedentary behaviours and higher obesity prevalence[140,141]. These findings were described as moderately consistent across observational studies, though strongly context dependent.

2- Specific external exposome and childhood obesity

2.1 Early Infant Feeding

Through our investigation, we reported that the primary protective variable withing this category, is the exposure to exclusive breastfeeding at least for the first six months of life. Different cohorts demonstrated that infants who exclusively breastfed have a lower BMI trajectories and reduced risk of childhood obesity when compared to those fed on formulas. A cumulative benefit is present whit longer breastfeeding durations. Studies adjusting for maternal BMI and socioeconomic factors generally retained a protective association, though effect sizes were attenuated[142,143].

Concerning human breast milk composition, some studies linked higher milk leptin or adiponectin levels to infant growth regulation, but results were inconsistent across cohorts, preventing firm conclusions.

2.2 Complementary Feeding Practices

The introduction of complementary foods before 4 to 6 months of age was associated with higher risk of overweight and obesity, particularly when combined with formula feeding. Some studies report that high intake of sugar-sweetened beverages in infancy and early childhood was consistently associated with higher BMI and adiposity. In contrast, the consumption of diets rich in fruits, vegetables, and minimally processed foods were associated with healthier weight trajectories, though fewer studies addressed diet quality comprehensively[144].

2.3 Children's Lifestyle Behaviours

Lifestyle factors such as PA, screen time, and sleep patterns were also emphasized. Higher levels of daily screen time (≥2 hours per day) were strongly correlated with higher BMI and obesity risk, whereas regular PA and appropriate sleep duration were associated with healthier weight trajectories. These associations were described as robust across populations, though predominantly observational[145].

2.4 Socioeconomic Status (SES)

In terms of SES influences, we observed obesity risk through complex pathways, including access to healthy foods, environments conducive to activity, and exposure to psychosocial stressors. In many high-income settings, lower SES was associated with higher childhood obesity risk, mediated by diet quality, environment, and access to activity. Though in some

low- and middle-income contexts, higher SES was associated with higher obesity prevalence, reflecting nutrition transition dynamics.

3- Internal Exposome: Biological Mechanisms

The first component we discussed within this category of exposures, is to better understand how prenatal exposures, particularly maternal nutrition, smoking, and EDCs, can trigger epigenetic modifications, altered hormonal signalling, and inflammatory pathways that predispose children to increased adipose tissue accumulation and metabolic dysregulation. Evidence framed oxidative stress and perturbations in endocrine signalling as mechanisms by which environmental and lifestyle exposures influence adipogenesis, insulin sensitivity, and energy balance early in life. Although this evidence is still evolving, interactions between the microbiome and metabolic signalling were reported as another internal determinant that could also be affected by external exposures such as diet and PA, as they may influence gut microbial composition, which in turn modulates inflammatory responses and metabolic pathways relevant to obesity[146,147].

We concluded that these internal processes likely mediate and expand the external exposures, which in turn reinforces obesity trajectories.

Across the exposome domains, we were able to identify a spectrum of protective and risk factors **(Figure 1)**. We emphasized that these 3 categories of exposures likely interact together on common biological mechanisms, including hormonal regulation, epigenetic remodelling, inflammatory signalling, and energy homeostasis mechanisms, and consequently influence childhood obesity risk.

This mechanistic framing reinforced the biological plausibility that early-life exposures can have sustained effects into later childhood and adulthood.

Within our investigation we revealed several limitations affecting the current evidence base, including the heterogeneity in exposure assessment, timing, and outcome measures across studies. Observational research was predominant and this limits causal inference. The effects of some exposures depend strongly on the surrounding circumstances.

Future direction must involve more longitudinal, multi-exposure, and mechanistic studies to strengthen causal interpretation and refine public health recommendations.

In conclusion, we can say that early-life exposures across multiple domains of the exposome significantly influence the risk childhood obesity. While some determinants like breastfeeding, adequate complementary feeding, PA, and reduced screen time are consistently protective, others such as EDCs exposure, air pollution, and maternal smoking contribute to obesogenic pathways.

Intervention to target childhood obesity must involve management for environmental conditions, lifestyle behaviours, and internal biological responses during critical developmental windows.

CHAPTER 3: EMPIRICAL STUDIES

In this chapter I am reporting the original empirical studies including, published and under submission papers. These analyses were conducted baseline data derived from the LIMIT[66] study. These studies investigate the association between maternal lifestyle factors, GWG, and neonatal microbiota composition.

I had the privilege to present both papers during my PhD journey the annual international congress on Obesity:

- Paper 1 at the 31rst European Congress on Obesity (ECO) which was held in Venice-Italy on May 2024.
- Paper 2 at the 32nd European Congress on Obesity (ECO) which was held in Malaga – Spain on May 2025

The articles are included same as published, except for minor paraphrasing and formatting adjustments.

Paper 1: Associations of Maternal Lifestyle Factors with Inadequate Pregnancy Weight Gain

In this cross-sectional analysis we aimed at investigating how maternal lifestyle choices could affect the adequacy of gestational weight gain, as it is a key driver for several pregnancy outcome with implications for maternal and child health.

published online at: https://doi.org/10.1007/s00394-024-03473-0

A. Introduction

GWG is an important factor during the prenatal period as it supports the health of the mother and her baby[56]. Inadequate GWG is when weight gained during pregnancy is outside the optimal ranges developed as guidelines by the Institute of Medicine (IOM). GWG range guidelines were provided in accordance with pre-BMI class as follow: underweight= 12.5-18 Kg, normal weight=11.5-16 Kg, overweight=7-11.5 Kg, and obesity=5-9 Kg[56,59]. The inadequacy in GWG is affecting more than half of the pregnant women population in most of the countries[148,149]. Not meeting these guidelines during pregnancy will increase health issues for mothers and their infants at the short- and long-terms. Some of these negative consequences are GDM and maternal weight retention after delivery which is usually followed

by obesity[56,150], having a large- or small-for-gestational-age infant[57,151], low or high-birth-weight infant (LBW, HBW)[151], risk of childhood obesity[150], and many other health problems[152,153]. As reported in the systematic review and meta-analysis of diverse international cohorts conducted by Goldstein et al (2017), 47% of a sample of more than 1 million pregnant women gained weight more than IOM recommendations, with a clear association with a higher risk of large-for-gestational-age, macrosomia, and cesarean delivery. While 23% of the sample gained less than the recommendations, with an obvious link with increased risk of small-for-gestational-age and preterm birth[57].

Other than maternal age, pre-BMI, and the physiological changes of pregnancy, lifestyle factors before and during pregnancy were found to have a great impact on GWG[59].

Evidence showed that inadequate GWG is connected to pre-BMI class[59,60], PA level[56,154], educational level[155], smoking habits[156], and adherence to a balanced diet[60].

Lifestyle interventions targeting eating habits and PA levels[56,157,158], before and during pregnancy, were widely studied and confirmed to have a positive impact in controlling GWG within the adequate ranges, and in reducing health risks in both mother and baby[59]. Other lifestyle factors have also shown a good effect on managing GWG. These include engaging in regular PA[46], having a higher educational level, and being non-smoker, as well as adhering to the MD[46,159,160]. Lower risks of GDM, overweight, and obesity are associated with these lifestyle factors[19], as well as lowers risks of facing preterm delivery, and other metabolic problems[35,57,161]. Adherence to the MD was shown to be also associated with a reduction in the health risks that may affect newborns and children, such as fetal growth alterations, inappropriate birth weight, prematurity, gastroschisis, and other problems[35].

Targeting GWG to stay within IOM recommendations is a public health challenge that aims at protecting the overall well-being of pregnant women, and their infants. Despite this, there is a paucity of evidence in Italy about the magnitude of GWG adequacy and its associated factors, particularly in the Lombardy region. Therefore, the primary goal of this study was to investigate the association of inadequate GWG with maternal lifestyle factors using baseline data from the LIMIT study[66,162].

B. Materials and methods

B.1 Study Design and Period

A cross-sectional study was conducted using baseline data from the ongoing LIMIT[66] (Lifestyle and Microbiome Interaction Early Adiposity Rebound in Children) prospective cohort project[160]. LIMIT[66] aims at identifying the longitudinal interplay between infant gut microbiome, infant/maternal lifestyle, and environmental variables, in children with early adiposity rebound, which is a risk factor for the development of childhood obesity. This assessment will be conducted at different points in time (T_0, at delivery; T_1, 1 month; T_2, 6 months; T_3, 12 months; T_4, 24 months; T_5, 36 months after birth).

B.2 Study Participants and Setting

In the ongoing LIMIT[66] prospective cohort study, a total of 200 pregnant women were enrolled between October 2022 and June 2024 during the pre-hospital care before birth at the UOC Neonatology and Neonatal Intensive Care, Fondazione IRCCS Policlinico San Matteo of Pavia (Pavia, Italy), of which 178 pregnant women were eligible and selected for this specific study. The overall selection was performed based on the inclusion and exclusion criteria previously defined in the LIMIT[66] study protocol[160] and the completeness of the data was added as an additional criterion for inclusion and exclusion. The study followed the Helsinki Declaration, and the participants signed a consent form.

B.3 Variables, Data source, and Measurements

At the delivery, women were investigated for i). sociodemographic characteristics (such as age, and educational level); ii). pre-pregnancy anthropometric data; iii). Lifestyle behaviours during pregnancy (such as eating habits including adherence to the MD, level of PA, and smoking habits) by using previously validated questionnaires and structured interviews.

B.4 Anthropometric measurements

Two trained dietitians collected maternal anthropometric data (GWG). Pre-gravid weight (Kg) was self-reported by the participants and a Harpenden stadiometer with a fixed vertical backboard and an adjustable headpiece was used to measure height in centimeters (cm). Participants were asked to stand barefooted on the platform of the stadiometer, in a Frankfort plane position for an accurate measurement of their height.

BMI was then calculated using the standard formula BMI = Weight (Kg)/Height (m^2), enabling the classification of participants into the class of women with low pre-pregnancy weight status (BMI<18.5 Kg/m^2); women with normal pre-pregnancy weight status (18.5≤BMI≤24.9 Kg/m^2), and women with overweight (25≤BMI≥29.9 Kg/m^2) or obesity (BMI≥30 Kg/m^2) statuses[23]. The total weight gained during pregnancy was also self-reported by the participants.

B.5 Adherence to the Mediterranean Diet

The maternal adherence to the MD was retrospectively investigated by two trained dietitians, using the MEDI-LITE **(Appendix)** score validated questionnaire [24]. However, since alcohol is not recommended during pregnancy, the MEDI-LITE score was adapted for the pregnant women target group, by removing its score as explained below. It evaluates the frequency of consumption of nine food groups.

- A score of 0 to 2 was assigned to indicate 2 for a high frequency, 1 for a moderate frequency, and 0 for a low frequency of consumption of five food groups: fruits, vegetables, cereals, legumes, and fish and fish products.
- For the groups of meat and meat products, and dairy products, a score of 2 was assigned for items consumed with low frequency, 1 for those consumed with moderate frequency, and 0 for those consumed with high frequency.
- For alcoholic consumption, a score of 2 was assigned to the middle category (1-2 units of alcohol/day), 1 for the lowest category (1 unit of alcohol/day), and 0 for the highest category (>2 units of alcohol/day). In our analysis, due to incomplete data as the participants are pregnant women, we excluded alcohol consumption from the MD adherence score calculation, and the total score was recalculated accordingly.
- The last section is for olive oil, a score of 2 was given for regular use, 1 for frequent use, and 0 for occasional use.

The overall score, after excluding the alcohol consumption score, ranged from 0 to 16, suggesting the greatest adherence for the highest score obtained. It was classified into tertiles, low, medium, and high adherence to the MD.

B.6 Physical Activity Level

A section of a previously developed and validated questionnaire for assessing lifestyle behaviours, including PA and sedentary behaviours among an Italian youth population[163], was adapted and administered to our adult participants. Questions referring to school-based PA were removed and only the items related to habitual activities were retained to capture general maternal PA habits during pregnancy. This adapted version was also pre-tested on a sample of 24 subjects and revised accordingly, to assess clarity and applicability. All answers were structured to quantify the time spent weekly in PA and sedentary leisure activity. This adaptation aimed to maintain the conceptual structure of the original instrument while ensuring applicability to pregnant adult participants. The questionnaire was composed of 5 multiple-choice questions that followed a Likert scale format comprising 4 choices ("Always", "Often", "Sometimes", and "Never") corresponding to a score between 0 and 3, where the highest score suggested the healthiest habit[163].

B.7 Smoking Habit

Smoking habits were collected through interviews, and the number of smoked cigarette packs per year was documented. Participants were categorized as those who had never smoked, quit smoking before or during pregnancy, or started or kept smoking during pregnancy.

B.8 Gestational Weight Gain (GWG)

Total GWG was calculated as the difference between the participant's weight at delivery time and the pre-pregnancy weight. Based on that, women were classified into 3 categories, according to the IOM (U.S.) and the National Research Council (U.S.) Committee to Reexamine IOM Pregnancy Weight Guidelines[56], as follows:

i. Women with Adequate GWG (AGWG; women with normal pre-BMI, gaining from 11.5 Kg to 16 Kg during pregnancy);

ii. Women with Excessive GWG (EGWG; women with pre-BMI indicating overweight or obesity, gaining more than 11.5 Kg or 9 Kg during pregnancy, respectively);

iii. Women with Low GWG (LGWG; women with low pre-pregnancy weight status, gaining less than 12.5 Kg).

B.9 Statistical Analysis

A descriptive analysis was performed to explore the distribution of variables across the study subjects by outcome status (AGWG, EGWG, LGWG) and summarized in terms of mean and

standard deviations for continuous variables. While frequency and percentage were used for categorical variables. Variables with a p-value of less than 0.2 ($p < 0.2$) in the univariate analysis were selected for the multinomial logistic regression model, aiming at identifying lifestyle factors associated with inadequate GWG. Education level, pre-BMI, PA score, MD adherence score, daily vegetable consumption, and daily consumption of meat and meat products fulfilled the criteria and were included as potential predictors in our multinomial logistic regressions. A forest plot with confidence interval and adjusted relative risk ratio (RRR) was used to visualize the presence and strength of association of those factors with inadequate GWG (EGWG and LGWG) considering AGWG as the reference category.

C. Results

Table 1 and **Figure 1** summarize the distribution of sociodemographic and lifestyle factors of the study participants based on the GWG category. More than half (61.2%) of the sample of study participants fell into the category of inadequate GWG, of which 60.5% and 39.4% were in the LGWG and EGWG categories, respectively.

Participants' mean age was reported for each category as AGWG (mean age: 34 [32-37] years), EGWG (mean age: 33 [30-35] years), and LGWG (mean age: 32 [30-36] years), showing no statistically significant relationship with GWG. Concerning the educational levels, a significant association was detected (P=0.0.011), where participants holding a university degree represented more than two-thirds of the AGWG (69%) and LGWG (79%) categories, compared to those with lower educational levels (31% of the AGWG; 21% of the LGWG). For the pre-BMI variable, a statistical significance was observed (P=0.0051), where the highest percentage of the participants in the EGWG category (63%), had a pre-BMI indicating overweight or obesity. **(Table 1)**

Regarding the adherence to MD which showed a significant association (P=0.008) with GWG, participants with highest and medium adherence scores represented almost half of the AGWG (52%) category and more than half of the LGWG (64%) category. On the other hand, those with the lowest adherence scores made up more than two-thirds of the EGWG (72%) category. **(Table 2).**

The individual items of the MEDI-Lite questionnaire were also considered; the results were summarized in **Table 2** and categorized based on the 3 GWG groups, showing a highly significant association with the average daily consumption of vegetables (P<0.001). Last, considering other lifestyle factors, participants with high and medium PA scores constituted

the highest percentage of the AGWG (68%) and LGWG (69%) categories, while those with the lowest scores accounted for almost half of the EGWG category. Despite these differences, the association was not observed between PA and GWG (P=0.25).

Table1. *Maternal sociodemographic and lifestyle factors related to gestational weight gain.*

	Gestational Weight Gain (GWG) Adequacy			p-value
	Adequate GWG	Excess GWG	Low GWG	
n	69 (38.8%)	43 (24.1%)	66 (37.1%)	
Age (year)	34.00 (32.00-37.00)	33.00 (30.00-35.00)	32.00 (30.00-36.00)	0.32
Mother's Educational Level				0.011
Lower or High School	21 (31%)	21 (49%)	14 (21%)	
University degree	47 (69%)	22 (51%)	52 (79%)	
Did you smoke during pregnancy?				0.10
NO	66 (96%)	40 (93%)	66 (100%)	
YES	3 (4%)	3 (7%)	0 (0%)	
Pregravid BMI category				0.005
Underweight	9 (13%)	1 (2%)	6 (9%)	
Normal weight	46 (67%)	26 (60%)	55 (83%)	
Overweight	8 (12%)	10 (23%)	4 (6%)	
Obesity	6 (9%)	6 (14%)	1 (2%)	
Physical Activity				0.25
Lower Score	22 (32%)	20 (47%)	20 (30%)	
Medium Score	23 (33%)	15 (35%)	22 (33%)	
Higher Score	24 (35%)	8 (19%)	24 (36%)	

*Legend. * Significance was set at p-value <0.05*

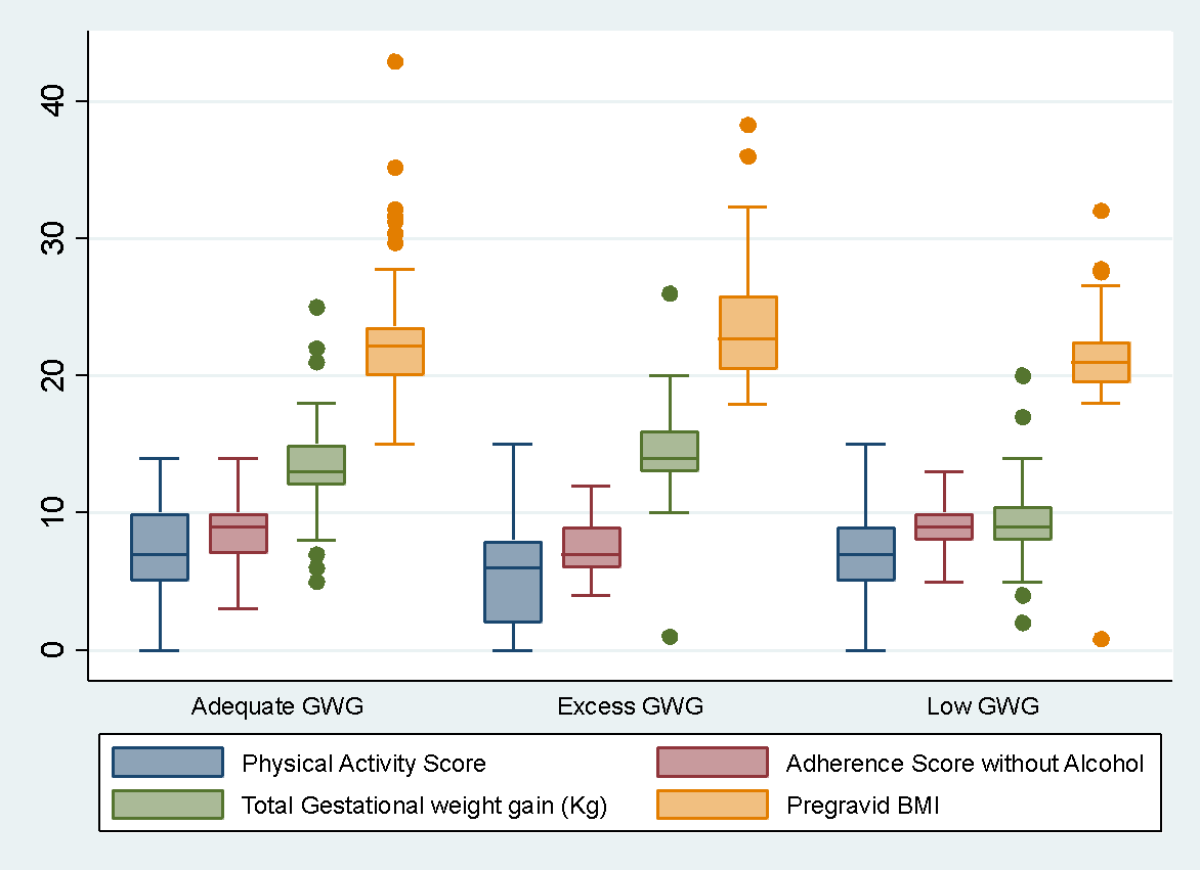


Figure 1. *Distribution of physical activity score, Adherence to MD score, total gestational weight gain and pre-gravid BMI across Gestational Weight Gain Adequacy using LIMIT data [0.055, 0.003, and 0.024 is the p-value for physical activity, pre-gravid BMI and adherence score, respectively]*

Figure 2 represents a significant association between average daily consumption of 1 to 1.5 portions of vegetables and lower risks of EGWG when compared to the reference line of AGWG (RRR=0.279, P=0.04). A significant association was also represented between the daily consumption of >1.5 portions of meat with higher risk of EGWG when compared to the reference line of AGWG (RRR=7.83, P=0.03). No significant evidence of any of the studied variables was detected in terms of LGWG in comparison with the reference line of AGWG.

Table 2. *Maternal diet consumption and adherence to the Mediterranean Diet by Gestational weight gain (GWG) adequacy.*

	Gestational weight gain (GWG) adequacy			p-value
	Adequate GWG	Excess GWG	Low GWG	
MID Adherence Score category without alcohol				0.008
Lower Score	33 (48%)	31 (72%)	24 (36%)	
Medium Score	13 (19%)	3 (7%)	15 (23%)	
Higher Score	23 (33%)	9 (21%)	27 (41%)	
Daily Fruits Consumption				0.57
Inadequate	21 (30%)	13 (33%)	13 (20%)	
Average	40 (58%)	22 (56%)	44 (68%)	
Adequate	8 (12%)	4 (10%)	8 (12%)	
Daily Vegetables Consumption				<0.001
Inadequate	7 (10%)	11 (28%)	8 (12%)	
Average	59 (87%)	23 (57%)	43 (65%)	
Adequate	2 (3%)	6 (15%)	15 (23%)	
Weekly Legumes Consumption				0.36
Inadequate	27 (40%)	15 (37%)	17 (26%)	
Average	29 (43%)	21 (51%)	39 (59%)	
Adequate	12 (18%)	5 (12%)	10 (15%)	
Daily CEREAL consumption				0.93
Inadequate	13 (19%)	8 (19%)	12 (18%)	
Average	39 (57%)	23 (53%)	33 (50%)	
Adequate	17 (25%)	12 (28%)	21 (32%)	
Weekly Fish Consumption				0.37
Inadequate	32 (49%)	17 (44%)	21 (33%)	
Average	31 (48%)	21 (54%)	39 (61%)	
Adequate	2 (3%)	1 (3%)	4 (6%)	
Daily Meat Consumption				0.11
Less than 1 portion	38 (56%)	19 (45%)	30 (46%)	
1 to 1.5 portion	28 (41%)	16 (38%)	31 (48%)	
>1.5 portion	2 (3%)	7 (17%)	4 (6%)	
Daily milk and milk product Consumption				0.25
Less than 1 portion	26 (38%)	15 (35%)	18 (28%)	
1 to 1.5 portion	35 (51%)	17 (40%)	36 (55%)	
>1.5 portion	8 (12%)	11 (26%)	11 (17%)	
Olive Oil Consumption				0.50
Occasional	7 (10%)	5 (12%)	4 (6%)	
Regular	50 (72%)	26 (60%)	45 (68%)	
Frequent	12 (17%)	12 (28%)	17 (26%)	

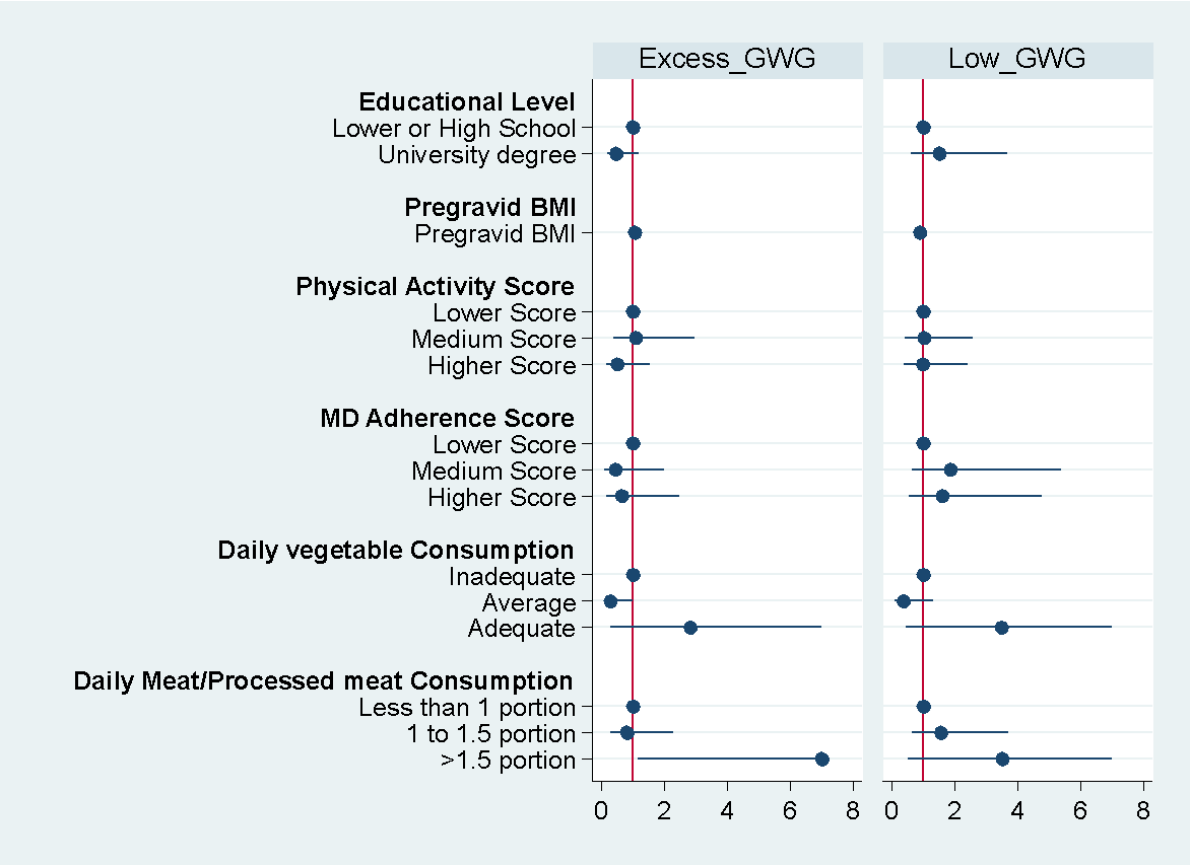


Figure 2. *Forest plot showing the results of the multinomial regression model using baseline data from the LIMIT prospective cohort study. Predictor's relative risk ratio and corresponding confidence intervals for Excess and Low GWG reported considering adequate GWG*

D. Discussion

This study showed an alarming increase in the prevalence of inadequate GWG accounting for 61.2% out of 178 participants. Our findings indicate that the adequacy of GWG could be influenced by maternal factors such as pre-BMI, educational level, adherence to the MD, and the average consumption of vegetables, as well as the excessive consumption of meat products. These findings may suggest the importance of promoting lifestyle interventions before and during pregnancy to ensure health and well-being for both, the mother and her infant.

No statistical significance supporting the evidence of the association between maternal age and inadequate GWG was observed. These results were inconsistent with a study conducted by Sun Y *et al.*[164] on a sample of 3172 pregnant women, where the authors found that an average age of 20-25 years old, is a protective factor for maintaining GWG within IOM guidelines against adverse health effects and malformations. The inconsistency in findings between their results and the current study could be influenced by the huge difference in sample size of both

studies. Furthermore, there was insufficient previous investigation about the effect of maternal age on GWG, which made it difficult to compare with other findings.

Current findings showed a significant association with maternal educational level, where 79% of the mothers who experienced an LGWG were holding a university degree. This result is not completely following the findings of a previous systematic review of observational studies that was conducted by O'Brien *et al.*[159], where pregnant women with low educational levels were less likely to maintain GWG within IOM recommendations. Further investigations must be performed on a larger sample targeting highly educated mothers to identify if their level of awareness about not gaining weight in excess during pregnancy, is negatively transformed into a GWG less than the recommendations.

Concerning pre-BMI, our findings suggested a statistically significant association with GWG, as participants with pre-pregnancy overweight or obesity were more likely to experience EGWG, increasing the risk of adverse health consequences for the mother and her infant. This finding was consistent with previous studies, where women with a pre-BMI value≥25 Kg/m^2, experienced EGWG and were at higher risk of developing GDM and/or pre-eclampsia, having preterm or cesarean birth, hemorrhage, infection and many other complications[59,152]. EGWG has been reported with an increased likelihood to retain weight after delivery, which is usually followed by a higher possibility of developing postpartum obesity and suffering from other difficulties during future pregnancies, as well as giving birth to large-for-gestational-age infants, who will also be at higher risk to develop childhood overweight or obesity and its associated consequences[151,153]. However, it was not possible to comment on the outcomes of our findings as this data was not yet collected.

In the present study, the adherence to the MD was significantly associated with GWG in comparison with IOM guidelines[56,59], suggesting a potential benefit for the health of both, the mother and her infant. Participants with low adherence were more likely to experience EGWG. These results were in line with previous studies where lower GWG was associated with higher adherence to the MD before and during pregnancy[165,166]. In another systematic review, some of the selected studies highlighted the importance of MD during pregnancy to prevent excessive GWG, while others confirmed its association with a decreased risk of maternal and fetal complications[35]. This alignment with the existing literature confirms the importance of dietary interventions in the management of GWG and prevention of its adverse health consequences. Moreover, for a better understanding of this association, it is highly

recommended to create or validate a questionnaire that is suitable for this population to detect adherence. Although our study did not measure the impact of MD adherence on newborns, the association with GWG suggests potential implications on the health of the infants and children. Previous studies also showed association between adherence to the MD and reduction of some pregnancy and childbirth complications as well as perinatal and childhood problems[35].

In terms of PA score, our findings imply no association with GWG, where score differences between GWG categories were relatively small, but higher among AGWG and LGWG indicating that more active pregnant women were less likely to gain excessive weight during pregnancy. This finding was in accordance with the systematic review results of O'Brien *et al.*[159] where PA was shown to be significantly inversely associated with GWG. It was also proposed by Teede *et al.*[157], that PA-based interventions are associated with reduced GWG as well as maternal and neonatal health risks. However, the highest mean value of PA score in our results was detected among the LGWG category, and this could also be linked to the high educational level of our participants. Perhaps, they tend to be more active due to their awareness of the importance of PA during pregnancy, leading to insufficient GWG. In 2019, O'Brien *et al.*[155] conducted a data meta-analysis about the impact of maternal education on GWG, indicating an increased risk of inadequate GWG among highly educated mothers following a mixed intervention of diet and PA-based interventions. Further investigations needed to be performed on a larger sample size, taking into consideration this relationship between educational level and PA and their association with GWG especially in terms of LGWG.

Maternal smoking habits were not associated with GWG. In contrast to our findings, a previously conducted systematic review by Zhou *et al.*[46], showed that smoking during pregnancy was associated with EGWG. However, our results may be influenced by the fact that only 6 participants from the whole sample were smokers, and those were equally distributed between AGWG and EGWG categories. Further examination is needed on a larger sample size.

Regarding the average daily consumption of vegetables that was shown in our study, to be associated with a lower risk of EGWG, our suggestion for future investigation is to identify if there is a link between high levels of maternal education and adequate consumption of vegetables on a larger sample size. This result could be considered following a previously

conducted cohort study by Hirko et al. [167], where women with obesity who consumed more fruits and vegetables during pregnancy, were less likely to experience EGWG.

As for the excessive daily consumption of red meat, a significant association was detected with an increased risk of EGWG. A study conducted by Maugeri et al.[168], showed an increase in the trend of EGWG that was linked to the consumption of western dietary pattern, which means a high consumption of red meat and fast-food products.

Our study encompasses some limitations. The sample size is considered small, which may hinder the detection or be the reason for some associations and therefore affect the statistical power of the study. For this reason, we recommend future investigations incorporating larger sample sizes to better explore these associations. Unfortunately, we did not include other potential factors that could influence GWG such as "income", since we did not collect this data, and "employment status" as there was a significant number of missing responses from our study participants. Additionally, pre-pregnancy weight and total GWG were self-reported by the participants and not measured by the trained dietitians, which increases the potential risk for bias by reducing the overall reliability of the data. Moreover, the lack of availability of a valid tool for assessing the adherence to the MD that is tailored to pregnant women, is a major limitation, as we had to adapt an existing questionnaire by removing the item of "alcohol consumption" and reducing the score, which affects its validity. Future research should focus on developing an assessment tool that is tailored to this specific population to enhance the robustness of the dietary assessment. However, this could also be considered as a strength of our study as we tried to make the measurement tool more relevant to our population to obtain meaningful data, as well as addressed a gap in the existing literature which is the absence of a valid questionnaire that measures adherence of the MD in pregnant women, taking into consideration the international guidelines in pregnancy which state the importance of avoiding alcohol within this critical period. Another key strength is the use of a section from the previously validated questionnaire by Turconi *et al.*[169] to assess PA levels among participants. Last but not least, our study provides a new region-specific data on GWG adequacy and its associated factors in the Lombardy region, offering insights to the policymakers and healthcare providers to develop new health interventions at the local level.

This study affirmed conclusions from previous research on the association between lifestyle factors including the adherence to the MD, before and during pregnancy, and the maintenance of GWG within IOM guidelines. An alarming increase in the rate of pregnant women with

inadequate GWG was revealed, indicating a significant association with maternal educational level, pre-BMI, adherence to the MD, average daily consumption of vegetables, and excessive consumption of meat products. The small sample size imposes a major limitation on our findings; however, the absence of a valid questionnaire that could assess MD adherence in pregnant women was detected as a gap in the literature and emphasized the importance of future research to fill it. Our results emphasize the importance of promoting lifestyle interventions during childbearing age as well as during pregnancy, as a crucial public health strategy to ensure health and well-being for two of the vulnerable groups, pregnant women, and infants.

Paper 2: Maternal BMI, gestational weight gain, lifestyle factors, and meconium microbiota structure

Under Submission

This study examines associations between maternal characteristics and neonatal meconium microbiota at delivery, addressing a critical gap in early-life microbiome research.

A. Introduction

The establishment of the gut microbiota during the early stages of life is a crucial process for maintaining overall human health. Initial microbial colonisation of the neonatal gastrointestinal tract plays a fundamental role in the development of the immune system, metabolism, and brain function.[69,70] Any disruption in this process may contribute to adverse short- and long-term health consequences, including necrotising enterocolitis (NEC), obesity, asthma, diabetes, inflammatory bowel disease, cancer, allergies, and neurological conditions related to the gut-brain axis[69].

Previously, the womb was believed to be sterile; however, growing evidence proposes that meconium, the first baby's stool, contains a unique microbial community. However, the prenatal gut microbiota is less diverse than the microbiota observed in the perinatal period, and mainly contains common bacterial groups such as *Firmicutes, Proteobacteria*, and *Bacteroidetes*. Among these groups, the most common types of bacteria are *Escherichia–Shigella, Enterococcus, Lactobacillu*s, and *Staphylococcus*[170,171].

Maternal microbiota during pregnancy and at delivery may influence fetal microbial and immune development through multiple interconnected pathways, including the transplacental transfer of microbial metabolites, exposure via the umbilical cord or amniotic fluid, fetal membranes, and gastrointestinal tract, as well as through vaginal delivery, skin contact, and mucosal membranes at birth[69]. Evidence indicates that microbiome-related metabolites originating from the maternal microbiota are transferred vertically to the fetus. Short-chain fatty acids (SCFAs) and secondary bile acids appear to play active roles in modulating the fetal immune system and preparing the gut for postnatal microbial exposure[71].

Other maternal and environmental factors also influence early-life microbiota development during the first 1000 days. These include pre-BMI, GWG, maternal dietary habits, delivery mode, infant feeding practices, antibiotic usage, and various environmental exposures. Mainly, research reports that maternal lifestyle factors, along with weight status, have been associated with variations in newborn gut microbial diversity and abundance[54,73–75].

An observational study of 935 mother-infant pairs found that infants born vaginally to mothers with overweight or obesity before pregnancy, had higher abundance of *Firmicutes*, especially the *Lachnospiraceae* family, and were three times more likely to develop overweight or obesity (95% CI, 1.49-7.41) by ages 1 and 3 years old, when compared to those born to mothers with normal pre-BMI[172]. This risk increased further among infants delivered by caesarean section to overweight mothers, who were five times more likely to become overweight (95% CI, 2.04-12.38) at the same ages[172]. Similarly, a study by Mueller NT *et al.*[173] involving 74

neonates to mothers with a pre-BMI indicating overweight or obesity, revealed changes in infants' microbiota, particularly among those born via vaginal delivery.[173] Another study conducted by Sasaki T. *et al.*[73], on 33 mother–infant pairs demonstrated that higher maternal adherence to a MD during pregnancy is associated with increased abundance of *Pasteurellaceae*, *Bacteroidaceae*, and other SCFAs-producing bacteria in neonatal meconium. In addition to microbiota changes, the study also found an association with epigenetic differences in placental and cord blood tissues, suggesting that maternal diet can influence early gut microbiota development not only through the direct transfer of microbes, but also by altering fetal gene activity by influencing the gut and immune system function[73].

Since meconium begins to develop in the fetal intestine during the late stages of gestation, it represents a valuable matrix for investigating early microbial and metabolic exposures. Therefore, analysing meconium provides a unique opportunity to understand better the prenatal microbial environment and its potential implications for the subsequent development of the gut microbiota during the first 1000 days of life[76]. Despite such findings, the influence of maternal factors during pregnancy on the composition of meconium microbiota is still lacking and needs further investigation.

In this context, using data from the LIMIT study[66], we investigated whether maternal pre-BMI, GWG adequacy, classified as adequate or inadequate, according to the Institute of Medicine guidelines[55], and maternal lifestyle factors during pregnancy (e.g. dietary habits, PA level, smoking habits) are associated with the composition of meconium microbiota.

B. Materials and methods

B.1 Study cohort description

The present research refers to the experimental hypothesis of the ongoing prospective cohort study LIMIT (www.clinicaltrials.gov NCT04960670)[66], which investigates the longitudinal interplay between infant gut microbiome, infant/maternal lifestyle exposure factors, and environmental variables at different follow-up (T_0, at delivery; T_1, 1 month; T_2, 6 months; T_3, 12 months; T_4, 24 months; T_5, 36 months after birth).

Ethical approval was granted by the Ethical Committee of IRCCS Policlinico San Matteo (Pavia) (protocol number: 0020200/22; Accepted: 11 April 2022).

A total of 200 pregnant women were enrolled between October 2022 and June 2024 during the pre-hospital care before birth at the UOC Neonatology and Neonatal Intensive Care, Fondazione IRCCS Policlinico San Matteo of Pavia (Pavia, Italy). The overall selection was performed based on the inclusion and exclusion criteria previously defined in the LIMIT study protocol[66]. Additionally, the completeness of the data and the availability of a neonatal meconium sample at T_0 for gut microbiota analysis were added as additional inclusion and exclusion criteria. Therefore, 168 mother-infant pairs were selected for this specific study.

The study adhered to the principles outlined in the Helsinki Declaration, and all participants signed a consent form. In the current analysis, we analysed meconium samples collected at

birth from newborns. At the delivery, women were investigated for potential modifiers of the microbial composition, including:

i) sociodemographic characteristics (such as age and educational level); ii) pre-pregnancy anthropometric data (pre-BMI) and GWG adequacy levels[56]; iii) lifestyle factors during pregnancy, including dietary habits, PA level, and smoking habits, were collected through the LIMIT study[66] by using previously validated questionnaires and structured interviews.

B.2 Maternal anthropometric measurements

Maternal pre-pregnancy weight and height were self-reported by the participants. BMI was calculated using weight (Kg) divided by height squared (m^2) and used to classify participants as underweight (BMI<18.5 Kg/m^2), normal weight (18.5≤BMI≤24.9 Kg/m^2), overweight (25≤BMI≥29.9 Kg/m^2) or with obesity (BMI≥30 Kg/m^2).[15] The total weight gained during pregnancy was also self-reported. Total GWG was calculated as the difference between pre-pregnancy weight and total weight at delivery. According to the IOM guidelines[56], participants were classified into i) AGWG: women who gained within the recommended range for their respective pre-BMI category; ii) excessive GWG (EGWG): women who gained more than the recommended range for their respective pre-BMI category; iii) low GWG (LGWG): women who gained less than the recommended range for their respective pre-BMI category.

B.3 Lifestyle variables assessment

Detailed descriptions of the questionnaires, scoring systems and interviews for lifestyle data collection are provided in the previous publication of the LIMIT study protocol[66].

In brief, Food consumption frequency (FFQ) and dietary habits (DH) were assessed using a previously validated self-administered dietary questionnaire. Two of the nine original sections, initially developed for an Italian adolescent population, were adapted to adults by two dietitians, as described elsewhere. The FFQ section (18 items) evaluated daily consumption of staple foods and beverages (e.g., bread, pasta, cereals, fruits, vegetables, milk, yoghurt, tea, coffee), and weekly intake of foods such as meat, fish, eggs, cheese, legumes, sweets, and alcohol. Responses were based on a four-point Likert scale (always, often, sometimes, never), scored from 0 to 3, with higher scores reflecting healthier choices. The DH section (14 items) investigated dietary habits, including breakfast frequency, number of daily meals, fruit and vegetable intake, and beverage consumption. Eight items use the same four-point scale, while six have alternative formats scored from 1 to 4. In both cases, the highest score indicates the healthiest behaviour[169,174]. Total scores were divided into tertiles: “inadequate”, “partially satisfactory,” and “satisfactory” dietary habits, aligned with the Italian National Dietary Guidelines[116].

The adherence to MD was evaluated through the MEDI-Lite score, a validated index that measures dietary patterns based on the frequency of consumption of nine key food groups characteristic of the MD, such as vegetables, fruits, legumes, cereals, and olive oil.[19] For this study, the scoring system was adapted to suit the specific nutritional needs and dietary habits of pregnant women[81]. Therefore, after excluding the alcohol component, the total score

ranged from 0 to 16, with higher values indicating greater adherence to the MD. Scores were categorised into tertiles representing low, medium, and high levels of adherence.

PA levels during pregnancy were assessed using a section of a previously developed and validated questionnaire for the Italian youth population, which was adapted for use with adults by removing items related to school-based physical activities. The modified version was pre-tested on a sample of 24 individuals and subsequently revised based on the feedback received[81,163]. The questionnaire aimed to assess PA patterns by quantifying the time spent weekly on various activities, including leisure-time PA and screen time.[163] It consisted of five multiple-choice items, each presented in a 4-point Likert scale format ("Always," "Often," "Sometimes," "Never"), with responses scored from 0 to 3. Higher scores indicated healthier lifestyle behaviours; scores were then categorised into tertiles representing low, medium, and high levels of PA.

Smoking habits were assessed through interviews, and participants were asked to report the number of cigarette packs smoked per year. Based on their responses, women were categorised as either smokers during pregnancy ("yes") or non-smokers during pregnancy ("no")

B.4 Meconium Sample Collection and DNA Extraction

Meconium samples were collected within the first 24 hours of birth using sterile protocols and stored at -80°C until analysis, as recommended by previous studies.[4,21] The faecal samples were shipped on dry ice and sequenced. DNA extraction was carried out using a revised DNeasy 96 PowerSoil Pro QIAcube HT Kit method as described by Jensen *et al.*[175] Modifications include mixing each sample with 500 µL of CD1 solution, followed by bead-beating at 1600 rpm for two minutes, repeated three times, with two-minute breaks on ice in between. After lysis, samples were spun at 3500 x g for ten minutes. The supernatant of each sample was transferred into a new S-block well and mixed with CD2 and nuclease-free water in the ratio 3:3:1 (V/V). Contents were thoroughly mixed, and samples were then spun again at 3500 x g for 10 min.

B.5 Bacteria 16S rRNA gene variable regions 1-8 (bV18-A) library preparation and sequencing

Meconium samples were first subjected to DNA extraction, followed by PCR amplification targeting the bacterial 16S rRNA gene variable regions 1–8 (bV18-A). The resulting amplicon libraries were sequenced using Oxford Nanopore technology. Sequencing reads were subsequently processed through a bioinformatic pipeline including quality filtering, taxonomic assignment against the GTDB reference database, and generation of OTU tables. Finally, microbial diversity and community structure were analysed using established ecological and statistical approaches.

Amplicon libraries for the bacteria 16S rRNA gene variable regions 1-8 (bV18-A) were prepared using a custom protocol. Up to 25 ng of extracted DNA was used as template for PCR amplification, and each PCR reaction (50 µL) contained 0.2 mM dNTP mix, 0.01 units of Platinum SuperFi DNA Polymerase (Thermo Fisher Scientific, USA), and 500 nM of each forward and reverse primer in the supplied SuperFi Buffer. PCR was done with the following

program: Initial denaturation at 98 ∘ C for 3 min, 25 cycles of amplification (98 ∘ C for 30 s, 62 ∘ C for 20 s, 72 ∘ C for 2 min) and a final elongation at 72 ∘ C for 5 min. The forward and reverse primers used include custom 24 nt barcode sequences followed by the sequences targeting bV18-A: [8F] AGRGTTYGATYMTGGCTCAG and [1391R] GACGGGCGGTGWGTRCA[176–179]. The resulting amplicon libraries were purified using the standard protocol for CleanNGS SPRI beads (CleanNA, NL) with a bead-to-sample ratio of 3:5. DNA was eluted in 25 µL of nuclease-free water (Qiagen, Germany). Sequencing libraries were prepared from the purified amplicon libraries using the SQK-LSK114 kit (Oxford Nanopore Technologies, UK) according to manufacturer protocol with the following modifications: 500 ng total DNA was used as input, and CleanNGS SPRI beads were used for library clean-up steps. DNA concentration was measured using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using Tapestation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of amplicon libraries.

The resulting sequencing library was loaded onto a PromethION R10.4.1 flowcell and sequenced using the MinKNOW 24.06.15 software (Oxford Nanopore Technologies, UK). Reads were basecalled and demultiplexed with MinKNOW Dorado 7.4.14 using the super accurate basecalling algorithm (config r10.4.1_400bps_sup.cfg) and custom barcodes.

B.6 Statistical analysis of maternal sociodemographic and lifestyle factors

Descriptive statistics were used to summarise the sociodemographic, anthropometric, and lifestyle characteristics of the study population. Continuous variables were reported as means and age range, while categorical variables were expressed as frequencies and percentages. Differences across GWG categories, classified as low (LGWG), adequate (AGWG), or excessive (EGWG), were assessed using the Kruskal–Wallis test for continuous variables and the Chi-square test for categorical variables. Since the sample related to each mother considered in the present analysis was substantially similar to that examined in our previous study[81], differing only by a few mother-infant pairs based on the availability of meconium samples at T0, the same multinomial logistic regression model was applied to identify factors associated with GWG categories, using AGWG as the reference category. This approach was adopted to confirm the consistency of the previously reported findings.

The results were expressed as relative risk ratios (RRRs) with corresponding 95% confidence intervals (CIs). All statistical tests were two-sided, and a p-value < 0.05 was considered indicative of statistical significance. Analyses were performed using StataCorp. 2024. Stata Statistical Software: Release 18.5. College Station, TX: StataCorp LLC.

B.7 Long read bioinformatic processing and statistical analyses

The sequencing reads in the demultiplexed and basecalled fastq files were filtered for length (320 - 2000 bp) and quality (phred score > 17) using a local implementation of filtlong v0.2.1 with the settings –min_length 320–max_length 2000 –min_mean_q 98. The filtered reads were mapped to the species-representative (reps) 16S rRNA (SSU) sequences from the GTDB release 220 database with minimap2 v2.24-r1122 using the -ax map-ont command[180] and

downstream processing using samtools v1.14.[180] The merged data set for bacteria and archaea entries (bac120_ssu_reps_r220.fna and ar53_ssu_reps_r220.fna) was filtered for archaea SSU > 450 bp, and bacteria SSU > 650 bp. Mapping results were filtered such that the query sequence length relative to the alignment length deviated < 5 %. Notably, low-abundant OTUs making up < 0.1 % of the total mapped reads within each sample were disregarded as a data denoising step.

Statistical analyses were conducted by using R Software v4.3.1[181]. The OTUs sequence was examined for non-standard nucleotide symbols, and any invalid characters were removed. Then it was loaded, together with the OTU count table and metadata, into an ampvis2 object using the "ampvis2" package v2.8.9.[182] The dataset was also cleaned of c-section-related samples, avoiding confounding and false positive effects. The β-diversity analyses were conducted by using the "vegan" package v2.6.10.[183] Differences in β-diversity were depicted in multidimensional space by Principal Coordinates Analysis (PCoA) produced using the *cmdscale* function, on the Bray-Curtis dissimilarity matrix. The α-diversity analysis was carried out using the *amp_alphadiv* function of the "ampvis2" package v2.8.9. Environmental fitting analyses were carried out by using the *envfit* function of the "vegan" package v2.6.10. Given the variety of statistical analyses performed, a detailed description of each analysis is provided in the Supplementary file.

C. Results

C.1 Maternal sociodemographic and lifestyle factors

Table 1 presents the distribution of sociodemographic and lifestyle factors of the study participants according to the GWG category.[14] Overall, 58.34% of women in the sample were classified as having inadequate GWG, of whom 68.37% were in the low GWG (LGWG) category and 31.64% in the excessive GWG (EGWG) category. The median age of participants was similar across GWG groups: 33 years (IQR 31–36) for AGWG, 32 years (IQR 29–36) for EGWG, and 33 years (IQR 30–36) for LGWG, with no statistically significant differences observed. Similarly, the educational level did not differ significantly among the groups. For the pre-BMI variable, a statistical significance was observed ($p < 0.001$); notably, 45% of the participants in the EGWG category had a pre-BMI indicating overweight (35%) or obesity (10%) (Table 1).

Concerning gestational lifestyle habits, no significant associations were observed between GWG categories and adherence to the MD. However, only 43% of women in the AGWG group reported medium or high adherence to the MD, compared to 55% and 52% in the LGWG and EGWG groups, respectively. Conversely, low adherence to the MD was more prevalent in the AGWG group, encompassing 57% of its participants **(Table 4**).

Concerning the FFQ score, a trend was observed ($p = 0.082$), suggesting an association between GWG categories and food frequency consumption. Indeed, among women with EGWG, 35% showed inadequate scores, 52% partially satisfactory, and only 13% satisfactory. In the AGWG

group, 40% had inadequate scores, 39% partially satisfactory, and 21% satisfactory. Conversely, among those with LGWG, a higher proportion (36%) achieved satisfactory scores, while 39% were partially satisfactory and 25% inadequate. A similar trend was observed for the DH Score ($p = 0.068$), where 48% of women in the EGWG group had inadequate scores, compared to 43% in the AGWG group and 30% in the LGWG group. The proportion of satisfactory scores was highest among women with LGWG (31%), followed by those with EGWG (29%) and AGWG (16%).

PA levels also showed no significant differences across the GWG categories. Nevertheless, medium or high PA scores were reported by 70% of participants in the AGWG group and 69% in the LGWG group **(Table 4)**. Finally, a statistically significant difference was found in smoking habits during pregnancy ($p < 0.001$). 13% of women in the EGWG group reported smoking while pregnant **(Table 4)**.

Table 4*. Maternal sociodemographic and lifestyle factors related to gestational weight gain.*

	EGWG	**AGWG**	**LGWG**	**P-value**
n	31	70	67	
Age (year)	32.00 (29.00-36.00)	33.00 (31.00-36.00)	33.00 (30.00-36.00)	0.20
Mother's Educational Level				0.061
Lower or High School	6 (20%)	7 (10%)	3 (5%)	
University degree	24 (80%)	63 (90%)	63 (95%)	
Pre-pregnancy BMI category				**<0.001**
Underweight (BMI<18.5 Kg/m^2)	0 (0%)	7 (10%)	9 (13%)	
Normal weight (18.5≤BMI≤24.9 Kg/m^2)	17 (55%)	54 (77%)	53 (79%)	
Overweight (25≤BMI≥29.9 Kg/m^2)	11 (35%)	5 (7%)	3 (4%)	
Obesity (BMI≥30 Kg/m^2)	3 (10%)	4 (6%)	2 (3%)	
Food Frequencies (FFQ)				0.082
Inadequate	11 (35%)	28 (40%)	17 (25%)	
Partially satisfactory	16 (52%)	27 (39%)	26 (39%)	
Satisfactory	4 (13%)	15 (21%)	24 (36%)	
Dietary Habits (DH)				0.068
Inadequate	15 (48%)	30 (43%)	20 (30%)	
Partially satisfactory	7 (23%)	29 (41%)	26 (39%)	
Satisfactory	9 (29%)	11 (16%)	21 (31%)	
Mediterranean Diet adherence Score category [without alcohol]				0.50
Lower Score	15 (48%)	40 (57%)	30 (45%)	
Medium Score	7 (23%)	18 (26%)	19 (28%)	
Higher Score	9 (29%)	12 (17%)	18 (27%)	

Physical Activity				0.45
Lower Score	15 (48%)	21 (30%)	21 (31%)	
Medium Score	8 (26%)	28 (40%)	24 (36%)	
Higher Score	8 (26%)	21 (30%)	22 (33%)	
Did you smoke during pregnancy?				**<0.001**
No	16 (52%)	24 (34%)	12 (18%)	
Yes	4 (13%)	3 (4%)	0 (0%)	

Notably, although FFQ and dietary habits DH were initially assessed using two validated sections of the Turconi *et al.* questionnaire[163], these variables were not analysed separately in Table S1 and then included in the final multivariable analyses. Instead, we focused exclusively on the MEDI-Lite score as a measure of adherence to the MD. This decision was based on both methodological and conceptual considerations.

First, the MEDI-Lite score is a comprehensive, validated index specifically designed to assess adherence to the Mediterranean dietary pattern, which was the primary dietary framework of interest in our study. Unlike the FFQ and DH sections, which provide more general insights into dietary behaviour and consumption frequency, the MEDI-Lite score directly reflects alignment with a well-characterised and evidence-based dietary model known to influence gut microbiota composition[184]. Second, including all three scores in the multivariate model would have introduced redundancy, as several components of the Turconi-derived scores overlap conceptually with the MEDI-Lite components (e.g., fruit and vegetable intake, frequency of sweets or meat consumption). This collinearity could have affected the robustness and interpretability of the regression models, resulting in misleading results.

No significant differences were observed in daily fruit consumption across GWG groups; most women reported average fruit intake, with proportions ranging from 55% in the EGWG group to 68% in the LGWG group. In contrast, a statistically significant difference emerged for daily vegetable consumption ($p = 0.031$); women in the AGWG group most frequently reported average vegetable intake (86%), whereas excessive and low GWG groups showed a higher prevalence of both inadequate (18% and 15%, respectively) and adequate (21% and 18%, respectively) consumption levels. Weekly legume consumption did not differ significantly among groups, although a higher proportion of women in the AGWG and LGWG categories reported average intake (55% and 58%, respectively), compared to only 37% in the EGWG group. Daily cereal consumption and weekly fish consumption were not significantly associated with GWG, and across all groups, with average intake reported by most participants in all groups. Daily meat consumption was evenly distributed among the three groups, with most women in each category consuming between 1 to 1.5 portions per day or less than one portion. Although not reaching statistical significance ($p = 0.054$), differences in daily milk and dairy product consumption approached significance and women with AGWG most frequently consumed 1 to 1.5 portions per day (60%), whereas both lower and excessive GWG groups had a higher proportion of women reporting consumption below or above this range. Lastly, regular use of olive oil was the most common across all GWG categories, particularly among women with AGWG (71%), showing no significant differences among the categories.

A multinomial regression analysis was conducted to identify dietary, anthropometric and social demographic predictors associated with GWG categories, using AGWG as the reference group. The results are presented in **Figure 1**.

Pre-BMI was significantly associated with an increased risk of EGWG compared to AGWG (Relative Risk Ratio [RRR]=1.162, p=0.007). Additionally, a trend toward a protective effect was observed for daily vegetable consumption: women reporting an average intake of 1 to 1.5 portions per day had a lower risk of EGWG compared to those with AGWG (RRR = 0.227, p=0.068), although this association did not reach conventional statistical significance.

No other variables included in the model were significantly associated with low GWG (LGWG) relative to the reference category.

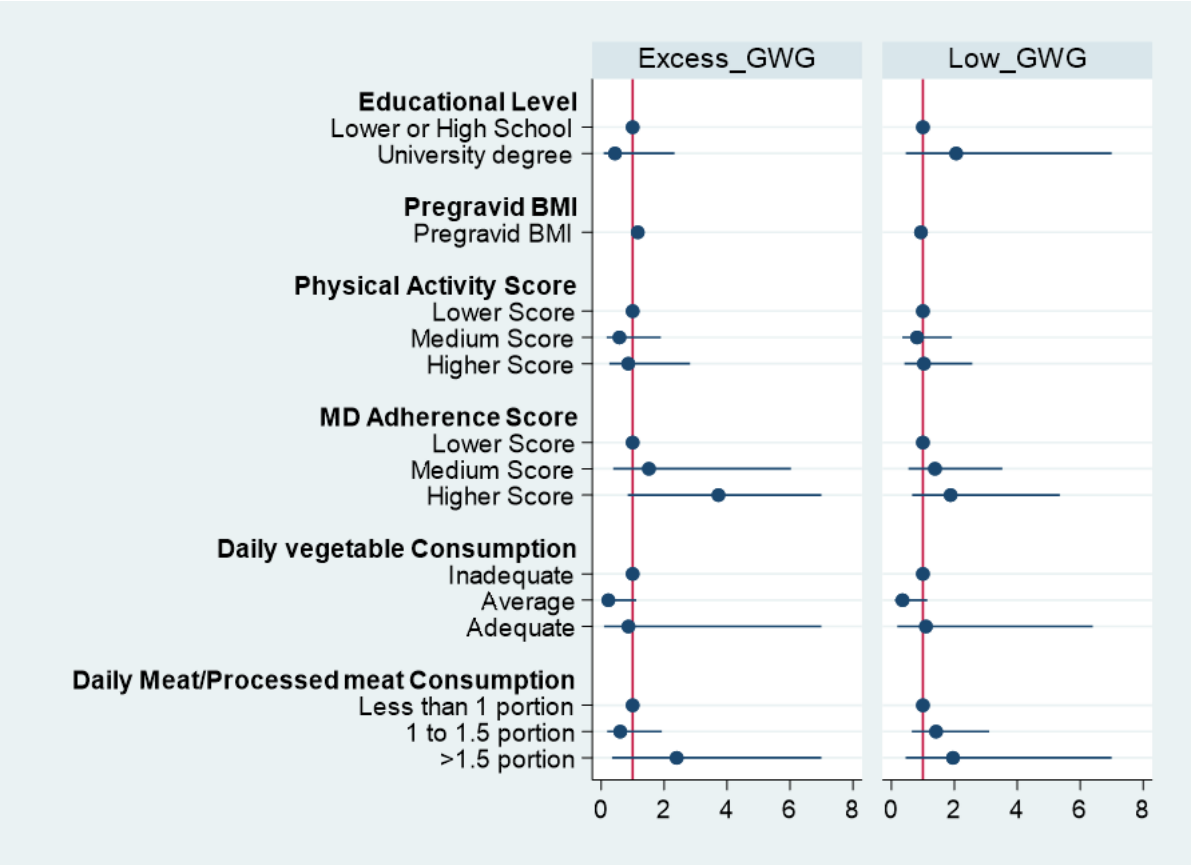


Figure 1. *The forest plot illustrates the results of the multinomial regression model based on maternal data from the LIMIT perspective cohort study. Relative risk ratios (RRRs) and corresponding confidence intervals for EGWG and LGWG are presented, using AGWG as the reference category.*

C.2 Meconium microbiota development according to maternal dietary parameters

The ordination analysis (PCoA based on Bray-Curtis distance; **Fig. 2a**) did not reveal any evident grouping of samples according to GWG categories. This observation was consistent with the results of the permutational multivariate analysis of variance (PERMANOVA; **Table S1**), which showed no significant effect of GWG on bacterial diversity. In other words, there were no significant differences in β-diversity among meconium samples from mothers with

AGWG, EGWG, or LGWG. A lack of significant effect on bacterial diversity was also observed for all main variables included in the model formula (GWG, gender, pre-BMI, GWG, Medi-Lite score, Medi-Lite score rank, celiac condition, PA, FFQ, and IPAQ), as assessed by adonis PERMANOVA **(Table S1)** and corroborated by comparing all group combinations by using pairwise adonis PERMANOVA **(Fig. 2b).** Environmental fitting analysis was also conducted to examine associations between main study variables (continuous variables) and community structure. Similarly, no environmental or lifestyle variable was found to significantly correlate with the primary axes of variation **(Fig. 3c and Table S2)**, suggesting that the observed sample distribution may reflect complex, multivariate factors not directly related to the studied variables. Furthermore, no significant differences in α-diversity were observed across GWG categories (adequate, excessive, and low) **(Fig. 2d).**

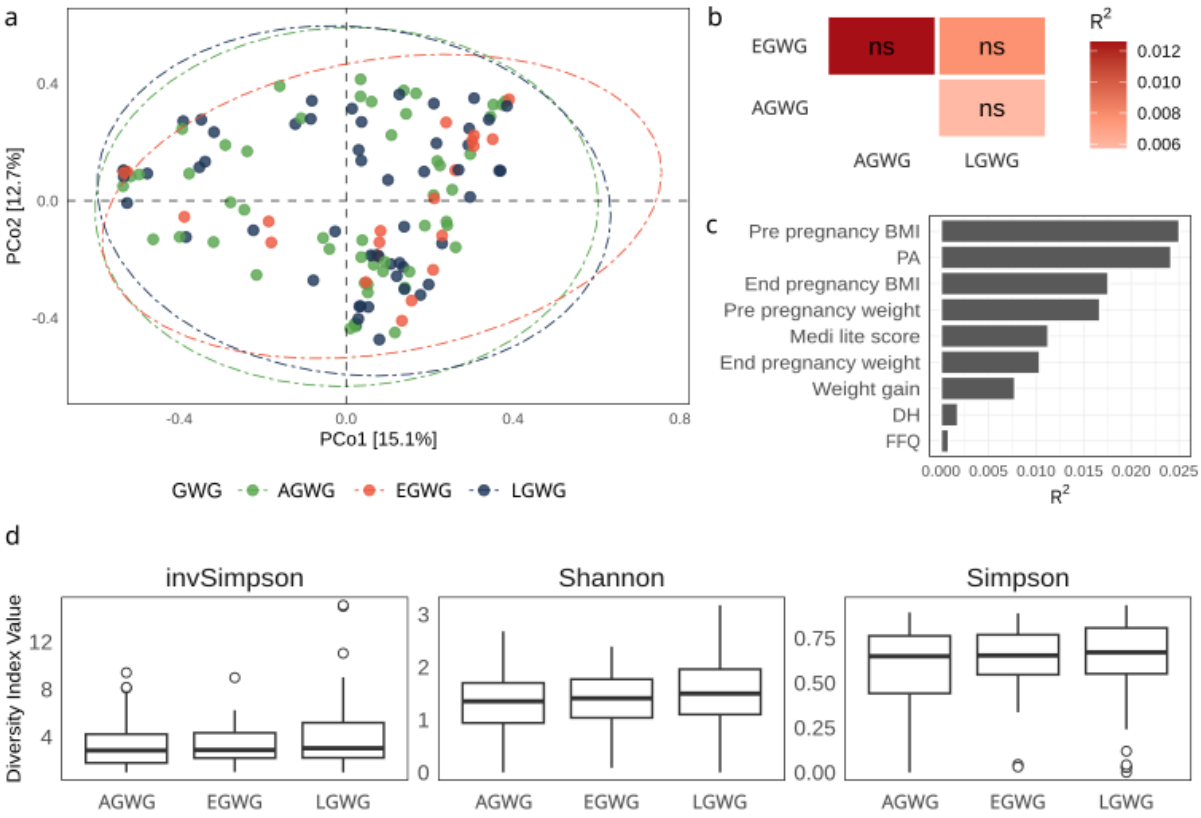


***Figure 2**. Effect of GWG on meconium bacterial diversity. (a) PCoA (Bray-Curtis distance) based on the relative abundance table depicted according to GWG by using a colour scheme. The first two principal coordinate axes (PCo1 and PCo2), which explained 15.1% and 12.7% of the total variation, respectively, were used to highlight a sample distribution. The 95% confidence ellipses were added using the dotted line. (b) Heatmaps report the results of pairwise Adonis PERMANOVA comparisons between GWG groups. The significance is annotated within each tile using annotation (ns: non-significant results). Each tile represented a pairwise comparison, coloured by the corresponding R^2 value. (c) Barplot reports the amount of variance (i.e. R2 from the envfit function) for each variable tested in the model formula. All variables tested display no significant effect ($p>0.05$). (d) Boxplot reports the α-diversity metrics, Inverse Simpson, Shannon and Simpson indices among different GWG categories. Differences between α-diversity metrics are assessed using the Kruskal-Wallis test.*

C.3 Unsupervised learning reveals distinct microbiota-type patterns.

As no significant associations were observed between bacterial diversity and the study-defined categories, we performed a deeper exploration of the dataset by performing an unsupervised learning analysis aimed at identifying coherent clusters independently of our study categories.

To identify natural groupings within the sample dataset, a partitioning around medoids (PAM) clustering approach was applied. The optimal number of clusters (k) was determined using average silhouette width (ASW), which peaked at an optimum of 5 clusters, indicating the most appropriate partitioning structure **(Fig. 3a)**. The unsupervised classification resulted in 5 distinct clusters reported by using ordination analysis (PCoA, Bray-Curtis distance in **Fig. 3b**), depicted different principal coordinate axes to highlight cluster separation better. Permutational multivariate analysis adonis PERMANOVA confirmed that the identified clusters accounted for a significant portion of the variation in microbial community composition ($R^2 = 0.349$, $p = 0.001$), highlighting that the clusters partitioning capture biologically meaningful structure in the dataset. The pairwise adonis PERMANOVA analysis further supported these distinctions: as expected all the inter-cluster comparisons were statistically significant, however, not all cluster comparisons explained the same amount of variance, as R^2 values ranging from 0.18 to 0.31, with highest amount reported for Cluster 3 vs Cluster 5 comparison and the lowest amount reported for Cluster 1 vs Cluster 3 and 4 comparison **(Fig. 3c)**. Therefore, a hierarchical clustering analysis was performed using the Bray-Curtis dissimilarity matrix and the average linkage method. The fidelity of the resulting dendrogram to the original distance matrix (used for the unsupervised analysis) was evaluated via cophenetic correlation ($r = 0.83$), indicating appropriate representational accuracy. The clustering structure was reported by using a dendrogram with cluster annotations, to highlight the correspondence between ordination-based and hierarchical groupings **(Fig. S4)**. To further highlight the distribution of OTUs based on abundance, a cluster analysis was performed using Euclidean distance and the Ward D2 linkage **(Figure 3d)**. The analysis was conducted on the 21 OTUs whose abundance significantly (Wilcoxon test with FDR correction) changed among the 5 clusters produced by the unsupervised learning. Despite the use of different cluster methods, the samples grouped coherently with the unsupervised cluster assignment, exhibiting consistent compositional profiles that confirmed the robustness of the clustering approach. Several OTUs displayed clear cluster-specific enrichment, with higher abundances concentrated in defined subsets of samples, whereas others showed a more heterogeneous distribution across clusters. Notably, low-abundance taxa substantially contributed to the observed differences, reflecting a less rich and polarized microbiota structure.

To investigate the contribution of specific OTUs to the observed community structure, we build PCoA ordination using an envfit-like approach. Statistically significant OTUs ($p < 0.05$) were then assigned to sample clusters by computing the cosine similarity between each OTU's vector of variation and the centroids of identified clusters in ordination space. This vector-based method revealed strong directional associations between subsets of OTUs and specific clusters, suggesting that distinct taxonomic signatures underlie the observed microbial community patterns, independently of measured environmental or lifestyle variables **(Fig. S3 and Table S5).**

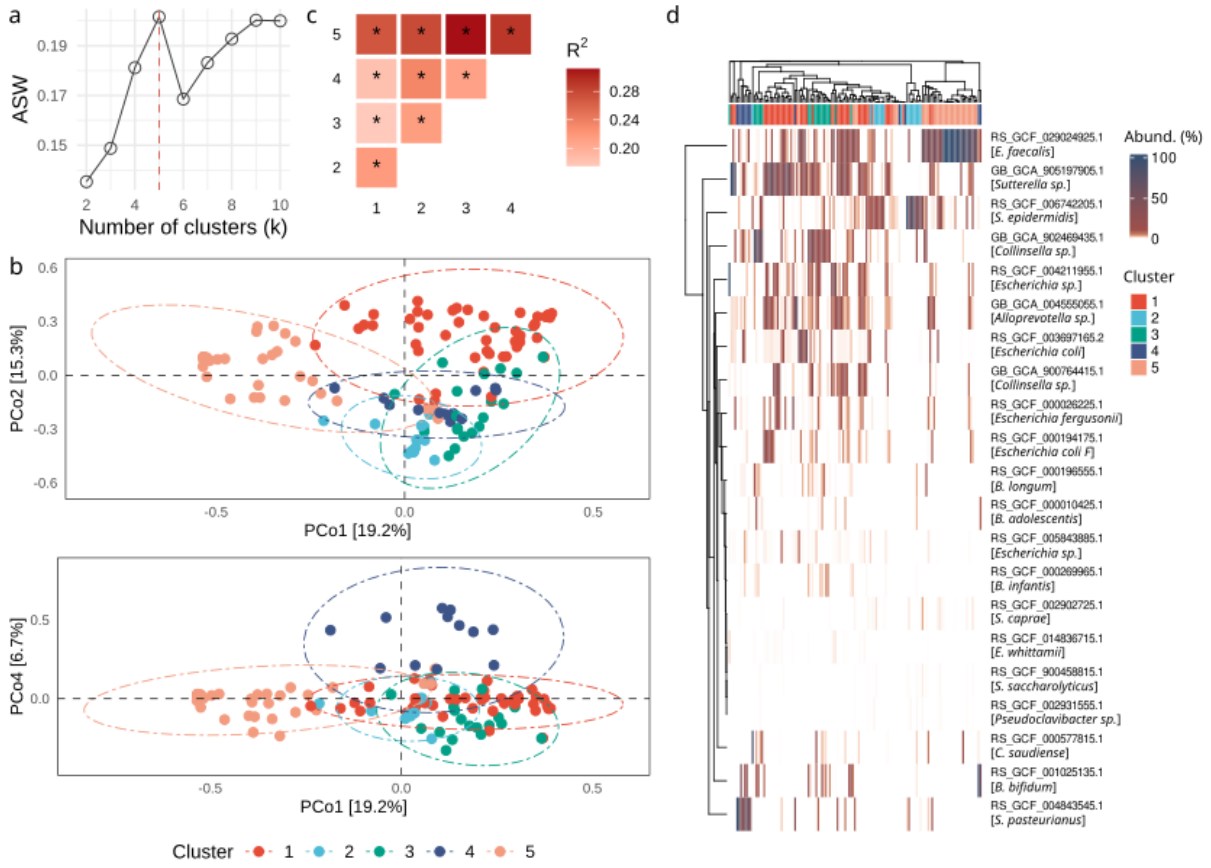


***Figure 3**. Assessment of different microbiota types through unsupervised learning analysis. . (a) The line chart reports the Average Silhouette Width (ASW) calculated for the interval between 2 and 10 clusters. Each point represents the ASW obtained for each cluster, and the vertical red dotted line highlights the optimum number of clusters. (b) PCoA based on Bray-Curtis distance, produced using the axis PCo1 vs PCo2 and PCo1 vs PCo4, highlights the sample distribution according to the clusters calculated by using the PAM algorithm. Clusters are represented using colour code in the legend, and ellipses represent 95% confidence intervals of the clusters. (c) The heatmap represents the results of cluster cross-comparisons produced by pairwise adonis PERMANOVA. Significant contrasts are reported using asterisks (*, $p<0.001$) while the R^2 values are reported using a colour gradient. (d) Heatmap reports the distribution of significantly different OTUs across clusters selected using the Wilcoxon test (FDR adjustment). The OTUs are reported in the rows, and each column corresponds to a sample. Percentage abundances are shown on a colour scale ranging from low (white) to high (dark red-blue). Top annotations indicate cluster membership, with colours assigned according to the colour scheme in the legend. Rows were hierarchically clustered using Euclidean distance and the Ward D2 linkage method. Each OTU label is reported together with the related taxonomic assignment, indicated below each OTU using brackets.*

The differences in the relative abundances of specific OTUs among the five clusters were assessed. Considering the most representative OTUs within the dataset (see *Taxonomic signature associated with the clusters section* in the supplementary file), we identified 21 OTUs whose relative abundances significantly changed among the five clusters **(Figure S5)**. Each of these OTUs exhibited significantly different abundance patterns when compared between each cluster by using post-hoc analyses **(Table S6)**. Each cluster showed prevalences in specific OTUs; Cluster 1 was characterised by species-level OTUs such as *Allo*prevotella *sp004555055*, *Collinsella sp900764415*, Sutterella sp90519795, *Escherichia fergusonii*, *Escherichia coli F*,

and *Escherichia coli*. These OTUs were predominant within cluster 1's microbiota, although some were also shared with other clusters, for instance, *Escherichia whittamii* and *Escherichia sp0044211955*, which were also representative of cluster 3. However, cluster 3 showed a significant association in species-level OTUs belonging to the *Bifidobacterium* genus, such as *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Bifidobacterium adolescentis*, the latter also being partly represented in cluster 4. Clusters 4, 2 and 5 were each associated with distinct OTU signatures that contributed substantially to their overall OTU abundance profiles. Specifically, *Streptococcus pasteurianus* was related to cluster 4, while *Staphylococcus epidermidis* was associated with cluster 2, and *Enterococcus faecalis* was associated with cluster 5. This overview of OTUs abundance variations highlights the presence of distinct microbiota types underlying each cluster, highlighting specific bacterial signatures in the meconium that appear to be independent of maternal diet and lifestyle habits.

D. Discussion

In this study, we investigated the influence of maternal GWG and other maternal lifestyle and physiological variables on the composition of neonatal meconium microbiota within the LIMIT study[66], applying a long-read sequencing approach to provide high-resolution microbial profiling.

Our analyses revealed that neither GWG categories nor maternal pre-BMI, MD adherence, or PA exerted a measurable effect on meconium microbial diversity. These findings suggest that maternal weight gain and lifestyle-related factors, although crucial for pregnancy outcomes and child health, may not directly shape the earliest microbial communities in newborns. The results are in line with previous work of Turunen *et al*[170], which pointed to the delivery mode as the strongest determinant of meconium microbial composition, thereby reinforcing the idea that gut microbiota establishment begins primarily at birth through exposures occurring during delivery and in the immediate postnatal period. Therefore, the lack of significant microbial community variation in meconium samples associated with GWG categories and other maternal factors, such as pre-BMI, MD adherence, and PA level, suggests that the initial establishment of the neonatal gut microbiota may be driven by complex, multifactorial processes not directly explained by the maternal lifestyle variables measured in this study.

At the same time, our results stand in contrast to other studies[185,186] reporting significant associations between maternal GWG and neonatal microbiota. For instance, evidence from Song and Liu[185] found that EGWG was linked to reduced alpha diversity and depletion of

specific microbial taxa. Another study conducted by Cho *et al.*[186], exploring the relationship between maternal pre-BMI, GWG, and early gut colonisation, revealed that maternal pre-BMI was associated with distinct microbial profiles in neonatal meconium. *Citrobacter* was more abundant in infants of mothers with normal pre-BMI, while higher *Lachnospira* levels were found in newborns of mothers experiencing underweight before pregnancy.

Additionally, maternal GWG was found to be linked to specific microbial signatures in neonatal meconium, with variations in bacterial composition observed across different levels of maternal weight gain.[186] Such discrepancies across studies likely reflect heterogeneity in study design, sample size, sequencing methodologies, and confounding control, and they highlight the complexity of disentangling maternal metabolic influences from other perinatal determinants.

Our study also considered maternal dietary patterns and lifestyle variables. Although maternal adherence to the MD and reported dietary habits showed no significant associations with meconium microbial diversity, this does not rule out their importance. Prior work by Sasaki *et al.* [73] demonstrated that higher adherence to the MD during pregnancy correlated with increased abundance of SCFAs-producing bacteria in meconium, and even with epigenetic changes in placental and cord blood tissues. The lack of associations in our cohort may be explained by methodological differences, by the exclusion of alcohol from the MD score during pregnancy, or by the fact that dietary influences may become more evident later in infancy during breastfeeding and complementary feeding, when maternal diet exerts a more direct impact. Similarly, no significant relationships were observed for maternal PA or smoking status, though these behaviours remain important for overall pregnancy and infant health. One of the strengths of this study lies in its use of unsupervised clustering, using the PAM algorithm, to move beyond predefined maternal categories and explore intrinsic microbial structures. This approach revealed five distinct and biologically meaningful clusters of meconium microbial communities, each defined by unique taxonomic signatures and differing in the relative abundance of key OTUs such as B*ifidobacterium, Streptococcus, Escherichia, Enterococcus, and Staphylococcus*. These findings suggested that neonatal microbiota may be organised into "types" or enterotype-like structures, reminiscent of clustering observed in adult gut microbiota studies, and align with the results of Chang *et al*,[35] who reported similar microbial community structures in meconium using long-read sequencing.

Our findings supported the notion that early-life microbial colonisation may follow distinct ecological trajectories, potentially shaped by perinatal exposures, genetic predispositions, or stochastic events during the gestational period and delivery, rather than specific maternal lifestyle or nutritional status. The presence of defined microbiota types at birth echoes similar observations in adult gut microbiota studies[187,188], where enterotype-like clustering has been proposed.

However, the determinants and long-term implications of these early microbial patterns remain to be elucidated. For instance, some studies[189,190][38,39] challenged the presence of a prenatal microbiome.

For example, Kennedy *et al.*[189] found no evidence of microbial presence in 20 prenatal meconium samples collected under sterile conditions, supporting the hypothesis that initial gut colonisation may begin only during or after delivery. Similarly, Santos Scott *et al.*[190] reported difficulties in identifying a distinct microbiome in 141 meconium samples from which DNA was extracted, also under sterile conditions to avoid contamination. These divergent findings may be due to differences in DNA extraction and sequencing protocols, contamination controls, or sensitivity of detection, and they underline the need for methodological rigour and standardisation in future research.

Taken together, our findings suggest that maternal GWG and lifestyle factors may not be the principal drivers of initial gut colonisation, which appears to be shaped by more complex and multifactorial influences.

The unsupervised clustering approach adopted here underscored the importance of exploring microbial community structures beyond traditional categorical variables. The application of unsupervised learning approaches in this context proved crucial for uncovering meaningful variation that would otherwise have remained hidden. This has significant implications for the study of microbiota development, highlighting the need for refined methodologies capable of capturing the complexity of host–microbe development in early life.

Limitations of this study must also be acknowledged. The analysis was restricted to meconium collected at birth, representing a single time point and therefore unable to capture the dynamic colonisation processes that occur immediately after delivery and during early infancy. The sample size, while larger than many previous studies, may still have been insufficient to detect subtle associations. The focus on the MEDI-Lite score,[19] although conceptually justified, may have reduced the granularity of dietary assessment. Additionally, although we excluded

caesarean-related samples to minimise confounding, other factors such as maternal stress or other unmeasured environmental variables may have influenced microbial signals.

Despite these limitations, our findings provide important insights into the complex and multifactorial nature of neonatal microbiota establishment. They suggest that GWG and maternal lifestyle, although important for other aspects of pregnancy and infant outcomes, may not be major determinants of meconium microbial structure. Instead, our identification of distinct microbiota clusters points to the possibility that neonatal colonisation follows specific ecological trajectories, the origins and implications of which remain to be fully elucidated.

Future research should adopt a longitudinal perspective, integrating repeated sampling across the first 1000 days of life and combining metagenomic, metabolomic, and transcriptomic approaches to determine whether early microbial clusters persist, converge, or diverge, and whether they predict outcomes such as growth trajectories, immune development, and susceptibility to metabolic or inflammatory diseases. The longitudinal design of the LIMIT study[66] provides a unique opportunity to address these questions.

In conclusion, while maternal GWG and lifestyle variables did not significantly influence meconium microbiota diversity, our unsupervised analyses revealed distinct and biologically relevant microbiota types in newborns' meconium. These findings suggest that neonatal microbial colonisation may be mainly influenced by unmeasured or stochastic factors than by maternal gestational characteristics alone. The entire longitudinal dataset will be essential to unravel the determinants and factors shaping these early microbial profiles.

CHAPTER 4: GENERAL DISCUSSION

This chapter provides a comprehensive discussion based on the findings of all the five included publications in my doctoral thesis. In the previous chapters, I presented the conducted papers in a chronological order, starting with a synthesis for the existing body of evidence[27,79,80] to understand what is well known and what gaps still exist, ending up with my original empirical studies to test some question that fill the same broader image, using samples of mother-infant dyads derived from the original cohort study namely LIMIT[66].

The transition between my conducted papers, I went from theoretical available evidence to population-based observations and early biological signals of infant microbiota at delivery, which allowed me to interpret how maternal lifestyle and metabolic factors, can influence pregnancy outcomes and microbiota-related pathways relevant to an increased risk of childhood obesity, while also identifying gaps and inconsistencies that require further investigation.

This chapter provides an integrated discussion connecting all the findings of the conducted studies in a sequential way to create a wider image that falls within the framework of the DOHaD. This framework suggests that exposures acting during critical periods of early development exert long-lasting effects on metabolic health and disease susceptibility across the life course. Within this framework, maternal lifestyle behaviours, pre-pregnancy adiposity, and gestational metabolic status function as early environmental stimuli capable of shaping fetal development through integrated hormonal, inflammatory, metabolic, epigenetic, and microbial pathways.[61,62] The first 1,000 days of life, spanning from preconception to the end of the second postnatal year, represent a phase of heightened developmental plasticity, during which these signals may permanently influence biological systems regulating energy balance and adiposity. Importantly, this window also represents a unique opportunity for targeted preventive interventions, as modifying maternal and early-life exposures during this period may mitigate the establishment of adverse metabolic trajectories and reduce the long-term risk of childhood obesity. Within this conceptual framework, the results of the present PhD thesis contribute to the growing evidence that maternal health and lifestyle before and during pregnancy represent critical leverage points for early prevention, as they may influence both maternal metabolic adaptation to pregnancy and early biological conditions at birth.

From a mechanistic standpoint, the observed associations between maternal lifestyle factors, GWG, and the composition of neonatal microbiota composition can be interpreted within

established pathways of metabolic and inflammatory programming. Maternal nutritional quality, PA, and metabolic status are known to influence insulin sensitivity, systemic inflammation, and hormonal signalling during pregnancy, all of which may shape the intrauterine milieu. These biological signals can affect fetal nutrient exposure and immune maturation, potentially influencing early microbial colonization patterns that are relevant to later metabolic regulation.

4.1 Insights from literature-based studies:

The first step in this journey included two narrative[27,79] and one systematic review[80], covering existing findings about maternal nutrition factors, lifestyle habits, and early biological exposures, with respect to the metabolic health of the offspring.

By looking at the findings of these three reviews, a consistency of evidence was presented supporting the impact of maternal lifestyle choices and metabolic status on pregnancy outcomes and infants' health, characterized by numerous pathways involving inflammation, glucose and lipid metabolisms, epigenetic conditions and hormonal regulations.

In the narrative review conducted on HBM microbiota[79] provide a wider frame for the potential role of maternal lifestyle factors on infant's health that goes beyond pregnancy to reach the different stages of lactation period, through enteromammary mechanisms. Similarly, the narrative review investigating the offspring exposomes[27] highlighted that that metabolic programming is not only shaped during the prenatal period, emphasizing the importance of lifestyle interventions across all stages of the critical first 1000 days window. The systematic review on FA supplementation[80], further emphasized this idea by showing how micronutrient exposures in the preconception stage or during pregnancy, can be altered depending on several factors involving maternal lifestyle behaviours and weight status.

As shown by these three reviews, one common message can be emerged demonstrating the role of maternal lifestyle and nutritional status as biological plausible moderators for maternal and neonatal metabolic health on the short- and long-terms.

On the other hand, inconsistencies and knowledge gaps were found in the literature, related to the differences in the timing of exposures measurement (during preconception, pregnancy, or postpartum); for the outcome's measurement (studying meconium or stool samples); or related to the difference in methodological analysis for the microbiota (sequencing and diversity reads

techniques). This proposes that maternal exposures act differently, based on the biological systems.

Our findings are in line with the DOHaD[61,62,191] framework and microbiome research[192], where the risks of developing any kind of diseases across life course, depends on the timing and biological system of the environmental exposures.

4.2 From Literature to Population Data: Maternal Lifestyle and GWG

Building on the first step of the literature synthesis, the second step involved two original analyses for the identified maternal factors, conducted on samples of mother-infant dyads derived from the original sample of the LIMIT study[66]. Both papers followed an observational cross-sectional design. The first one assessed the relationship between maternal lifestyle factors and GWG adequacy. The second one investigated the relationship between maternal weight status, lifestyle behaviours, and environmental exposures, with the meconium microbial profile via analysing meconium samples at delivery.

The first empirical study[81], I conducted within the LIMIT study[66] examined associations between maternal lifestyle factors and GWG adequacy.

The findings of this study[81] confirmed the associations suggested in the reviews, determining how maternal lifestyle choices can significantly affect the adequacy of GWG. Dietary patterns, PA, and pre-pregnancy weight class were all associated with maintaining GWG within recommended ranges.

With these results, we were able to link the narrative and systematic reviews with real-world cohort data, providing additional evidence on how maternal lifestyle factors are measurable determinants of biological and metabolic health outcomes.

Similar observations between maternal lifestyle behaviours and GWG adequacy were previously reported in large cohorts and meta-analyses including European populations, showing a strong connection with adverse health outcomes at the level of mothers and offspring[57,193].

These findings are connected at a common point highlighting that GWG represents a key intermediate phenotype linking maternal lifestyle exposures with metabolic health during pregnancy. In this context, dietary quality and PA emerge as central modifiable determinants

that may influence both maternal metabolic adaptation and downstream pregnancy outcomes, reinforcing the relevance of lifestyle-based preventive strategies during this critical period.

Although several covariates were included in the statistical models, the possibility of some residual confounding cannot be excluded. These confounding variables could be related to additional lifestyle behaviours, socioeconomic conditions, environmental exposures, or maternal metabolic characteristics. Moreover, some analyses were conducted using complete-case datasets after excluding participants with missing values in key variables. While this approach improves interpretability of multivariable analyses, it may introduce bias if missing data are not completely random.

4.3 Maternal Lifestyle and Early Microbial Signals: Insights from Meconium Microbiota Analysis

The second empirical study was conducted as a further investigation targeting meconium microbiota, as it is considered the first measurable biological signal in infants.

The microbiome-related pathways potentially linking prenatal exposures to later obesity risk include the production of short-chain fatty acids (SCFAs), modulation of immune development, and regulation of inflammatory and metabolic signalling. SCFAs such as acetate, propionate, and butyrate play key roles in energy homeostasis, gut barrier integrity, and immune tolerance. Altered early microbial profiles may influence these pathways during critical windows of development, thereby contributing to long-term metabolic programming. Additionally, early-life microbial exposures are increasingly recognised as modulators of systemic inflammation and insulin sensitivity, mechanisms that are central to obesity pathophysiology.

Despite the expectations derived from the literature reviews, maternal weight status, adherence to the MD, and engaging in PA, did not show any association with the overall microbial diversity in meconium. These results support the idea previously discussed regarding the different effects of maternal exposures depending on the timing of measurement and the measured biological systems. In other words, the influence of maternal lifestyle factors may not susbstantially affect the composition of the meconium microbiota at birth. In addition, the microbial analysis of meconium may be influenced by environmental contamination.

Rather than being interpreted solely as negative findings, these results may reflect the specific biological nature of meconium as a very early intrauterine snapshot. From a DOHaD

perspective, these findings may also reflect the timing of biological programming processes during early development. The DOHaD framework emphasizes that the effects of prenatal exposures depend not only on the exposure itself but also on the developmental window during which it occurs. In this context, the absence of detectable associations between maternal lifestyle factors and meconium microbial diversity at birth does not necessarily contradict developmental programming hypotheses. Instead, it may indicate that microbiome-related programming mechanisms emerge progressively after birth, when environmental exposures such as feeding practices, antibiotic use, and early-life nutrition begin to shape microbial colonization more strongly. Therefore, the evaluation of microbial trajectories across early infancy may provide a more informative perspective for understanding how prenatal exposures contribute to long-term metabolic risk within the DOHaD framework. Meconium captures late prenatal exposures before postnatal factors begin to exert a strong influence on microbial development. It is considered a low-biomass microbial environment and the detection of microbial DNA in it does not necessarily indicate the presence of viable bacteria. For this reason, the microbial environment of meconium should be interpreted with caution. Growing evidence suggests that lifestyle-related microbial alterations may emerge more clearly later in infancy, when breastfeeding practices, complementary feeding, antibiotic exposure, and environmental contacts dynamically shape gut microbial communities. Moreover, ongoing scientific debate regarding the presence and stability of a detectable microbiota in meconium prior to birth may further explain inconsistencies across studies.

Following these findings, it is important to consider both the timing of measurement and the biological context when interpreting microbiome related associations. This conclusion aligns with previously reported inconsistencies and weak associations when assessed at birth compared with later in infancy[70].

Moreover, the absence of associations at T0, does not cancel the effect of maternal lifestyle factors, but since meconium provides a very early snapshot for the intrauterine exposures, the absence of associations in our results suggests that microbial alteration may occur predominantly later during infancy. This was reported in other longitudinal studies, which confirmed that infant gut microbiota is rapidly and strongly shaped after being exposed to several factors such as breastfeeding, complementary feeding, antibiotic use, and any environmental contact with siblings or pets[74,194].

4.4 Early Microbiota Profile: the five clusters

Although we did not find any association with microbial diversity, the PAM algorithm (unsupervised analysis) identified the presence of five different meconium microbiota profiles composed of various taxonomic compositions.

This result determines a new observation within the topic of my thesis, early-life microbial communities differ naturally between infants, and are not easy to measure through the studied maternal lifestyle variables or environmental exposures.

Going back to the findings of the narrative review conducted on offspring exposomes[27], we can see the alignment with this new observation, indicating that early-life biological systems may be shaped by multifactorial stimuli, some of which are not yet known or unable to be measured by standard exposures.

These findings align with other emerging microbiome studies, which suggest that early-microbial development is influenced by unexplained multifaceted colonisations[195], maternal microbial reservoirs[196], and in utero microbial exposures[171,197] that may influence early microbial development.

The identification of these distinct microbial clusters therefore represents an original contribution of this thesis, suggesting that heterogeneity in neonatal microbial profiles at birth may reflect complex and partially unmeasured biological processes. This observation supports the need for longitudinal follow-up and integrative exposure assessment to better understand how early microbial patterns evolve and relate to later metabolic outcomes.

4.5 Conclusion

Within the whole framework of my thesis, I can finally conclude that maternal lifestyle factors and nutritional status can significantly affect pregnancy outcomes and maternal and offspring health, while their observed impact on early microbiota at birth appears to be limited. The integration of reviews with population-based analysis underscores the importance for future investigations, taking into account the timing effect and the studied biological system on the resulted measure. From a broader perspective, these findings can also be interpreted within the offspring exposome framework, which recognises that metabolic programming results from cumulative environmental exposures acting across early life. In addition to lifestyle behaviours, chemical exposures such as EDCs (e.g., phthalates and bisphenols) [131–137]. may interact with biological systems during sensitive developmental windows, contributing to obesity susceptibility through shared inflammatory, endocrine, and metabolic pathways.

Within the LIMIT cohort, the baseline microbial and lifestyle profiles described in this thesis provide a reference point for subsequent longitudinal analyses. These T0 findings inform hypotheses regarding how early microbial patterns may evolve across infancy and interact with postnatal lifestyle exposures at T1–T5, ultimately influencing growth trajectories and the timing of adiposity rebound. By characterizing early-life starting conditions, this work supports a developmental interpretation of future findings related to early adiposity rebound and childhood obesity risk.

A major strength of this thesis falls within its conceptual and transitional design. Some limitations are linked to the restricted analysis of T0 data and the inability to follow-up on the postnatal effects. However, these will be assessed later throughout the LIMIT cohort[66].

An additional methodological limitation relates to challenges inherent to microbiota analysis, including variability in sequencing approaches and diversity metrics, particularly in low-biomass samples such as meconium. Future longitudinal data from the LIMIT cohort will allow for the investigation of postnatal microbial trajectories and their relationship with early adiposity rebound and metabolic outcomes.

From a clinical and public health perspective, these findings reinforce the importance of preconception and prenatal care as key opportunities for obesity prevention. Integrating lifestyle medicine approaches into maternal care—including nutritional counselling, promotion of PA, smoking cessation, and stress management—may support healthier metabolic and microbial environments early in life. Such strategies have the potential to reduce intergenerational transmission of obesity risk and improve long-term metabolic and obstetric trajectories. Emphasizing early prevention rather than postnatal treatment aligns with emerging public health priorities and supports more sustainable approaches to addressing the global burden of obesity.

References:

1. world obesity. (2022). *Prevalence of Obesity*. World Obesity. https://www.who.int/health-topics/obesity/obesity?#tab=tab_1

2. Kerr, J. A., Patton, G. C., Cini, K. I., Abate, Y. H., Abbas, N., Abd Al Magied, A. H. A., Abd ElHafeez, S., Abd-Elsalam, S., Abdollahi, A., Abdoun, M., Abdulah, D. M., Abdulkader, R. S., Abdullahi, A., Abeywickrama, H. M., Abie, A., Abiodun, O., Abohashem, S., Abtahi, D., Abualruz, H., … Azzopardi, P. S. (2025). Global, regional, and national prevalence of child and adolescent overweight and obesity, 1990–2021, with forecasts to 2050: a forecasting study for the Global Burden of Disease Study 2021. *The Lancet*, *405*(10481), 785–812. https://doi.org/10.1016/S0140-6736(25)00397-6

3. World Health Organization (WHO). (n.d.). *Obesity*. Retrieved February 2, 2021, from https://www.who.int/health-topics/obesity/obesity?#tab=tab_1

4. World Health Organization (WHO). (2022). *Obesity among children and adolescents, BMI > +2 standard deviations above the median, prevalence (crude estimate) (%)*. https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-obesity-among-children-and-adolescents-bmi-2-standard-deviations-above-the-median-(crude-estimate)-(-)

5. National Academies of Sciences, E. and M. H. and M. D. F. and N. B. R. on O. S., & Callahan, E. A. (2023). The Science, Strengths, and Limitations of Body Mass Index. *Translating Knowledge of Foundational Drivers of Obesity into Practice*. https://doi.org/10.17226/26942

6. Swinburn, B. A., Kraak, V. I., Allender, S., Atkins, V. J., Baker, P. I., Bogard, J. R., Brinsden, H., Calvillo, A., De Schutter, O., Devarajan, R., Ezzati, M., Friel, S., Goenka, S., Hammond, R. A., Hastings, G., Hawkes, C., Herrero, M., Hovmand, P. S., Howden, M., … Dietz, W. H. (2019). The Global Syndemic of Obesity, Undernutrition, and Climate Change: The Lancet Commission report. *The Lancet*, *393*(10173), 791–846. https://doi.org/10.1016/S0140-6736(18)32822-8

7. Popkin, B. M., & Ng, S. W. (2021). The nutrition transition to a stage of high obesity and noncommunicable disease prevalence dominated by ultra-processed foods is not inevitable. *Obesity Reviews*, *23*(1), e13366. https://doi.org/10.1111/OBR.13366

8. World Health Organization (WHO). (n.d.). *The double burden of malnutrition: policy brief*. Retrieved January 13, 2026, from https://www.who.int/publications/i/item/WHO-NMH-NHD-17.3

9. Popkin, B. M., Corvalan, C., & Grummer-Strawn, L. M. (2020). Dynamics of the double burden of malnutrition and the changing nutrition reality. *The Lancet*, *395*(10217), 65–74. https://doi.org/10.1016/S0140-6736(19)32497-3

10. The OECD (Organisation for Economic Co-operation and Development). (n.d.). *Health at a Glance 2025 | OECD*. Retrieved January 13, 2026, from

https://www.oecd.org/en/publications/health-at-a-glance-2025_8f9e3f98-en.html

11. World Health Organization (WHO) COSI. (n.d.). *WHO European Childhood Obesity Surveillance Initiative (COSI): report on the sixth round of data collection, 2022– 2024*. Retrieved January 13, 2026, from https://www.who.int/europe/publications/i/item/WHO-EURO-2025-11788-51560-78769

12. World Health Organization (WHO). (n.d.). *Childhood obesity and overweight remain a major concern across the Region, new WHO report finds*. Retrieved January 13, 2026, from https://www.who.int/europe/news/item/04-11-2025-childhood-obesity-and-overweight-remain-a-major-concern-across-the-region--new-who-report-finds

13. Phelps, N. H., Singleton, R. K., Zhou, B., Heap, R. A., Mishra, A., Bennett, J. E., Paciorek, C. J., Lhoste, V. P., Carrillo-Larco, R. M., Stevens, G. A., Rodriguez-Martinez, A., Bixby, H., Bentham, J., Di Cesare, M., Danaei, G., Rayner, A. W., Barradas-Pires, A., Cowan, M. J., Savin, S., … Ezzati, M. (2024). Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *The Lancet*, *403*(10431), 1027–1050. https://doi.org/10.1016/S0140-6736(23)02750-2

14. Società Italiana di Nutrizione Umana. (n.d.). *Adherence to the Mediterranean diet in Italy from 2019 to 2022: results from an observational study of over 10,000 adults* . Retrieved January 13, 2026, from https://sinu.it/wp-content/uploads/2024/04/3eng.pdf

15. Buscemi, S., Corleo, D., Galvano, F., & De Lorenzo, A. (2020). The Mediterranean diet and its individual components: Linking with obesity in Italy. *The Mediterranean Diet: An Evidence-Based Approach*, 285–292. https://doi.org/10.1016/B978-0-12-818649-7.00026-6

16. Simmonds, M., Llewellyn, A., Owen, C. G., & Woolacott, N. (2016). Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. *Obesity Reviews : An Official Journal of the International Association for the Study of Obesity*, *17*(2), 95–107. https://doi.org/10.1111/OBR.12334

17. Geserick, M., Vogel, M., Gausche, R., Lipek, T., Spielau, U., Keller, E., Pfäffle, R., Kiess, W., & Körner, A. (2018). *Acceleration of BMI in Early Childhood and Risk of Sustained Obesity*. *379*(14), 1303–1312. https://www.nejm.org/doi/pdf/10.1056/NEJMoa1803527

18. Bray, G. A. (2002). *Predicting obesity in adults from childhood and adolescent weight 1,2*. www.ajcn.org

19. World Health Organization. (2022). *WHO European Regional Obesity Report 2022*. 1–220. http://apps.who.int/bookorders.%0Ahttps://www.who.int/europe/publications/i/item/9789289057738

20. Abdi, F., Alizadeh, S., Roozbeh, N., Montazeri, F., Banaei, M., Mehrnoush, V., & Darsareh, F. (2025). Prepregnancy overweight and obesity and the risk of adverse pregnancy outcomes. *Scientific Reports 2025 15:1*, *15*(1), 16846-.

https://doi.org/10.1038/s41598-025-02113-9

21. Nowak, M., Kalwa, M., Oleksy, P., Marszalek, K., Radon-Pokracka, M., & Huras, H. (2019). The relationship between pre-pregnancy BMI, gestational weight gain and neonatal birth weight: a retrospective cohort study. *Ginekologia Polska*, *90*(1), 50–54. https://doi.org/10.5603/GP.2019.0008

22. Heslehurst, N., Vieira, R., Akhter, Z., Bailey, H., Slack, E., Ngongalah, L., Pemu, A., & Rankin, J. (2019). The association between maternal body mass index and child obesity: A systematic review and meta-analysis. *PLoS Medicine*, *16*(6). https://doi.org/10.1371/JOURNAL.PMED.1002817

23. Frontier in Nutrition. (n.d.). *Frontiers | Maternal Metabolic Health: From Preconception to Postpartum*. Retrieved January 13, 2026, from https://www.frontiersin.org/research-topics/66195/maternal-metabolic-health-from-preconception-to-postpartum/articles

24. Deng, Y., Tam, C. H. T., Yang, A., Shi, M., Yuen, L. Y., Ng, N. Y. H., Tsang, A. Y. T., Tsoi, K. Y., Ozaki, R., Li, A. M., Chow, E., Hui, L. L., Chan, J. C. N., Wang, C. C., Tam, W. H., & Ma, R. C. W. (2025). Association of maternal overweight and gestational diabetes mellitus with offspring adiposity trajectory: from birth to early adolescence. *Diabetologia 2025 68:10*, *68*(10), 2194–2204. https://doi.org/10.1007/S00125-025-06468-6

25. Matvienko-Sikar, K., Cooney, J., Flannery, C., Murphy, J., Khashan, A., & Huizink, A. (2021). Maternal stress in the first 1000 days and risk of childhood obesity: a systematic review. *Journal of Reproductive and Infant Psychology*, *39*(2), 180–204. https://doi.org/10.1080/02646838.2020.1724917

26. Voerman, E., Santos, S., Golab, B. P., Amiano, P., Ballester, F., Barros, H., Bergström, A., Charles, M. A., Chatzi, L., Chevrier, C., Chrousos, G. P., Corpeleijn, E., Costet, N., Crozier, S., Devereux, G., Eggesbø, M., Ekström, S., Fantini, M. P., Farchi, S., … Jaddoe, V. W. V. (2019). Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Medicine*, *16*(2), e1002744. https://doi.org/10.1371/JOURNAL.PMED.1002744

27. Maccarini, B., Loperfido, F., Bianco, I., Sottotetti, F., El Masri, D., Ferrara, C., Verme, F., Cangelosi, E., Meriggi, N., De Filippo, C., Cena, H., & De Giuseppe, R. (2025). Offspring's exposome: a narrative review on the influence of early-life factors on childhood obesity risk. *Frontiers in Nutrition*, *12*, 1597746. https://doi.org/10.3389/FNUT.2025.1597746/FULL

28. Pérez-Muñoz, C., Carretero-Bravo, J., Ortega-Martín, E., Ramos-Fiol, B., Ferriz-Mas, B., & Díaz-Rodríguez, M. (2022). Interventions in the first 1000 days to prevent childhood obesity: a systematic review and quantitative content analysis. *BMC Public Health*, *22*(1). https://doi.org/10.1186/S12889-022-14701-9

29. Hao, Y., Wang, N., Hong, S., Liu, Y., Lin, G., Xu, X., Zhou, Y., Wen, X., Sun, B., Wang, H., Huang, M., Wang, J., Chen, Y., & Jiang, Q. (2025). Relationship between serum

mineral levels in the second and third trimester of pregnancy and the risk of gestational diabetes mellitus: a retrospective cohort study. *Frontiers in Nutrition*, *12*, 1634419. https://doi.org/10.3389/FNUT.2025.1634419/BIBTEX

30. Wu, Y., Xiao, H., Chen, L., Qin, J., & Wang, T. (2025). Pre-pregnancy body mass index and risk of macrosomia: glycemic status-specific thresholds and subgroup interactions in a prospective cohort. *Frontiers in Nutrition*, *12*, 1633088. https://doi.org/10.3389/FNUT.2025.1633088/BIBTEX

31. Kardelen, A. D., & Darendeliler, F. (2025). The Role of the Intrauterine Environment in Shaping Childhood and Adolescence Metabolic Outcomes. *Metabolites*, *15*(4), 252. https://doi.org/10.3390/METABO15040252

32. Stephenson, J., Heslehurst, N., Hall, J., Schoenaker, D. A. J. M., Hutchinson, J., Cade, J. E., Poston, L., Barrett, G., Crozier, S. R., Barker, M., Kumaran, K., Yajnik, C. S., Baird, J., & Mishra, G. D. (2018). Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. *The Lancet*, *391*(10132), 1830–1841. https://doi.org/10.1016/S0140-6736(18)30311-8

33. the Lancet. (n.d.). *The Lancet: Diet and lifestyle of both parents before conception play a key role in health of offspring | EurekAlert!* Retrieved January 13, 2026, from https://www.eurekalert.org/news-releases/846749

34. UNICEF. (n.d.). *Maternal nutrition | UNICEF*. Retrieved January 13, 2026, from https://www.unicef.org/nutrition/maternal

35. Zaragoza-Martí, A., Ruiz-Ródenas, N., Herranz-Chofre, I., Sánchez-SanSegundo, M., Serrano Delgado, V. de la C., & Hurtado-Sánchez, J. A. (2022). *Adherence to the Mediterranean Diet in Pregnancy and Its Benefits on Maternal-Fetal Health: A Systematic Review of the Literature*. *9*, 813942. https://pubmed.ncbi.nlm.nih.gov/35433794/

36. Hill, D. J., & Hill, T. G. (2024). Maternal diet during pregnancy and adaptive changes in the maternal and fetal pancreas have implications for future metabolic health. *Frontiers in Endocrinology*, *15*, 1456629. https://doi.org/10.3389/FENDO.2024.1456629/FULL

37. Chia, A. R., Chen, L. W., Lai, J. S., Wong, C. H., Neelakantan, N., Van Dam, R. M., & Chong, M. F. F. (2019). Maternal Dietary Patterns and Birth Outcomes: A Systematic Review and Meta-Analysis. *Advances in Nutrition*, *10*(4), 685. https://doi.org/10.1093/ADVANCES/NMY123

38. Gurumurthy, G., & K. Agrawal, D. (2025). Impact of Maternal Ultra-Processed Food Consumption and Preterm Birth on the Development of Metabolic Disorders in Offspring. *Journal of Pediatrics, Perinatology and Child Health*, *9*(2). https://doi.org/10.26502/JPPCH.74050214

39. Sharma, S., Esposito, S., Di Castelnuovo, A., Gialluisi, A., De Domenico, P., de Gaetano, G., Bonaccio, M., & Iacoviello, L. (2025). Impact of Mediterranean Diet Adherence During Pregnancy on Preeclampsia, Gestational Diabetes Mellitus, and Excessive

Gestational Weight Gain: A Systematic Review of Observational Studies and Randomized Controlled Trials. *Nutrients* , *17*(10), 1723. https://doi.org/10.3390/NU17101723/S1

40. Márquez Ibarra, A. A., Barrera Hernández, L. F., Gregorio, E. V., Olivas Aguirre, F. J., & Teresa de Jesús Quintero Vargas, J. (2025). Microbiota during pregnancy, childbirth, and postpartum, and its relationship with health and disease states. *Medicine in Microecology*, *25*, 100141. https://doi.org/10.1016/J.MEDMIC.2025.100141

41. Davenport, M. H., Ruchat, S. M., Poitras, V. J., Jaramillo Garcia, A., Gray, C. E., Barrowman, N., Skow, R. J., Meah, V. L., Riske, L., Sobierajski, F., James, M., Kathol, A. J., Nuspl, M., Marchand, A. A., Nagpal, T. S., Slater, L. G., Weeks, A., Adamo, K. B., Davies, G. A., … Mottola, M. F. (2018). Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *British Journal of Sports Medicine*, *52*(21), 1367–1375. https://doi.org/10.1136/BJSPORTS-2018-099355

42. Ruchat, S. M., Mottola, M. F., Skow, R. J., Nagpal, T. S., Meah, V. L., James, M., Riske, L., Sobierajski, F., Kathol, A. J., Marchand, A. A., Nuspl, M., Weeks, A., Gray, C. E., Poitras, V. J., Jaramillo Garcia, A., Barrowman, N., Slater, L. G., Adamo, K. B., Davies, G. A., … Davenport, M. H. (2018). Effectiveness of exercise interventions in the prevention of excessive gestational weight gain and postpartum weight retention: a systematic review and meta-analysis. *British Journal of Sports Medicine*, *52*(21), 1347–1356. https://doi.org/10.1136/BJSPORTS-2018-099399

43. Worska, A., Maciaszek, J., Ciążyńska, J., & Szumilewicz, A. (2025). Contradictions and convergences in recommendations on physical activity in pregnancy in different countries after the publication of the WHO guidelines in 2020—a scoping review. *Frontiers in Public Health*, *13*, 1540355. https://doi.org/10.3389/FPUBH.2025.1540355/BIBTEX

44. Xie, W., Zhang, L., Cheng, J., Wang, Y., Kang, H., & Gao, Y. (2024). Physical activity during pregnancy and the risk of gestational diabetes mellitus: a systematic review and dose–response meta-analysis. *BMC Public Health 2024 24:1*, *24*(1), 594-. https://doi.org/10.1186/S12889-024-18131-7

45. Phillips, J. K., Skelly, J. M., King, S. E., Bernstein, I. M., & Higgins, S. T. (2017). Associations of Maternal Obesity and Smoking Status with Perinatal Outcomes. *The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *31*(12), 1620. https://doi.org/10.1080/14767058.2017.1322950

46. Zhou, M., Peng, X., Yi, H., Tang, S., & You, H. (2022). Determinants of excessive gestational weight gain: a systematic review and meta-analysis. *Archives of Public Health = Archives Belges de Sante Publique*, *80*(1). https://doi.org/10.1186/S13690-022-00864-9

47. Dejong, K., Olyaei, A., & Lo, J. O. (2019). Alcohol Use in Pregnancy. *Clinical Obstetrics*

and Gynecology, *62*(1), 142. https://doi.org/10.1097/GRF.0000000000000414

48. Harskamp-Van Ginkel, M. W., Ierodiakonou, D., Margetaki, K., Vafeiadi, M., Karachaliou, M., Kogevinas, M., Vrijkotte, T. G. M., & Chatzi, L. (2020). Gestational sleep deprivation is associated with higher offspring body mass index and blood pressure. *Sleep*, *43*(12), zsaa110. https://doi.org/10.1093/SLEEP/ZSAA110

49. Wart, R. (2023). Issue 3 1 Opinion Citation: Robby W. Exploring the impact of maternal stress on pregnancy outcomes. *J Preg Neonatal Med*, *7*(3), 144. https://doi.org/10.35841/aapnm

50. Lim, S. X., Lai, J. S., Chen, L. W., & Chia, A. (2023). Editorial: Maternal dietary and lifestyle patterns with pregnancy, birth, and child health outcomes. *Frontiers in Nutrition*, *10*, 1266598. https://doi.org/10.3389/FNUT.2023.1266598/BIBTEX

51. Schnurr, T. M., Ängquist, L., Nøhr, E. A., Hansen, T., Sørensen, T. I. A., & Morgen, C. S. (2022). Smoking during pregnancy is associated with child overweight independent of maternal pre-pregnancy BMI and genetic predisposition to adiposity. *Scientific Reports 2022 12:1*, *12*(1), 3135-. https://doi.org/10.1038/s41598-022-07122-6

52. Derme, M., Briante, M., Ceccanti, M., Giannini, G., Vitali, M., Messina, M. P., Piccioni, M. G., Mattia, A., Nicotera, S., & Crognale, A. (2024). Prenatal Alcohol Exposure and Metabolic Disorders in Pediatrics: The Role of the Oxidative Stress—A Review of the Literature. *Children 2024, Vol. 11, Page 269*, *11*(3), 269. https://doi.org/10.3390/CHILDREN11030269

53. Lamichhane, N., Olsen, N. J., Mortensen, E. L., Obel, C., Heitmann, B. L., & Händel, M. N. (2020). Associations between maternal stress during pregnancy and offspring obesity risk later in life-A systematic literature review. *Obesity Reviews : An Official Journal of the International Association for the Study of Obesity*, *21*(2). https://doi.org/10.1111/OBR.12951

54. Grech, A., Collins, C. E., Holmes, A., Lal, R., Duncanson, K., Taylor, R., & Gordon, A. (2021). Maternal exposures and the infant gut microbiome: a systematic review with meta-analysis. *Gut Microbes*, *13*(1), 1897210. https://doi.org/10.1080/19490976.2021.1897210

55. Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines; Rasmussen KM, Yaktine AL, editors. W. (DC): N. A. P. (US); (2009). *Weight Gain During Pregnancy: Reexamining the Guidelines - Chapter 4: Determinants of Gestational Weight Gain - Weight Gain During Pregnancy - NCBI Bookshelf*. https://www.ncbi.nlm.nih.gov/books/NBK32811/

56. Rasmussen, K. M., & Yaktine, A. L. (2009). Committee to Reexamine IOM Pregnancy Weight Guidelines. *Food and Nutrition Board, Board on Children, Youth and Families, Institute of Medicine, National Research Council: Weight Gain During Pregnancy: Reexamining the Guidelines*, 71–110. https://europepmc.org/books/n/nap12584/nap12584.s2/

57. Goldstein, R. F., Abell, S. K., Ranasinha, S., Misso, M., Boyle, J. A., Black, M. H., Li, N.,

Hu, G., Corrado, F., Rode, L., Kim, Y. J., Haugen, M., Song, W. O., Kim, M. H., Bogaerts, A., Devlieger, R., Chung, J. H., & Teede, H. J. (2017). Association of Gestational Weight Gain With Maternal and Infant Outcomes: A Systematic Review and Meta-analysis. *JAMA*, *317*(21), 2207–2225. https://doi.org/10.1001/JAMA.2017.3635

58. Li, Y., Yuan, J., Lan, K., Li, Y., Li, X., & Zhao, B. (2025). Association between body composition and weight retention from early pregnancy to 42 days postpartum: a retrospective cohort study. *BMJ Open*, *15*(5). https://doi.org/10.1136/BMJOPEN-2024-092439

59. KM, R., & AL, Y. (2009). *Weight Gain During Pregnancy: Reexamining the Guidelines-Background*. https://doi.org/10.17226/12584

60. Suliga, E., Rokita, W., Adamczyk-Gruszka, O., Pazera, G., Cieśla, E., & Głuszek, S. (2018). Factors associated with gestational weight gain: A cross-sectional survey. *BMC Pregnancy and Childbirth*, *18*(1), 1–11. https://doi.org/10.1186/S12884-018-2112-7/TABLES/4

61. Lacagnina, S. (2019). The Developmental Origins of Health and Disease (DOHaD). *American Journal of Lifestyle Medicine*, *14*(1), 47. https://doi.org/10.1177/1559827619879694

62. Barker, D. J. P. (2004). The developmental origins of adult disease. *Journal of the American College of Nutrition*, *23*(6 Suppl), 588S-595S. https://doi.org/10.1080/07315724.2004.10719428

63. Gkintoni, E., Papachatzi, E., Efthymiadou, E., Magriplis, E., & Vantarakis, A. (2025). Neurodevelopmental Pathways from Maternal Obesity to Offspring Outcomes: An Umbrella Review of Cognitive and Behavioral Consequences Across Development. *Healthcare (Switzerland)*, *13*(20), 2653. https://doi.org/10.3390/HEALTHCARE13202653/S1

64. Hanson, M. A., & Gluckman, P. D. (2014). Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiological Reviews*, *94*(4), 1027–1076. https://doi.org/10.1152/PHYSREV.00029.2013

65. Li Pomi, A., Pepe, G., Aversa, T., Corica, D., Valenzise, M., Francesca Messina, M., Anna Morabito, L., Stagi, S., & Wasniewska, M. (n.d.). *Early adiposity rebound: predictors and outcomes*. https://doi.org/10.1186/s13052-024-01671-4

66. De Giuseppe, R., Loperfido, F., Cerbo, R. M., Monti, M. C., Civardi, E., Garofoli, F., Angelini, M., Maccarini, B., Sommella, E., Campiglia, P., Bertuzzo, L., Chieppa, M., Ghirardello, S., & Cena, H. (2022). *LIMIT: LIfestyle and Microbiome InTeraction Early Adiposity Rebound in Children, a Study Protocol*. *12*(9), 809. https://doi.org/10.3390/METABO12090809

67. Goławski, K., & Wojtyła, C. (2022). Impact of Physical Activity of Pregnant Women on Obstetric Outcomes. *International Journal of Environmental Research and Public Health*, *19*(19), 12541. https://doi.org/10.3390/IJERPH191912541

68. Calcaterra, V., Cena, H., Pirazzi, A., Sottotetti, F., Cordaro, E., Cavallo, C., Milanta, C., El Masri, D., Conti, M. V., Vandoni, M., & Zuccotti, G. (2025). From Pregnancy to Breastfeeding: The Role of Maternal Exercise in Preventing Childhood Obesity. *Nutrients 2025, Vol. 17, Page 660*, *17*(4), 660. https://doi.org/10.3390/NU17040660

69. Yao, Y., Cai, X., Ye, Y., Wang, F., Chen, F., & Zheng, C. (2021). The Role of Microbiota in Infant Health: From Early Life to Adulthood. *Frontiers in Immunology*, *12*, 708472. www.frontiersin.org

70. Stiemsma, L. T., & Michels, K. B. (2018). The Role of the Microbiome in the Developmental Origins of Health and Disease. *Pediatrics*, *141*(4). https://doi.org/10.1542/PEDS.2017-2437

71. Wang, W., Gu, W., Schweitzer, R., Koren, O., Khatib, S., Tseng, G., & Konnikova, L. (2025). In utero human intestine contains maternally derived bacterial metabolites. *Microbiome*, *13*(1). https://doi.org/10.1186/S40168-025-02110-0

72. Indrio, F., Martini, S., Francavilla, R., Corvaglia, L., Cristofori, F., Mastrolia, S. A., Neu, J., Rautava, S., Spena, G. R., Raimondi, F., & Loverro, G. (2017). Epigenetic matters: The link between early nutrition, microbiome, and long-term health development. *Frontiers in Pediatrics*, *5*, 286554. https://doi.org/10.3389/FPED.2017.00178/FULL

73. Sasaki, T., Kawamura, M., Okuno, C., Lau, K., Riel, J., Lee, M. J., & Miller, C. (2024). Impact of Maternal Mediterranean-Type Diet Adherence on Microbiota Composition and Epigenetic Programming of Offspring. *Nutrients*, *16*(1), 47. https://doi.org/10.3390/NU16010047/S1

74. O'Neill, I. J., Sanchez Gallardo, R., Saldova, R., Murphy, E. F., Cotter, P. D., McAuliffe, F. M., & van Sinderen, D. (2020). Maternal and infant factors that shape neonatal gut colonization by bacteria. *Expert Review of Gastroenterology & Hepatology*, *14*(8), 651–664. https://doi.org/10.1080/17474124.2020.1784725

75. Cömert, T. K., Akpinar, F., Erkaya, S., Durmaz, B., & Durmaz, R. (2022). The effect of pre-pregnancy obesity on gut and meconium microbiome and relationship with fetal growth. *Journal of Maternal-Fetal and Neonatal Medicine*, *35*(26), 10629–10637. https://doi.org/10.1080/14767058.2022.2148098

76. Senn, V., Bassler, D., Choudhury, R., Scholkmann, F., Righini-Grunder, F., Vuille-dit-Bile, R. N., & Restin, T. (2020). Microbial Colonization From the Fetus to Early Childhood—A Comprehensive Review. *Frontiers in Cellular and Infection Microbiology*, *10*, 573735. https://doi.org/10.3389/FCIMB.2020.573735/PDF

77. Bekhti, N., Castelli, F., Paris, A., Guillon, B., Junot, C., Moiron, C., Fenaille, F., & Adel-Patient, K. (2022). The Human Meconium Metabolome and Its Evolution during the First Days of Life. *Metabolites*, *12*(5), 414. https://doi.org/10.3390/METABO12050414/S1

78. Liu, C., Zheng, W., Wang, J., Yuan, X., Zhang, Y., Wang, Y., Ma, X., & Li, G. (2025). Multiple perinatal characteristics affect the association between maternal diabetes status and early neonatal gut microbiota. *MSphere*, *10*(6).

https://doi.org/10.1128/MSPHERE.00914-24;PAGEGROUP:STRING:PUBLICATION

79. Bianco, I., Ferrara, C., Romano, F., Loperfido, F., Sottotetti, F., El Masri, D., Vincenti, A., Cena, H., & De Giuseppe, R. (2024). The Influence of Maternal Lifestyle Factors on Human Breast Milk Microbial Composition: A Narrative Review. *Biomedicines*, *12*(11). https://doi.org/10.3390/BIOMEDICINES12112423

80. Loperfido, F., Sottotetti, F., Bianco, I., El Masri, D., Maccarini, B., Ferrara, C., Limitone, A., Cena, H., & De Giuseppe, R. (2025). REVIEW Open Access Folic acid supplementation in European women of reproductive age and during pregnancy with excessive weight: a systematic review. *Reproductive Health*, *22*, 13. https://doi.org/10.1186/s12978-025-01953-y

81. El Masri, D., Alemayohu, M. A., Loperfido, F., Bianco, I., Ferrara, C., Cerbo, R. M., Ghirardello, S., Monti, M. C., Maccarini, B., Sottotetti, F., Civardi, E., Garofoli, F., Angelini, M., Cena, H., & De Giuseppe, R. (2024). Associations of maternal lifestyle factors with inadequate pregnancy weight gain: findings from the baseline data of the LIMIT prospective cohort study. *European Journal of Nutrition*, *63*(8), 2911. https://doi.org/10.1007/S00394-024-03473-0

82. Selma-Royo, M., Calvo Lerma, J., Cortés-Macías, E., & Collado, M. C. (2021). Human milk microbiome: From actual knowledge to future perspective. *Seminars in Perinatology*, *45*(6), 151450. https://doi.org/10.1016/J.SEMPERI.2021.151450

83. Kumari, P., Raval, A., Rana, P., & Mahto, S. K. (2023). Regenerative Potential of Human Breast Milk: A Natural Reservoir of Nutrients, Bioactive Components and Stem cells. *Stem Cell Reviews and Reports*, *19*(5), 1307–1327. https://doi.org/10.1007/S12015-023-10534-0

84. Yi, D. Y., & Kim, S. Y. (2021). Human Breast Milk Composition and Function in Human Health: From Nutritional Components to Microbiome and MicroRNAs. *Nutrients*, *13*(9). https://doi.org/10.3390/NU13093094

85. Doare, K. Le, Holder, B., Bassett, A., & Pannaraj, P. S. (2018). Mother's Milk: A Purposeful Contribution to the Development of the Infant Microbiota and Immunity. *Frontiers in Immunology*, *9*(FEB). https://doi.org/10.3389/FIMMU.2018.00361

86. Mackie, R. I., Sghir, A., & Gaskins, H. R. (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. *The American Journal of Clinical Nutrition*, *69*(5), 1035S-1045S. https://doi.org/10.1093/AJCN/69.5.1035S

87. Notarbartolo, V., Giuffre, M., Montante, C., Corsello, G., & Carta, M. (2022). Composition of Human Breast Milk Microbiota and Its Role in Children's Health. *Pediatric Gastroenterology, Hepatology & Nutrition*, *25*(3), 194–210. https://doi.org/10.5223/PGHN.2022.25.3.194

88. Lopez Leyva, L., Brereton, N. J. B., & Koski, K. G. (2021). Emerging frontiers in human milk microbiome research and suggested primers for 16S rRNA gene analysis. *Computational and Structural Biotechnology Journal*, *19*, 121–133.

https://doi.org/10.1016/J.CSBJ.2020.11.057

89. Singh, P., Al Mohannadi, N., Murugesan, S., Almarzooqi, F., Kabeer, B. S. A., Marr, A. K., Kino, T., Brummaier, T., Terranegra, A., McGready, R., Nosten, F., Chaussabel, D., & Al Khodor, S. (2023). Unveiling the dynamics of the breast milk microbiome: impact of lactation stage and gestational age. *Journal of Translational Medicine*, *21*(1). https://doi.org/10.1186/S12967-023-04656-9

90. Groer, M. W., Morgan, K. H., Louis-Jacques, A., & Miller, E. M. (2020). A Scoping Review of Research on the Human Milk Microbiome. *Journal of Human Lactation*, *36*(4), 628–643. https://doi.org/10.1177/0890334420942768;WEBSITE:WEBSITE:SAGE;JOURNAL:JOURNAL:JHLA;REQUESTEDJOURNAL:JOURNAL:JHLA;ISSUE:ISSUE:DOI

91. Biagi, E., Quercia, S., Aceti, A., Beghetti, I., Rampelli, S., Turroni, S., Faldella, G., Candela, M., Brigidi, P., & Corvaglia, L. (2017). The Bacterial Ecosystem of Mother's Milk and Infant's Mouth and Gut. *Frontiers in Microbiology*, *8*(JUN). https://doi.org/10.3389/FMICB.2017.01214

92. Selvamani, S., Dailin, D. J., Gupta, V. K., Wahid, M., Keat, H. C., Natasya, K. H., Malek, R. A., Haque, S., Sayyed, R. Z., Abomoelak, B., Sukmawati, D., Varzakas, T., & Enshasy, H. A. El. (2021). An Insight into Probiotics Bio-Route: Translocation from the Mother's Gut to the Mammary Gland. *Applied Sciences 2021, Vol. 11, Page 7247*, *11*(16), 7247. https://doi.org/10.3390/APP11167247

93. Vélez-Ixta, J. M., Juárez-Castelán, C. J., Ramírez-Sánchez, D., Lázaro-Pérez, N. del S., Castro-Arellano, J. J., Romero-Maldonado, S., Rico-Arzate, E., Hoyo-Vadillo, C., Salgado-Mancilla, M., Gómez-Cruz, C. Y., Krishnakumar, A., Piña-Escobedo, A., Benitez-Guerrero, T., Pizano-Zárate, M. L., Cruz-Narváez, Y., & García-Mena, J. (2024). Post Natal Microbial and Metabolite Transmission: The Path from Mother to Infant. *Nutrients*, *16*(13), 1990. https://doi.org/10.3390/NU16131990/S1

94. Juárez-Castelán, C. J., Vélez-Ixta, J. M., Corona-Cervantes, K., Piña-Escobedo, A., Cruz-Narváez, Y., Hinojosa-Velasco, A., Landero-Montes-de-Oca, M. E., Davila-Gonzalez, E., González-del-Olmo, E., Bastida-Gonzalez, F., Zárate-Segura, P. B., & García-Mena, J. (2022). The Entero-Mammary Pathway and Perinatal Transmission of Gut Microbiota and SARS-CoV-2. *International Journal of Molecular Sciences*, *23*(18). https://doi.org/10.3390/IJMS231810306

95. Fehr, K., Moossavi, S., Sbihi, H., Boutin, R. C. T., Bode, L., Robertson, B., Yonemitsu, C., Field, C. J., Becker, A. B., Mandhane, P. J., Sears, M. R., Khafipour, E., Moraes, T. J., Subbarao, P., Finlay, B. B., Turvey, S. E., & Azad, M. B. (2020). Breastmilk Feeding Practices Are Associated with the Co-Occurrence of Bacteria in Mothers' Milk and the Infant Gut: the CHILD Cohort Study. *Cell Host and Microbe*, *28*(2), 285-297.e4. https://doi.org/10.1016/j.chom.2020.06.009

96. Moossavi, S., Sepehri, S., Robertson, B., Bode, L., Goruk, S., Field, C. J., Lix, L. M., de Souza, R. J., Becker, A. B., Mandhane, P. J., Turvey, S. E., Subbarao, P., Moraes, T. J.,

Lefebvre, D. L., Sears, M. R., Khafipour, E., & Azad, M. B. (2019). Composition and Variation of the Human Milk Microbiota Are Influenced by Maternal and Early-Life Factors. *Cell Host and Microbe*, *25*(2), 324-335.e4. https://doi.org/10.1016/j.chom.2019.01.011

97. Gopalakrishna, K. P., & Hand, T. W. (2020). Influence of Maternal Milk on the Neonatal Intestinal Microbiome. *Nutrients*, *12*(3). https://doi.org/10.3390/NU12030823

98. Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., Ross, M. C., Lloyd, R. E., Doddapaneni, H. V., Metcalf, G. A., Muzny, D., Gibbs, R. A., Vatanen, T., Huttenhower, C., Xavier, R. J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A. G., … Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, *562*(7728), 583–588. https://doi.org/10.1038/S41586-018-0617-X

99. Aya, V., Flórez, A., Perez, L., & Ramírez, J. D. (2021). Association between physical activity and changes in intestinal microbiota composition: A systematic review. *PloS One*, *16*(2). https://doi.org/10.1371/JOURNAL.PONE.0247039

100. Cataldi, S., Bonavolontà, V., Poli, L., Clemente, F. M., De Candia, M., Carvutto, R., Silva, A. F., Badicu, G., Greco, G., & Fischetti, F. (2022). The Relationship between Physical Activity, Physical Exercise, and Human Gut Microbiota in Healthy and Unhealthy Subjects: A Systematic Review. *Biology*, *11*(3). https://doi.org/10.3390/BIOLOGY11030479

101. Dorelli, B., Gallè, F., De Vito, C., Duranti, G., Iachini, M., Zaccarin, M., Preziosi Standoli, J., Ceci, R., Romano, F., Liguori, G., Romano Spica, V., Sabatini, S., Valeriani, F., & Cattaruzza, M. S. (2021). Can Physical Activity Influence Human Gut Microbiota Composition Independently of Diet? A Systematic Review. *Nutrients*, *13*(6). https://doi.org/10.3390/NU13061890

102. Bressa, C., Bailén-Andrino, M., Pérez-Santiago, J., González-Soltero, R., Pérez, M., Montalvo-Lominchar, M. G., Maté-Muñoz, J. L., Domínguez, R., Moreno, D., & Larrosa, M. (2017). Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PloS One*, *12*(2). https://doi.org/10.1371/JOURNAL.PONE.0171352

103. Sun, J., Fang, D., Wang, Z., & Liu, Y. (2023). Sleep Deprivation and Gut Microbiota Dysbiosis: Current Understandings and Implications. *International Journal of Molecular Sciences*, *24*(11). https://doi.org/10.3390/IJMS24119603

104. Marsh, A. J., Azcarate-Peril, M. A., Aljumaah, M. R., Neville, J., Perrin, M. T., Dean, L. L., Wheeler, M. D., Hines, I. N., & Pawlak, R. (2022). Fatty acid profile driven by maternal diet is associated with the composition of human milk microbiota. *Frontiers in Microbiomes*, *1*, 1041752. https://doi.org/10.3389/FRMBI.2022.1041752/BIBTEX

105. Cortes-Macías, E., Selma-Royo, M., García-Mantrana, I., Calatayud, M., González, S., Martínez-Costa, C., & Collado, M. C. (2021). Maternal Diet Shapes the Breast Milk Microbiota Composition and Diversity: Impact of Mode of Delivery and Antibiotic

Exposure. *Journal of Nutrition*, *151*(2), 330–340. https://doi.org/10.1093/jn/nxaa310

106. Padilha, M., Danneskiold-Samsøe, N. B., Brejnrod, A., Hoffmann, C., Cabral, V. P., Iaucci, J. de M., Sales, C. H., Fisberg, R. M., Cortez, R. V., Brix, S., Taddei, C. R., Kristiansen, K., & Saad, S. M. I. (2019). The Human Milk Microbiota is Modulated by Maternal Diet. *Microorganisms*, *7*(11). https://doi.org/10.3390/MICROORGANISMS7110502

107. Londoño-Sierra, D. C., Mesa, V., Guzmán, N. C., Bolívar Parra, L., Montoya-Campuzano, O. I., & Restrepo-Mesa, S. L. (2023). Maternal Diet May Modulate Breast Milk Microbiota-A Case Study in a Group of Colombian Women. *Microorganisms*, *11*(7). https://doi.org/10.3390/MICROORGANISMS11071812

108. Shenker, N. S., Perdones-Montero, A., Burke, A., Stickland, S., McDonald, J. A. K., Alexander-Hardiman, K., Flanagan, J., Takats, Z., & Cameron, S. J. S. (2020). Metabolomic and Metataxonomic Fingerprinting of Human Milk Suggests Compositional Stability over a Natural Term of Breastfeeding to 24 Months. *Nutrients*, *12*(11), 1–19. https://doi.org/10.3390/NU12113450

109. Browne, P. D., Aparicio, M., Alba, C., Hechler, C., Beijers, R., Rodríguez, J. M., Fernández, L., & de Weerth, C. (2019). Human Milk Microbiome and Maternal Postnatal Psychosocial Distress. *Frontiers in Microbiology*, *10*(OCT). https://doi.org/10.3389/FMICB.2019.02333

110. Nuti, F., Fernández, F. R., Severi, M., Traversi, R., Fanos, V., Street, M. E., Palanza, P., Rovero, P., & Papini, A. M. (2024). Study of Endocrine-Disrupting Chemicals in Infant Formulas and Baby Bottles: Data from the European LIFE-MILCH PROJECT. *Molecules*, *29*(22), 5434. https://doi.org/10.3390/MOLECULES29225434/S1

111. Fernández-Tuñas, M. del C., Pérez-Muñuzuri, A., Trastoy-Pena, R., Pérez del Molino, M. L., & Couce, M. L. (2023). Effects of Maternal Stress on Breast Milk Production and the Microbiota of Very Premature Infants. *Nutrients*, *15*(18). https://doi.org/10.3390/NU15184006

112. Olga, L., van Diepen, J. A., Chichlowski, M., Petry, C. J., Vervoort, J., Dunger, D. B., Kortman, G. A. M., Gross, G., & Ong, K. K. (2023). Butyrate in Human Milk: Associations with Milk Microbiota, Milk Intake Volume, and Infant Growth. *Nutrients*, *15*(4). https://doi.org/10.3390/NU15040916

113. Salih, M. A., Murshid, W. R., & Seidahmed, M. Z. (2014). Epidemiology, prenatal management, and prevention of neural tube defects. *Saudi Medical Journal*, *35*(Suppl 1), S15. /pmc/articles/PMC4362104/

114. European Commission. (n.d.). *Folic Acid and Neural Tube Defects | EU RD Platform*. Retrieved October 16, 2024, from https://eu-rd-platform.jrc.ec.europa.eu/eurocat/prevention-and-risk-factors/folic-acid-neural-tube-defects_en

115. World Health Organization (WHO). (2023). *Periconceptional folic acid supplementation*

to prevent neural tube defects. https://www.who.int/tools/elena/interventions/folate-periconceptional

116. Food and Nutrition - CREA. (n.d.). *Linee guida per una sana alimentazione 2018 - Linee guida per una sana alimentazione 2018 - Alimenti e Nutrizione - CREA*. Retrieved October 16, 2024, from https://www.crea.gov.it/web/alimenti-e-nutrizione/-/linee-guida-per-una-sana-alimentazione-2018

117. Dunlap, B., Shelke, K., Salem, S. A., & Keith, L. G. (2011). Folic acid and human reproduction—ten important issues for clinicians. *Journal of Experimental & Clinical Assisted Reproduction*, *8*. /pmc/articles/PMC3183498/

118. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Systematic Reviews*, *10*(1). https://doi.org/10.1186/S13643-021-01626-4

119. Mohd-Shukri, N. A., Duncan, A., Denison, F. C., Forbes, S., Walker, B. R., Norman, J. E., & Reynolds, R. M. (2015). Health Behaviours during Pregnancy in Women with Very Severe Obesity. *Nutrients 2015, Vol. 7, Pages 8431-8443*, *7*(10), 8431–8443. https://doi.org/10.3390/NU7105403

120. Linnell, A., Murphy, N., Godwin, J., & Cremona, A. (2022). An evaluation of adherence to folic acid supplementation in pregnant women during early gestation for the prevention of neural tube defects. *Public Health Nutrition*, *25*(11), 3025–3035. https://doi.org/10.1017/S1368980022001574

121. Kancherla, V. (2023). *Neural tube defects: a review of global prevalence, causes, and primary prevention*. *39*(7), 1703–1710. https://pubmed.ncbi.nlm.nih.gov/36882610/

122. Redfern, K. M., Hollands, H. J., Welch, C. R., Pinkney, J. H., & Rees, G. A. (2022). Dietary Intakes of Folate, Vitamin D and Iodine during the First Trimester of Pregnancy and the Association between Supplement Use and Demographic Characteristics amongst White Caucasian Women Living with Obesity in the UK. *Nutrients*, *14*(23). https://doi.org/10.3390/NU14235135

123. Cawley, S., Mullaney, L., McKeating, A., Farren, M., McCartney, D., & Turner, M. J. (2016). An analysis of folic acid supplementation in women presenting for antenatal care. *Journal of Public Health (Oxford, England)*, *38*(1), 122–129. https://doi.org/10.1093/PUBMED/FDV019

124. A. Malvasi, F. Casciaro, M.M. Minervini, I. Kosmas, O.A. Mynbaev, E. Pacella, V. Monti Condesnitt, A. Creanza, G.C. Di Renzo, A. T. (2014). *Myo-inositol, D-chiro-inositol, folic acid and manganese in second trimester of pregnancy: a preliminary investigation*. https://www.europeanreview.org/article/6605

125. 2025, istituto superiore di sanita. (n.d.). *Obesità news*. Retrieved January 14, 2026, from

https://www.epicentro.iss.it/obesita/aggiornamenti

126. Bendor, C. D., Bardugo, A., Pinhas-Hamiel, O., Afek, A., & Twig, G. (2020). Cardiovascular morbidity, diabetes and cancer risk among children and adolescents with severe obesity. *Cardiovascular Diabetology*, *19*(1). https://doi.org/10.1186/S12933-020-01052-1

127. Horesh, A., Tsur, A. M., Bardugo, A., & Twig, G. (2021). Adolescent and Childhood Obesity and Excess Morbidity and Mortality in Young Adulthood-a Systematic Review. *Current Obesity Reports*, *10*(3), 301–310. https://doi.org/10.1007/S13679-021-00439-9

128. Wild, C. P. (2005). Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *14*(8), 1847–1850. https://doi.org/10.1158/1055-9965.EPI-05-0456

129. D'Errico, A., Maritano, S., Moccia, C., Isaevska, E., Pizzi, C., Moirano, G., & Popovic, M. (2023). [Exposome: from definition to future challenges]. *Recenti Progressi in Medicina*, *114*(6), 349–354. https://doi.org/10.1701/4042.40227

130. Vrijheid, M. (2014). The exposome: a new paradigm to study the impact of environment on health. *Thorax*, *69*(9), 876–878. https://doi.org/10.1136/THORAXJNL-2013-204949

131. La Merrill, M. A., Vandenberg, L. N., Smith, M. T., Goodson, W., Browne, P., Patisaul, H. B., Guyton, K. Z., Kortenkamp, A., Cogliano, V. J., Woodruff, T. J., Rieswijk, L., Sone, H., Korach, K. S., Gore, A. C., Zeise, L., & Zoeller, R. T. (2020). Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nature Reviews. Endocrinology*, *16*(1), 45–57. https://doi.org/10.1038/S41574-019-0273-8

132. Calcaterra, V., Cena, H., Loperfido, F., Rossi, V., Grazi, R., Quatrale, A., De Giuseppe, R., Manuelli, M., & Zuccotti, G. (2024). Evaluating Phthalates and Bisphenol in Foods: Risks for Precocious Puberty and Early-Onset Obesity. *Nutrients*, *16*(16). https://doi.org/10.3390/NU16162732

133. Dong, Y., Gao, D., Li, Y., Yang, Z., Wang, X., Chen, M., Wang, Z., Song, Y., Zou, Z., & Ma, J. (2022). Effect of childhood phthalates exposure on the risk of overweight and obesity: A nested case-control study in China. *Environment International*, *158*. https://doi.org/10.1016/j.envint.2021.106886

134. Gutiérrez-Torres, D. S., Barraza-Villarreal, A., Hernandez-Cadena, L., Escamilla-Nuñez, C., & Romieu, I. (2018). Prenatal Exposure to Endocrine Disruptors and Cardiometabolic Risk in Preschoolers: A Systematic Review Based on Cohort Studies. *Annals of Global Health*, *84*(2), 239–249. https://doi.org/10.29024/AOGH.911

135. Frangione, B., Birk, S., Benzouak, T., Rodriguez-Villamizar, L. A., Karim, F., Dugandzic, R., & Villeneuve, P. J. (2024). Exposure to perfluoroalkyl and polyfluoroalkyl substances and pediatric obesity: a systematic review and meta-analysis. *International Journal of*

Obesity (2005), *48*(2), 131–146. https://doi.org/10.1038/S41366-023-01401-6

136. Symeonides, C., Aromataris, E., Mulders, Y., Dizon, J., Stern, C., Barker, T. H., Whitehorn, A., Pollock, D., Marin, T., & Dunlop, S. (2024). An Umbrella Review of Meta-Analyses Evaluating Associations between Human Health and Exposure to Major Classes of Plastic-Associated Chemicals. *Annals of Global Health*, *90*(1). https://doi.org/10.5334/AOGH.4459

137. Ribeiro, C. M., Beserra, B. T. S., Silva, N. G., Lima, C. L., Rocha, P. R. S., Coelho, M. S., Neves, F. D. A. R., & Amato, A. A. (2020). Exposure to endocrine-disrupting chemicals and anthropometric measures of obesity: a systematic review and meta-analysis. *BMJ Open*, *10*(6). https://doi.org/10.1136/BMJOPEN-2019-033509

138. Ji, N., Johnson, M., Eckel, S. P., Gauderman, W. J., Chavez, T. A., Berhane, K., Faham, D., Lurmann, F., Pavlovic, N. R., Grubbs, B. H., Lerner, D., Habre, R., Farzan, S. F., Bastain, T. M., & Breton, C. V. (2023). Prenatal ambient air pollution exposure and child weight trajectories from the 3rd trimester of pregnancy to 2 years of age: a cohort study. *BMC Medicine*, *21*(1). https://doi.org/10.1186/S12916-023-03050-Y

139. Zheng, J., Zhang, H., Shi, J., Li, X., Zhang, J., Zhang, K., Gao, Y., He, J., Dai, J., & Wang, J. (2024). Association of air pollution exposure with overweight or obesity in children and adolescents: A systematic review and meta–analysis. *Science of the Total Environment*, *910*. https://doi.org/10.1016/j.scitotenv.2023.168589

140. Kabisch, N., van den Bosch, M., & Lafortezza, R. (2017). The health benefits of nature-based solutions to urbanization challenges for children and the elderly – A systematic review. *Environmental Research*, *159*, 362–373. https://doi.org/10.1016/j.envres.2017.08.004

141. Heo, S., Afanasyeva, Y., Liu, M., Mehta-Lee, S., Yang, W., Trasande, L., Bell, M. L., & Ghassabian, A. (2025). Prenatal exposure to residential greenness, fetal growth, and birth outcomes: a cohort study in New York City. *American Journal of Epidemiology*, *194*(9), 2621–2630. https://doi.org/10.1093/AJE/KWAE436

142. Alejandre, J. C., & Lynch, M. (2020). "Kids Get in Shape with Nature": A Systematic Review Exploring the Impact of Green Spaces on Childhood Obesity. *Journal of Nutritional Science and Vitaminology*, *66*(Supplement), S129–S133. https://doi.org/10.3177/JNSV.66.S129

143. Jia, P., Shi, Y., Jiang, Q., Dai, S., Yu, B., Yang, S., Qiu, G., & Yang, S. (2023). Environmental determinants of childhood obesity: a meta-analysis. *The Lancet. Global Health*, *11*, S7. https://doi.org/10.1016/S2214-109X(23)00092-X

144. Soares, R. M., Antunes Nunes, M. A., Schmidt, M. I., Giacomello, A., Manzolli, P., Camey, S., Buss, C., Drehmer, M., Melere, C., Hoffman, J., Ozcariz, S., Manenti, C. N., Pinheiro, A. P., & Duncan, B. B. (2009). Inappropriate eating behaviors during pregnancy: prevalence and associated factors among pregnant women attending primary care in southern Brazil. *The International Journal of Eating Disorders*, *42*(5), 387–393.

https://doi.org/10.1002/EAT.20643

145. Liu, J., Li, L., Chen, X., Lu, Y., & Wang, D. (2019). Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: a novel idea for monitoring and evaluation of heat stress — A review. *Asian-Australasian Journal of Animal Sciences*, *32*(9), 1332–1339. https://doi.org/10.5713/AJAS.18.0743

146. Part, C., Filippi, V., Cresswell, J. A., Ganaba, R., Hajat, S., Nakstad, B., Roos, N., Kadio, K., Chersich, M., Lusambili, A., Kouanda, S., & Kovats, S. (2022). How do high ambient temperatures affect infant feeding practices? A prospective cohort study of postpartum women in Bobo-Dioulasso, Burkina Faso. *BMJ Open*, *12*(10). https://doi.org/10.1136/BMJOPEN-2022-061297

147. Manser, C. N., Paul, M., Rogler, G., Held, L., & Frei, T. (2013). Heat waves, incidence of infectious gastroenteritis, and relapse rates of inflammatory bowel disease: a retrospective controlled observational study. *The American Journal of Gastroenterology*, *108*(9), 1480–1485. https://doi.org/10.1038/AJG.2013.186

148. Centers for Disease Control and Prevention. (n.d.). *Weight Gain During Pregnancy | Pregnancy | Maternal and Infant Health | CDC*. Retrieved October 30, 2023, from https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pregnancy-weight-gain.htm

149. Deputy, N. P., Sharma, A. J., Kim, S. Y., & Hinkle, S. N. (2015). Prevalence and characteristics associated with gestational weight gain adequacy. *Obstetrics and Gynecology*, *125*(4), 773–781. https://doi.org/10.1097/AOG.0000000000000739

150. Darling, A. M., Wang, D., Perumal, N., Liu, E., Wang, M., Ahmed, T., Christian, P., Dewey, K. G., Kac, G., Kennedy, S. H., Subramoney, V., Briggs, B., & Fawzi, W. W. (2023). Risk factors for inadequate and excessive gestational weight gain in 25 low- and middle-income countries: An individual-level participant meta-analysis. *PLOS Medicine*, *20*(7), e1004236. https://doi.org/10.1371/JOURNAL.PMED.1004236

151. Yu, Z., Han, S., Zhu, J., Sun, X., Ji, C., & Guo, X. (2013). Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: a systematic review and meta-analysis. *PloS One*, *8*(4). https://doi.org/10.1371/JOURNAL.PONE.0061627

152. Heslehurst, N., Simpson, H., Ells, L. J., Rankin, J., Wilkinson, J., Lang, R., Brown, T. J., & Summerbell, C. D. (2008). The impact of maternal BMI status on pregnancy outcomes with immediate short-term obstetric resource implications: a meta-analysis. *Obesity Reviews : An Official Journal of the International Association for the Study of Obesity*, *9*(6), 635–683. https://doi.org/10.1111/J.1467-789X.2008.00511.X

153. Cresswell, J. A., Campbell, O. M. R., De Silva, M. J., & Filippi, V. (2012). Effect of maternal obesity on neonatal death in sub-Saharan Africa: multivariable analysis of 27 national datasets. *Lancet (London, England)*, *380*(9850), 1325–1330. https://doi.org/10.1016/S0140-6736(12)60869-1

154. Harrison, C. L., Bahri Khomami, M., Enticott, J., Thangaratinam, S., Rogozińska, E., &

Teede, H. J. (2023). Key Components of Antenatal Lifestyle Interventions to Optimize Gestational Weight Gain: Secondary Analysis of a Systematic Review. *JAMA Network Open*, *6*(6), E2318031. https://doi.org/10.1001/JAMANETWORKOPEN.2023.18031

155. O'Brien, E. C., Segurado, R., Geraghty, A. A., Alberdi, G., Rogozinska, E., Astrup, A., Barakat Carballo, R., Bogaerts, A., Cecatti, J. G., Coomarasamy, A., De Groot, C. J. M., Devlieger, R., Dodd, J. M., El Beltagy, N., Facchinetti, F., Geiker, N., Guelfi, K., Haakstad, L., Harrison, C., … McAuliffe, F. M. (2019). Impact of maternal education on response to lifestyle interventions to reduce gestational weight gain: individual participant data meta-analysis. *BMJ Open*, *9*(8). https://doi.org/10.1136/BMJOPEN-2018-025620

156. Suzuki, M., Wakayama, R., Yamagata, Z., & Suzuki, K. (2022). Effect of maternal smoking during pregnancy on gestational weight gain and birthweight: A stratified analysis by pregestational weight status. *Tobacco Induced Diseases*, *20*(1 January). https://doi.org/10.18332/TID/143952

157. Teede, H. J., Bailey, C., Moran, L. J., Bahri Khomami, M., Enticott, J., Ranasinha, S., Rogozińska, E., Skouteris, H., Boyle, J. A., Thangaratinam, S., & Harrison, C. L. (2022). Association of Antenatal Diet and Physical Activity-Based Interventions With Gestational Weight Gain and Pregnancy Outcomes: A Systematic Review and Meta-analysis. *JAMA Internal Medicine*, *182*(2), 106–114. https://doi.org/10.1001/JAMAINTERNMED.2021.6373

158. International Weight Management in Pregnancy (i-WIP) Collaborative Group. (2017). Effect of diet and physical activity based interventions in pregnancy on gestational weight gain and pregnancy outcomes: meta-analysis of individual participant data from randomised trials. *BMJ (Clinical Research Ed.)*, *358*, j3119. https://doi.org/10.1136/BMJ.J3119

159. O'Brien, E. C., Alberdi, G., & McAuliffe, F. M. (2018). The influence of socioeconomic status on gestational weight gain: a systematic review. *Journal of Public Health (Oxford, England)*, *40*(1), 41–55. https://doi.org/10.1093/PUBMED/FDX038

160. De Giuseppe, R., Bocchi, M., Maffoni, S., Del Bo, E., Manzoni, F., Cerbo, R. M., Porri, D., & Cena, H. (2021). Mediterranean Diet and Lifestyle Habits during Pregnancy: Is There an Association with Small for Gestational Age Infants? An Italian Single Centre Experience. *Nutrients*, *13*(6), 1941. https://doi.org/10.3390/NU13061941

161. Biagi, C., Di Nunzio, M., Bordoni, A., Gori, D., & Lanari, M. (2019). Effect of Adherence to Mediterranean Diet during Pregnancy on Children's Health: A Systematic Review. *Nutrients*, *11*(5). https://doi.org/10.3390/NU11050997

162. Lumbanraja, S., Lutan, D., & Usman, I. (2013). Maternal Weight Gain and Correlation with Birth Weight Infants. *Procedia - Social and Behavioral Sciences*, *103*, 647–656. https://doi.org/10.1016/J.SBSPRO.2013.10.383

163. Turconi, G., Celsa, M., Rezzani, C., Biino, G., Sartirana, M. A., & Roggi, C. (2003). Reliability of a dietary questionnaire on food habits, eating behaviour and nutritional

knowledge of adolescents. *European Journal of Clinical Nutrition*, *57*(6), 753–763. https://doi.org/10.1038/SJ.EJCN.1601607

164. Sun, Y., Shen, Z., Zhan, Y., Wang, Y., Ma, S., Zhang, S., Liu, J., Wu, S., Feng, Y., Chen, Y., Cai, S., Shi, Y., Ma, L., & Jiang, Y. (2020). Effects of pre-pregnancy body mass index and gestational weight gain on maternal and infant complications. *BMC Pregnancy and Childbirth*, *20*(1). https://doi.org/10.1186/S12884-020-03071-Y

165. H. Al Wattar, B., Dodds, J., Placzek, A., Beresford, L., Spyreli, E., Moore, A., Gonzalez Carreras, F. J., Austin, F., Murugesu, N., Roseboom, T. J., Bes-Rastrollo, M., Hitman, G. A., Hooper, R., Khan, K. S., & Thangaratinam, S. (2019). Mediterranean-style diet in pregnant women with metabolic risk factors (ESTEEM): A pragmatic multicentre randomised trial. *PLoS Medicine*, *16*(7). https://doi.org/10.1371/JOURNAL.PMED.1002857

166. Silva-del Valle, M. A., Sánchez-Villegas, A., & Serra-Majem, L. (2013). Association between the adherence to the Mediterranean diet and overweight and obesity in pregnant women in Gran Canaria. *Nutricion Hospitalaria*, *28*(3), 654–659. https://doi.org/10.3305/NH.2013.28.3.6377

167. Hirko, K. A., Comstock, S. S., Strakovsky, R. S., & Kerver, J. M. (2020). Diet during Pregnancy and Gestational Weight Gain in a Michigan Pregnancy Cohort. *Current Developments in Nutrition*, *4*(8). https://doi.org/10.1093/CDN/NZAA121

168. Maugeri, A., Barchitta, M., Favara, G., La Rosa, M. C., Mastra, C. La, San Lio, R. M., & Agodi, A. (2019). Maternal Dietary Patterns Are Associated with Pre-Pregnancy Body Mass Index and Gestational Weight Gain: Results from the "Mamma & Bambino" Cohort. *Nutrients*, *11*(6). https://doi.org/10.3390/NU11061308

169. Turconi, G., Bazzano, R., Roggi, C., & Cena, H. (2010). Reliability and relative validity of a quantitative food-frequency questionnaire for use among adults in Italian population. *International Journal of Food Sciences and Nutrition*, *61*(8), 846–862. https://doi.org/10.3109/09637486.2010.495329

170. Turunen, J., Tejesvi, M. V., Paalanne, N., Hekkala, J., Lindgren, O., Kaakinen, M., Pokka, T., Kaisanlahti, A., Reunanen, J., & Tapiainen, T. (2021). Presence of distinctive microbiome in the first-pass meconium of newborn infants. *Scientific Reports 2021 11:1*, *11*(1), 1–12. https://doi.org/10.1038/s41598-021-98951-4

171. Kaisanlahti, A., Turunen, J., Byts, N., Samoylenko, A., Bart, G., Virtanen, N., Tejesvi, M. V., Zhyvolozhnyi, A., Sarfraz, S., Kumpula, S., Hekkala, J., Salmi, S., Will, O., Korvala, J., Paalanne, N., Erawijantari, P. P., Suokas, M., Medina, T. P., Vainio, S., … Reunanen, J. (2023). Maternal microbiota communicates with the fetus through microbiota-derived extracellular vesicles. *Microbiome*, *11*(1). https://doi.org/10.1186/S40168-023-01694-9

172. Tun, H. M., Bridgman, S. L., Chari, R., Field, C. J., Guttman, D. S., Becker, A. B., Mandhane, P. J., Turvey, S. E., Subbarao, P., Sears, M. R., Scott, J. A., & Kozyrskyj, A. L. (2018). Roles of Birth Mode and Infant Gut Microbiota in Intergenerational

Transmission of Overweight and Obesity From Mother to Offspring. *JAMA Pediatrics*, *172*(4), 368. https://doi.org/10.1001/JAMAPEDIATRICS.2017.5535

173. Mueller, N. T., Shin, H., Pizoni, A., Werlang, I. C., Matte, U., Goldani, M. Z., Goldani, H. A. S., & Dominguez-Bello, M. G. (2016). Birth mode-dependent association between pre-pregnancy maternal weight status and the neonatal intestinal microbiome. *Scientific Reports*, *6*, 23133. https://doi.org/10.1038/SREP23133

174. Di Napoli, I., Tagliaferri, S., Sommella, E., Salviati, E., Porri, D., Raspini, B., Cena, H., Campiglia, P., La Rocca, C., Cerbo, R. M., & De Giuseppe, R. (2021). Lifestyle habits and exposure to bpa and phthalates in women of childbearing age from northern italy: A pilot study. *International Journal of Environmental Research and Public Health*, *18*(18), 9710. https://doi.org/10.3390/IJERPH18189710/S1

175. Jensen, T. B. N., Dall, S. M., Knutsson, S., Karst, S. M., & Albertsen, M. (2024). High-throughput DNA extraction and cost-effective miniaturized metagenome and amplicon library preparation of soil samples for DNA sequencing. *PLOS ONE*, *19*(4), e0301446. https://doi.org/10.1371/JOURNAL.PONE.0301446

176. Brosius, J., Palmer, M. L., Kennedy, P. J., & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(10), 4801–4805. https://doi.org/10.1073/PNAS.75.10.4801

177. Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M., & Tiedje, J. M. (2007). The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic Acids Research*, *35*(Database issue). https://doi.org/10.1093/NAR/GKL889

178. Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1). https://doi.org/10.1093/NAR/GKS808

179. Karst, S. M., Ziels, R. M., Kirkegaard, R. H., Sørensen, E. A., McDonald, D., Zhu, Q., Knight, R., & Albertsen, M. (2021). High-accuracy long-read amplicon sequences using unique molecular identifiers with Nanopore or PacBio sequencing. *Nature Methods*, *18*(2), 165–169. https://doi.org/10.1038/S41592-020-01041-Y

180. Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, *34*(18), 3094–3100. https://doi.org/10.1093/BIOINFORMATICS/BTY191

181. *R Core Team (2023) R A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. - References - Scientific Research Publishing*. (n.d.). Retrieved January 14, 2026, from https://www.scirp.org/reference/referencespapers?referenceid=3582659

182. Andersen, K. S., Kirkegaard, R. H., Karst, S. M., & Albertsen, M. (2018). ampvis2: an R

package to analyse and visualise 16S rRNA amplicon data. *BioRxiv*, 299537. https://doi.org/10.1101/299537

183. *GitHub - vegandevs/vegan: R package for community ecologists: popular ordination methods, ecological null models & diversity analysis*. (n.d.). Retrieved January 14, 2026, from https://github.com/vegandevs/vegan

184. Ross, F. C., Patangia, D., Grimaud, G., Lavelle, A., Dempsey, E. M., Ross, R. P., & Stanton, C. (2024). The interplay between diet and the gut microbiome: implications for health and disease. *Nature Reviews. Microbiology*, *22*(11), 671–686. https://doi.org/10.1038/S41579-024-01068-4

185. Song, Z., & Liu, H. (2023). The investigation of the association of pregnancy weight gain on maternal and neonatal gut microbiota composition and abundance using 16sRNA sequencing. *BMC Pregnancy and Childbirth 2023 23:1*, *23*(1), 109-. https://doi.org/10.1186/S12884-022-05289-4

186. Cho, K. H., Kwon, Y., Kasani, P. H., Lee, S. G., & Jeong, S. J. (2025). Influence of Maternal Weight Dynamics Prior to and Throughout Gestation on Early Infant Gut Microbiome Colonization. *Microbial Ecology 2025 88:1*, *88*(1), 32-. https://doi.org/10.1007/S00248-025-02520-5

187. Arumugam, M., Raes, J., Pelletier, E., Paslier, D. Le, Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., … Zeller, G. (2011). Enterotypes of the human gut microbiome. *Nature 2011 473:7346*, *473*(7346), 174–180. https://doi.org/10.1038/nature09944

188. Costea, P. I., Hildebrand, F., Manimozhiyan, A., Bäckhed, F., Blaser, M. J., Bushman, F. D., De Vos, W. M., Ehrlich, S. D., Fraser, C. M., Hattori, M., Huttenhower, C., Jeffery, I. B., Knights, D., Lewis, J. D., Ley, R. E., Ochman, H., O'Toole, P. W., Quince, C., Relman, D. A., … Bork, P. (2017). Enterotypes in the landscape of gut microbial community composition. *Nature Microbiology 2017 3:1*, *3*(1), 8–16. https://doi.org/10.1038/s41564-017-0072-8

189. Kennedy, K. M., Gerlach, M. J., Adam, T., Heimesaat, M. M., Rossi, L., Surette, M. G., Sloboda, D. M., & Braun, T. (2021). Fetal meconium does not have a detectable microbiota before birth. *Nature Microbiology 2021 6:7*, *6*(7), 865–873. https://doi.org/10.1038/s41564-021-00904-0

190. Santos, S. J. Dos, Pakzad, Z., Elwood, C. N., Albert, A. Y. K., Gantt, S., Manges, A. R., Dumonceaux, T. J., Maan, E. J., Hill, J. E., Money, D. M., Maternal, T., Legacy, M., & Team, P. (2021). Early Neonatal Meconium Does Not Have a Demonstrable Microbiota Determined through Use of Robust Negative Controls with cpn60-Based Microbiome Profiling. *Microbiology Spectrum*, *9*(2). https://doi.org/10.1128/SPECTRUM.00067-21

191. Godfrey, K. M., Gluckman, P. D., & Hanson, M. A. (2010). Developmental origins of metabolic disease: life course and intergenerational perspectives. *Trends in*

Endocrinology & Metabolism, *21*(4), 199–205. https://doi.org/10.1016/J.TEM.2009.12.008

192. Koletzko, B., Godfrey, K. M., Poston, L., Szajewska, H., Van Goudoever, J. B., De Waard, M., Brands, B., Grivell, R. M., Deussen, A. R., Dodd, J. M., Patro-Golab, B., & Zalewski, B. M. (2019). Nutrition During Pregnancy, Lactation and Early Childhood and its Implications for Maternal and Long-Term Child Health: The Early Nutrition Project Recommendations. *Annals of Nutrition & Metabolism*, *74*(2), 93–106. https://doi.org/10.1159/000496471

193. Deputy, N. P., Sharma, A. J., Kim, S. Y., & Hinkle, S. N. (2015). Prevalence and Characteristics Associated With Gestational Weight Gain Adequacy. *Obstetrics and Gynecology*, *125*(4), 773. https://doi.org/10.1097/AOG.0000000000000739

194. Korpela, K., Salonen, A., Saxen, H., Nikkonen, A., Peltola, V., Jaakkola, T., de Vos, W., & Kolho, K. L. (2020). Antibiotics in early life associate with specific gut microbiota signatures in a prospective longitudinal infant cohort. *Pediatric Research 2020 88:3*, *88*(3), 438–443. https://doi.org/10.1038/s41390-020-0761-5

195. Bautista, J., & López-Cortés, A. (2025). Microbial beginnings: determinants and disruptions of the neonatal microbiome. *Frontiers in Microbiology*, *16*, 1672780. https://doi.org/10.3389/FMICB.2025.1672780/FULL

196. Zhang, Z., Jia, L., Liu, B., Liu, Y., Zhao, J., Wang, Y., Zhang, M., Qiao, W., Yang, B., Luo, L., & Chen, L. (2025). Maternal gut microbiota and placenta-derived tissues microbes are important for initial gut microbial colonization in infants. *Frontiers in Microbiology*, *16*, 1631590. https://doi.org/10.3389/FMICB.2025.1631590/FULL

197. Miller, C., Luu, K., Mikami, B., Riel, J., Qin, Y., Khadka, V., & Lee, M. J. (2024). Temporal Investigation of the Maternal Origins of Fetal Gut Microbiota. *Microorganisms*, *12*(9), 1865. https://doi.org/10.3390/MICROORGANISMS12091865/S1

Appendices

Appendix A	The Influence of Maternal Lifestyle Factors on Human Breast Milk Microbial Composition: A Narrative Review
Appendix B	Folic acid supplementation in European women of reproductive age and during pregnancy with excessive weight: a systematic review
Appendix C	Offspring's exposome: a narrative review on the influence of early-life factors on childhood obesity risk
Appendix D	Questionnaires used in Paper 1 Empirical
Appendix E	Supplementary materials for Paper 2 Empirical

Appendix A

The Influence of Maternal Lifestyle Factors on Human Breast Milk Microbial Composition: A Narrative Review

biomedicines

MDPI

Review

The Influence of Maternal Lifestyle Factors on Human Breast Milk Microbial Composition: A Narrative Review

Irene Bianco [1,†], Chiara Ferrara [1,†], Francesca Romano [1], Federica Loperfido [1], Francesca Sottotetti [1,*], Dana El Masri [1], Alessandra Vincenti [1], Hellas Cena [1,2,‡] and Rachele De Giuseppe [1,‡]

[1] Laboratory of Dietetics and Clinical Nutrition, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, 27100 Pavia, Italy; irene.bianco@unipv.it (I.B.); chiara.ferrara01@unipv.it (C.F.); romanofrancesca93@gmail.com (F.R.); federica.loperfido@unipv.it (F.L.); dana.elmasri01@universitadipavia.it (D.E.M.); alessandra.vincenti@unipv.it (A.V.); hellas.cena@unipv.it (H.C.); rachele.degiuseppe@unipv.it (R.D.G.)

[2] Clinical Nutrition Unit, General Medicine, Istituti Clinici Scientifici (ICS) Maugeri, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), 27100 Pavia, Italy

* Correspondence: francesca.sottotetti@unipv.it

† These authors contributed equally to this work.

‡ These authors also contributed equally to this work.

Abstract: Human breast milk (HBM) is considered the gold standard for infant nutrition due to its optimal nutrient profile and complex composition of cellular and non-cellular components. Breastfeeding positively influences the newborn's gut microbiota and health, reducing the risk of conditions like gastrointestinal infections and chronic diseases (e.g., allergies, asthma, diabetes, and obesity). Research has revealed that HBM contains beneficial microbes that aid gut microbiota maturation through mechanisms like antimicrobial production and pathogen exclusion. The HBM microbiota composition can be affected by several factors, including gestational age, delivery mode, medical treatments, lactation stage, as well as maternal lifestyle habits (e.g., diet, physical activity, sleep quality, smoking, alcohol consumption, stress level). Particularly, lifestyle factors can play a significant role in shaping the HBM microbiota by directly modulating the microbial composition or influencing the maternal gut microbiota and influencing the HBM microbes through the enteromammary pathway. This narrative review of current findings summarized how maternal lifestyle influences HBM microbiota. While the influence of maternal diet on HBM microbiota is well-documented, indicating that dietary patterns, especially those rich in plant-based proteins and complex carbohydrates, can positively influence HBM microbiota, the impact of other lifestyle factors is poorly investigated. Maintaining a healthy lifestyle during pregnancy and breastfeeding is crucial for the health of both mother and baby. Understanding how maternal lifestyle factors influence microbial colonization of HBM, along with their interactions and impact, is key to developing new strategies that support the beneficial maturation of the infant's gut microbiota.

Keywords: human breast milk; microbiota; maternal dietary pattern; lifestyle; breastfeeding

Citation: Bianco, I.; Ferrara, C.; Romano, F.; Loperfido, F.; Sottotetti, F.; El Masri, D.; Vincenti, A.; Cena, H.; De Giuseppe, R. The Influence of Maternal Lifestyle Factors on Human Breast Milk Microbial Composition: A Narrative Review. *Biomedicines* **2024**, *12*, 2423. https://doi.org/10.3390/biomedicines12112423

Academic Editor: Farhad Vahid

Received: 16 September 2024
Revised: 17 October 2024
Accepted: 18 October 2024
Published: 22 October 2024

1. Introduction

Human breast milk (HBM) is widely regarded as the gold standard of infant nutrition, offering an unparalleled source of essential nutrients tailored specifically for the newborn during the crucial neonatal period [1]. This remarkable fluid is highly complex, comprising diverse cellular components and bioactive molecules, all working together to support the infant's growth, immune development, and overall health [2]. "Human milk" refers to milk produced by lactating parents and includes (i) breast milk produced by a parent that is given directly to infants through the breast or expressed by the breastfeeding parent and then given to the infant; (ii) donor milk produced by breastfeeding individuals is stored and then donated (e.g., human milk banks) or given to infants other than their

children [3]. Breastfeeding is one of the exposure factors that can modify the composition of the newborn's gut microbiota and, therefore, perpetuate the state of newborn health [4]. Notably, breastfed children exhibit a decreased risk of developing diseases in their lifespan, such as gastrointestinal infection, acute otitis media, and infectious diarrhea, compared to infants fed with formula milk [5,6].

As widely described, HBM is recognized as one of the most important postpartum elements modulating metabolic and immunologic programming related to the child's health [7,8]; this is partly due to the presence of a specific HBM microbiota, a source of beneficial, non-pathogenic, and probiotic bacteria that may positively affect the infant gut microbiota maturation [9]. Several studies have also shown that breastfed babies had a lower risk of chronic diseases such as allergies, asthma, diabetes, and obesity in childhood and adulthood [10–12]. All of this could be related to the composition of the HBM microbiota and its multiple mechanisms [13], including the production of antimicrobial compounds against pathogenic bacteria, the exclusion of competitors, the prevention of adherence of pathogenic bacteria to the intestinal epithelium, and the increase in intestinal mucosal production [14].

Alterations in the composition of the HBM microbiota can be ascribed to several exposure factors, such as maternal health (presence of certain pathologies including obesity, allergies, celiac disease, mastitis) [15–17], maternal dietary habits and lifestyle factors [18], gestational age (term vs. preterm birth) [19], delivery mode (vaginal birth vs. C-section birth) [7], medical treatment and antibiotic exposure [7], lactation period (colostrum vs. transitional milk vs. mature milk) [20]. Furthermore, it is widely believed that the bacteria colonizing HBM originate from the mother's skin and the newborn's oral cavity, with the infant being exposed to the mother's vaginal and intestinal microbiota during childbirth [9].

Particularly, among these factors, it is important to highlight the crucial role that the adoption of adequate lifestyle plays in periconception and prenatal and postnatal care in maintaining general health and preventing lifestyle-related diseases, including non-communicable diseases (NCDs), throughout the entire life course [21].

Given the growing interest in identifying modifiable maternal factors that may influence the HBM microbiota, to the best of our knowledge, the present narrative review is the first to provide an overview of maternal lifestyle elements, including dietary habits, physical activity, sleep quality, smoking, and stress levels, that are associated with shaping the HBM microbiota. Special attention is given to maternal diet during lactation and its potential impact on the infant's health. By understanding the effect of lifestyle on the formation of the HBM microbiota, it is possible to generate personalized advice to adapt to the maternal profile and encourage the adoption of lifestyle changes that can influence the composition of the newborn's intestinal microbiota and preserve its health.

2. The Human Breast Milk Composition

The HBM is a dynamic, bioactive fluid that changes in composition from colostrum to late lactation and varies within feeds, diurnally, and between mothers [2]. It consists of cellular and non-cellular components coming from different sources (i.e., lactocytes) [2].

2.1. Non-Cellular Components of the Human Breast Milk

The non-cellular components of HBM encompass macro- and micronutrients, as well as a plethora of active compounds like immunoglobulins, human milk oligosaccharides (HMOs), growth factors, cytokines, chemokines, and epithelial and immune cells, which provide nutritional, immunological, and developmental benefits to the newborn, as well as having a key role in anti-oncogenicity, neurocognitive development, cellular communication, and differentiation [9,22,23].

HBM has a unique composition that constantly changes based on neonatal needs [24]. For instance, concerning macronutrients, colostrum, which is produced in the first few days postpartum, is rich in protein (14–16 g/L), lipids (15–20 g/L), and carbohydrates (50–60 g/L), particularly HMO (20–24 g/L), and other cellular components; transitional

milk, which is produced approximately 5–14 days after giving birth and is similar in composition to colostrum [2,4]; mature milk differs completely from the previous lactation stages and consists of 90% water, 3.8% fat, 1.0% protein, and 7% lactose [20]. Regarding the micronutrients present in human breast milk, they constitute the only source of nutrients for a newborn, at least for the first six months of life [2]. Although the composition of breast milk is highly conserved, the amount of nutrients can vary depending on the mother's diet and body reserves. Since maternal dietary habits are not always optimal, the use of supplements is recommended during breastfeeding as vitamins, minerals, and trace elements are essential for infant development, including hormone synthesis, immune system function, and antioxidant activity [2]. Besides macro and micronutrient content, HBM contains human milk oligosaccharides (HMOs), complex sugars found in significant concentrations and with unique structural diversity [25]. Although they are the fourth most abundant component of human milk after water, lipids, and lactose, they provide no direct nutritional value to the newborn [25]. However, HMOs have a prebiotic effect, and through their features, they are responsible for the microbiota composition of the baby and the composition of the bacterial community of the milk itself [2,25]. Indeed, HMOs serve as a selective food source for beneficial bacteria, particularly *Bifidobacteria* and *Lactobacilli*, which have specific enzymes that can break down HMOs, allowing them to proliferate [25]. At the same time, HMOs can prevent pathogenic bacteria from binding to the intestinal lining by mimicking the receptor structures that these pathogens would normally bind to; this reduces the colonization of harmful bacteria both in the baby's intestines and, to some extent, in the breast milk itself [25].

2.2. Cellular Components of the Human Breast Milk: The Microbial Composition

The HBM is not only rich in nutrients and antibodies, which play a fundamental role in the immunological development of newborns, but also includes types of cells derived from blood and breast, including immune cells, lactocytes, stem cells, and epithelial cells [2]. These cells are accompanied by the presence of some types of bacterial communities, which influence the health and development of the infant by colonizing and shaping the gut microbiota in early life [26].

In recent years, culture-independent methods, such as 16S ribosomal RNA (16S rRNA) sequencing, clone libraries, meta taxonomy, as well as next-generation sequencing technologies, have been developed, enabling accurate identification of microbial communities in HBM [27,28] and showing high inter- and intra-individual specific profiles [1].

The microbiota of breast milk comprises over 820 taxa at a species level, predominantly from the *Proteobacteria* and *Firmicutes* phyla, with *Streptococcus* and *Staphylococcus* being the most prevalent genera [1]. Studies indicate that the majority of bacterial communities in HBM are anaerobic [28]. Consequently, the HBM microbiota serves as the second source of microbes for newborns, following exposure to the birth canal during vaginal delivery [28]. Furthermore, it has been reported that by consuming approximately 800 mL of HBM per day, an infant ingests between 10^5 and 10^7 bacteria daily [1].

In general, the bacteriome core of HBM includes nine genera, such as *Staphylococcus*, *Streptococcus*, *Serratia*, *Pseudomonas*, *Corynebacterium*, *Ralstonia*, *Propionibacterium*, *Sphingomonas*, and *Bradyrhizobium*, representing half of the microbial milk community, although their abundance can vary across milk samples [28]. Currently, the shape of the HBM microbiota is still unclear; however, two conceivable mechanisms have been proposed to elucidate the composition of the milk microbiota [29].

First, the notion of "retrograde transfer" describes the external influx of bacteria coming from the periareolar skin and the baby's mouth, which can also influence the composition of the infant's gut microbiota shaping [29,30]. For instance, a study conducted by Biagi and colleagues [31] on 36 healthy mother–child pairs found several operational taxonomic units (OTUs) belonging to the genera *Bifidobacterium*, *Streptococcus*, and *Staphylococcus* in both the oral and fecal microbiota of the child as well as in the HBM microbiota. This could support the hypothesis that the baby's mouth can play a bidirectional role: on

the one hand, it could influence the composition of the newborn's intestinal microbiota throughout swallowing; on the other hand, it could, instead, influence the colonization of the maternal milk ducts during suckling [31].

Second, the most widely accepted hypothesis, known as the "enteromammary pathway" (also known as the Gut–Breast Axis), illustrates the intricate connection between the mother's gut health and the quality of her breast milk, emphasizing the role of lifestyle factors in shaping maternal and infant health outcomes [32,33]. In this pathway, where the translocation of internal bacteria from the mother's gastrointestinal (GI) tract into the mammary gland, driven by immune cells during the late stages of pregnancy, the dendritic cells of the maternal intestinal mucosa engulf the mother's intestinal bacteria and translocate through the lymphatic or circulatory system to the mammary gland [32,33]. In this manner, the maternal GI tract is a source of bacteria for the HBM microbiota, and the maternal gut microbiota is vertically transferred to the infant via HBM [34]; *Lactobacilli*, *Staphylococci*, *Enterococci*, and *Bifidobacteria* would be transferred through the milk from the mother to the newborn, then boosting the growth of the same bacteria in the newborn's intestine, influencing different functions such as the development of the gastrointestinal tract, the immune system, and the central nervous system of the baby [35]. However, it is noteworthy that although the "enteromammary pathway" process can establish a good microbiome environment, it also has the potential to move infectious agents such as immunodeficiency virus, herpes, and cytomegalovirus [36].

Breastfeeding shapes the development of the infant's gut microbiota in the first years of life, both directly through the infant's contact with the breast milk microbiota and indirectly through bioactive factors and compounds in breast milk that influence the growth and metabolism of bacteria [37]. Several studies have analyzed whether breast milk feeding practices (e.g., breastfeeding exclusivity, nursing at the breast, feeding pumped breastmilk from a bottle) may influence the transmission of bacteria from HBM to the newborn's intestine [38,39]. For instance, the analysis conducted on 1249 mothers belonging to the CHILD (Canadian Healthy Infant Longitudinal Development) cohort study demonstrated that *Streptococcus* spp. and *Veillonella dispar* were simultaneously found in both HBM and baby's feces and were reduced if babies received pumped HBM or if they began weaning [38]. Others [40] found that in *Bifidobacteria* (*Bifidobacterium bifidum* and *B. animalis*), most taxa shared between breast milk and newborn feces were found in 48% of direct breastfeeding compared to 30% of indirect breastfeeding. Again, the infant gut microbiota composition is strongly associated with the exclusivity and duration of breastfeeding [41]. Stewart et al., in the TEDDY (The Environmental Determinants of Diabetes in the Young) cohort study [42], showed that the gut microbiota of exclusively breastfed infants was related to a lower diversity but higher bacterial counts, and higher levels of *Lactobacillus* spp., *Bifidobacterium breve*, and *B. bifidum*, when compared to formula-fed infants; furthermore, the cessation of breastfeeding resulted in faster maturation of the gut microbiome, as marked by the *phylum Firmicutes* [42]. Fehr and colleagues [38] found increased co-occurrence of amplicon sequencing variant (ASV), both in HBM and infant stool, in still-breastfed babies at 12 months, suggesting that prolonged breastfeeding promoted continued transfer of bacteria via HBM. Cabrera R. and colleagues [7] also investigated HM composition in 18 women during different stages of lactation; the analysis revealed that *Weisella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus* genera were predominant in colostrum samples, while in 1- and 6-months milk samples, *Veillonella*, *Leptotrichia*, and *Prevotella*, typical of the oral cavity, increased significantly [7].

3. Human Breast Milk Microbiota Is Influenced by Maternal Lifestyle Habits

Maternal lifestyle habits, such as diet, physical activity and exercise, sleep quality, smoking, alcohol consumption, and psychological distress (including stress, anxiety, or depression), during pregnancy and lactation can affect the health of both mother and child [43]. Currently, it has been established that the microbial diversity of HBM varies during the lactation stages, depending on many factors, such as genetics, health, antibiotic usage, demography, environmental differences, and mode of delivery [31].

Regarding lifestyle factors, maternal dietary habits [44–51], smoking habits [40], alcohol consumption [52], and psychological distress [53,54] were evaluated for their direct effect on the composition of the HBM microbiota; physical activity/exercise and sleep quality [55–59] were investigated for their influence on the maternal intestinal microbiota composition, hypothesizing their potential effect on the HBM microbiota through the enteromammary pathway. Nonetheless, even if the topic is still poorly investigated, a healthy lifestyle during pregnancy and breastfeeding is essential and should be recommended to ensure the shaping of an HBM microbiota that promotes the health and well-being of the newborn; the choices a mother makes regarding her diet, physical activity, stress management, and avoidance of harmful substances directly influence the microbial composition of her breast milk, which plays a critical role in the development of her child's immune system, gut health, and overall long-term health.

3.1. Maternal Dietary Habits

Maternal dietary habits could play an important role in shaping the HBM microbiota by influencing microbiota diversity through changes in maternal gut microbiota diversity [44]; indeed, the mother's gut and breast can undergo certain bacterial migration through the enteromammary pathway [45]. This could explain the origin of bacteria in milk that are not only found on the maternal skin or in the newborn's mouth (e.g., contamination hypothesis), described as potential bacterial sources that shape the human milk microbiota [44]. Thanks to this evidence, intervention in the maternal diet during lactation may affect the composition of the maternal gut microbiota and, thus, regulate breast milk microbiota, as well as the intestinal microbiota of breastfed infants [45]. Studies [44,46–51] reported how different maternal dietary patterns, as well as single dietary nutrients, can influence the HBM microbiota shaping (Table 1).

For instance, considering the maternal diet as a whole, Marsh et al. [46] conducted a cross-sectional microbiome diversity analysis of 72 human milk samples to investigate the relationship between different maternal dietary patterns (e.g., vegan, vegetarian, and omnivore) and HBM microbiota composition [46]. No significant differences were observed between the diet groups at the phylum level [46]. However, statistically significant differences were observed between the vegan and omnivore groups at the genus level where of the 18 genera that were statistically different between the two groups, *Mycobacterium*, *Rothia*, *Geobacillus*, *Actinomyces*, and a genus of the family *Vermiphilaceae* were present in more than 30 samples, and differences in their relative abundance were significant after correction for false discovery rate [46]. Moreover, species indicator analysis (SIA) revealed several species to be significant positive indicators of omnivore, vegetarian, and vegan dietary patterns, and *Rothia mucilaginosa* was the most significant indicator associated with the omnivore group [46]. Similarly, Cortes-Macias and colleagues [47] conducted a cross-sectional study to investigate the influence of maternal diet and specific nutrients during pregnancy on HBM microbiota from healthy mothers (MAternal Microbes—MAMI, Spanish cohort), divided into the group with a high maternal intake of fiber, plant protein, and carbohydrates (Cluster I) and the group with a high maternal intake of animal protein and lipids (Cluster II) [47]. Contrary to what Marsh et al. [46] have reported, the authors highlighted that HBM microbiota was shaped by maternal dietary clusters with important contributions coming from dietary fiber and both plant and animal protein intakes [47]. Indeed, Cluster I showed (i) at the phylum level, a higher relative abundance of both *Bacteroidetes* and *Actinobacteria*; (ii) at the genus level, a higher relative abundance of *Staphylococcus* and

Lactobacillus, *Bifidobacterium*, and *Sediminibacterium*; (iii) at the genus level, a lower relative abundances of *Bacteroides* and *Escherichia/Shigella*, when compared with Cluster II [47]. Moreover, LEfSe [linear discriminant analysis (LDA) > 2.5] analysis showed that Cluster II was characterized by *Bacteroides* and *Escherichia/Shigella* genera, whereas *Staphylococcus* spp. was characteristic of Cluster I [47]. Interestingly, the discrepancies between the findings highlighted by Marsh et al. [46] and Cortes-Macias et al. [47] may be due to the wide range of postpartum periods during which milk samples in the Marsh et al. [47] study were collected (2 weeks–over 2 years). It is known that the lactation phase (i.e., colostrum, transitional or mature milk) influences the microbiota of HBM, and although longitudinal studies characterizing human milk throughout the phase are lacking, that study showed that *Sphingobium* and *Pseudomonas* were enriched later during breastfeeding [60].

Regarding the contribution of maternal nutrient intake in shaping the HBM of lactating mothers, again, Marsh and colleagues in the same study previously described [46], classified HBM samples into low and high groups according to the content of fatty acids: saturated fats (SF, lower/greater than 40%), unsaturated fats (UF, lower/greater than 60%), and trans-unsaturated fatty acids (TF, lower/greater than 0.7%) [46]. The authors reported that TF breast milk content differed by dietary groups and highlighted the relationship between maternal diet and the microbial profile of human milk [46]. Indeed, the microbiota composition of HBM containing a greater content of TF differed significantly from that containing low TF; furthermore, differences in diversity between the high and low groups within both the SF and UF categories were noted, although these differences did not achieve statistical significance. [46]. SIA also identified 20 taxa as representative of the high UF group, including *Lactobacillus rhamnosus* and undetermined species of *Lactobacillus*; 21 taxa as representative of the high SF, including *Streptococcus* and *Bifidobacterium*, and 19 taxa as representative of the TF group, including *L. rhamnosus* and *Lactobacillus agilis* [46]. At the same time, Cortes-Macias et al. [47] reported an association between HBM microbiota and specific dietary nutrients; among these, it is noteworthy to mention that *Staphylococcus* and *Bifidobacterium* were associated with carbohydrate intake, whereas the *Streptococcus* genus was associated with intakes of the n–3 PUFAs (EPA and docosapentaenoic acid, 22:5ω-3).

Padilha and colleagues [44] explored in a cross-sectional study the effect of the maternal diet during pregnancy and the first month of lactation on the human milk microbiota profile of healthy lactating Brazilian women volunteers with uncomplicated pregnancies. The authors reported that the effects of maternal dietary habits appeared to be different for diet during pregnancy and the breastfeeding period, as diet during pregnancy had a greater impact on bacterial community structure than diet during the first month of breastfeeding, where specific nutrients, on the contrary, have been shown to influence the abundance of specific bacterial genera differentially [44]. Particularly, the vitamin C intake during pregnancy was positively correlated with the *Staphylococcus* genus, while PUFAs and linoleic fatty acid correlated positively with the relative abundance of the genera *Bifidobacterium* during the lactation period [44]. Moreover, *Pseudomonas* correlated negatively with sugars and positively with vitamin B_9, while *Enterococcus* correlated negatively with vitamins B_1, B_2, and B_9 [44].

Table 1. Studies evaluating the association between maternal dietary patterns and macro- and micro-nutrient intake during pregnancy and lactation and HBM microbiota composition.

Maternal Dietary Habits and HBM Microbiota Composition						
	Type of Dietary Pattern or Nutrients	**Author(s) and Year**	**Type of the Study**	**Aim of the Study**	**HBM Samples Collection and Study Population**	**Microbiome Analysis Method**	**Results**
Dietary pattern	• Vegan • Vegetarian • Omnivore	Marsh et al., 2022 [46]	Observational and cross-sectional study	To investigate the relationship between different maternal dietary patterns (e.g., vegan, vegetarian, and omnivore) and HBM microbiota composition	A total of 72 HBM samples from healthy lactating mothers (mean age of the mothers: 32.1 ± 4.9 years; infants' age range: from 3.5 to 186 weeks)	16S rRNA amplicon sequencing	(i) At the phylum level: no significant differences between the diet groups; (ii) At the genus level: significant differences in 18 genera between vegan and omnivore groups. *Mycobacterium*, *Rothia*, *Geobacillus*, *Actinomyces*, and a genus of the family *Vermiphilaceae* differed significantly ($p < 0.001$) in their relative abundance after correction for false discovery rate; (iii) At the species level: Species indicator analysis (SIA) revealed species to be significant positive indicators of • omnivore ($n = 9$ species); • vegetarian ($n = 9$ species); • vegan ($n = 12$ species).
	• High intake of plant protein, fiber, and carbohydrates (Cluster I) • High intake of animal protein, lipids (Cluster II)	Cortès-Macias et al., 2021 [47]	Observational and cross-sectional study	To assess whether maternal diet and specific nutrients during pregnancy would shape the HBM microbiota	A total of 120 HBM samples (collected during 7–15 days after birth) from healthy lactating mothers (MAMI Spanish cohort)	16S rRNA amplicon sequencing	• Cluster I showed (i) at the phylum level: higher relative abundance of *Bacteroidetes* ($p < 0.001$), *Actinobacteria* ($p = 0.014$); (ii) at the genus level: higher relative abundance of *Staphylococcus* ($p = 0.036$) and *Lactobacillus* ($p = 0.022$), *Bifidobacterium* ($p = 0.026$), *Sediminibacterium* ($p < 0.001$); a lower relative abundance of *Bacteroides* ($p = 0.023$) and *Escherichia/Shigella* ($p = 0.023$), when compared with Cluster II. • LEfSe [linear discriminant analysis (LDA) > 2.5] analysis: - Cluster I was characterized by *Staphylococcus* spp.; - Cluster II was characterized by *Bacteroides* and *Escherichia/Shigella* genera.
	Gluten-free diet	Olshan et al., 2021 [51]	Case-control and cross-sectional study	To analyze the HBM microbiome integrated with metabolome profiling of subjects with CD on a gluten-free diet	A total of 36 HBM samples (collected at 7–14 days post-partum) from 20 mothers with CD on a gluten-free diet and 16 healthy mothers ingesting gluten	Multi-omics approach with shotgun metagenomics	• No significant differences in α- or • β-diversity between groups at the species or strain level (gluten-free diet vs. gluten ingestion); • Significantly higher abundance of *Acinetobacter ursingii*, *Rothia mucilaginosa*, and *Acintobacter* sp. (p-value < 0.05) in women adhering to a gluten-free diet.

Table 1. *Cont.*

Maternal Dietary Habits and HBM Microbiota Composition							
	Type of Dietary Pattern or Nutrients	**Author(s) and Year**	**Type of the Study**	**Aim of the Study**	**HBM Samples Collection and Study Population**	**Microbiome Analysis Method**	**Results**
Macro and micronutrient intake	Fatty acids - SF, lower/greater than 40%; - UF, lower/greater than 60%; - TF, lower/greater than 0.7%	Marsh et al., 2022 [46]	Observational and cross-sectional study	To investigate the relationship between different maternal dietary patterns (e.g., vegan, vegetarian, and omnivore) and HBM microbiota composition	A total of 72 HBM samples from healthy lactating mothers (mean age of the mothers: 32.1 ± 4.9 years; infants' age range: from 3.5–186 weeks)	16S rRNA amplicon sequencing	• The microbiota composition of HBM containing a greater content of TF differed significantly from that containing low TF, • Species indicator analysis (SIA) identified: - 20 taxa representative of the high UF group, including *Lactobacillus rhamnosus* and undetermined species of *Lactobacillus*; - 21 taxa representative of the high SF, including *Streptococcus* and *Bifidobacterium*; - 19 taxa representative of the TF group, including *L. rhamnosus* and *Lactobacillus agilis*.
	• Carbohydrate • n–3 PUFAs	Cortès-Macias et al., 2021 [47]	Observational and cross-sectional study	To assess whether maternal diet and specific nutrients during pregnancy would shape the HBM microbiota	A total of 120 HBM samples (collected during 7–15 days after birth) from healthy lactating mothers (MAMI Spanish cohort)	16S rRNA amplicon sequencing	• Carbohydrate intake was associated with *Staphylococcus* and *Bifidobacterium;* • n–3 PUFAs (EPA and docosapentaenoic acid, 22:5ω-3) intake was associated with the *Streptococcus* genus.
	• Vitamin C (during pregnancy) • PUFA/linoleic fatty acid (during lactation) • Sugars • Vitamin B group (e.g., B_1, B_2, B_9) • Lycopene (during pregnancy) • Pectin (during pregnancy)	Padilha et al., 2019 [44]	Observational and cross-sectional study	To evaluate the effect of maternal diet during pregnancy, the first month of lactation on the HBM microbiota	A total of 94 HBM samples (collected at 30 ± 4) days after delivery) from healthy lactating Brazilian women volunteers with uncomplicated pregnancy	16S rRNA amplicon sequencing	• The vitamin C intake was positively correlated ($p = 0.029$) with *Staphylococcus* genus and differed significantly between Cluster I (driven by *Streptococcus)* and Cluster II (driven by *Staphylococcus)* ($p = 0.0249$); • PUFAs ($p = 0.005$) and linoleic fatty acid ($p = 0.007$) correlated positively with the relative abundance of the genera *Bifidobacterium*; • *Pseudomonas* correlated negatively with sugars ($p = 0.0085$) and positively with vitamin B_9 ($p = 0.0053$); • *Enterococcus* correlated negatively with vitamin B_1 ($p = 0.0002$), B_2 ($p = 0.0084$), and B_9 ($p = 0.00001$); • Cluster 2 showed higher levels of • pectin ($p = 0.053$) and lycopene ($p = 0.058$).

Table 1. *Cont.*

Maternal Dietary Habits and HBM Microbiota Composition							
	Type of Dietary Pattern or Nutrients	**Author(s) and Year**	**Type of the Study**	**Aim of the Study**	**HBM Samples Collection and Study Population**	**Microbiome Analysis Method**	**Results**
Macro and micronutrient intake	• Carbohydrates • Total, monounsaturated, saturated fat • Total protein • Cholesterol • Fibre • B vitamin group (B_1, B_2, B_3, B_9) • Vitamin A • Vitamin C • Pantothenic acid • Magnesium	Londoño-Sierra et al., 2023 [48]	Observational and cross-sectional study	To analyze the effects of food and nutritional status during gestation and the first trimester of lactation on the microbiota of HBM	A total of 30 HBM samples from healthy women from Colombia in their first trimester of lactation	16S rRNA amplicon sequencing	• Carbohydrate intake during pregnancy and lactation positively correlated with *Enterobacter* spp. ($p \leq 0.01$ and $p \leq 0.01$, respectively) and negatively with the genus *Bifidobacterium* spp. ($p \leq 0.01$) during lactation; • Total fat ($p = 0.03$), saturated fat ($p = 0.03$), and monounsaturated fat ($p = 0.02$) intake during lactation correlated positively with the genus *Eubacterium* spp.; • Total protein ($p = 0.01$), total carbohydrates ($p \leq 0.01$), cholesterol ($p \leq 0.01$), and fiber ($p \leq 0.\ 01$) negatively correlated with the genus *Aerococcus* spp. during lactation; • Positive correlation for (i) folic acid intake and *Akkermansia* spp. ($p \leq 0.01$) during lactation; (ii) B complex vitamins, including B_1 ($p = 0.01$), B_2 ($p \leq 0.01$), B_3 ($p \leq 0.01$), and genus *Gemella* spp., during lactation; (iii) vitamin A, genera *Bifidobacterium* spp. ($p = 0.047$), *Corynebacterium* spp. ($p = 0.01$), and *Ruminococcus UCG.009* spp. ($p \leq 0.01$) during lactation; • Negative correlation between genus *Aerococcus* spp. and vitamin A ($p = 0.01$), vitamin C ($p \leq 0.01$), folic acid ($p \leq 0.01$), pantothenic acid ($p \leq 0.01$), and magnesium ($p \leq 0.01$) during lactation; • Positive correlations between the intake of simple ($p \leq 0.01$) and total carbohydrates ($p = 0.02$), saturated fat ($p = 0.03$), and total protein ($p = 0.04$) with the genus *Enterobacter* spp. during pregnancy; • Saturated fat also positively correlated with the genus *Halomonas* spp. ($p = 0.02$) during pregnancy; • Zinc and vitamin C positively correlated with *Pseudomonas* spp. ($p = 0.03$) and *Rothia* spp. ($p = 0.01$) during pregnancy, respectively; • Protein ($p = 0.01$) and saturated fat ($p \leq 0.01$) were negatively correlated with *Bifidobacterium* spp. during pregnancy; • Fibre negatively correlated with *Ruminiclostridium 9* spp. ($p = 0.02$), *Ruminococcaceae UCG.005* ($p = 0.03$), *Ruminococcaceae UCG.014* ($p = 0.02$), and *Ruminococcus 1* spp. ($p \leq 0.01$) during pregnancy.

Table 1. *Cont.*

Maternal Dietary Habits and HBM Microbiota Composition							
	Type of Dietary Pattern or Nutrients	**Author(s) and Year**	**Type of the Study**	**Aim of the Study**	**HBM Samples Collection and Study Population**	**Microbiome Analysis Method**	**Results**
Macro and micronutrient intake	• Non-digestible carbohydrates (e.g., fibers and resistant starch)	Olga et al., 2023 [49]	Prospective longitudinal study (Cambridge Baby Growth and Breastfeeding study, CBGS-BF)	To provide evidence on the origin of HBM butyrate by examining its associations with HBM microbiota composition	A total of 47 HBM samples (collected at 6 weeks infant age) from healthy mothers of the CBGS-BF study	16S rRNA amplicon sequencing	• Non-digestible carbohydrates promoted the metabolism of the *Clostridiales*-dominant microbiota, resulting in the fermentation of SCFAs (e.g., butyrate); • HBM butyrate content was associated with HBM microbiota composition ($p = 0.036$).
	• Fiber from grains • Fat (Polyunsaturated and Trans)	LeMay-Nedjelski et al., 2021 [50]	Observational and cross-sectional study	To assess the association between lactating diet and HBM microbiota at 3 months post-partum in mothers with gestational glucose intolerance	A total of 93 HBM samples (collected at 3 months post-partum) from mothers with different degrees of gestational glucose intolerance	16S rRNA amplicon sequencing	• Polyunsaturated fats ($p = 0.047$) and fiber from grains ($p = 0.048$) positively correlated with α-diversity (Shannon Index) and negatively correlated with *Acinetobacter* incidence; • Fiber from grains was positively correlated ($p = 0.04$) with β-diversity (Bray–Curtis dissimilarity index) and associated with a reduced incidence of Streptococcus; • Trans fats showed a positive association with both S*taphylococcus* and *Gemella* incidence.

Legend. HBM: human breast milk; SF: saturated fatty acids; UF: unsaturated fatty acids; TF: trans-unsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SCFAs: short-chain fatty acids.

Again, a recent cross-sectional study conducted by Londoño-Sierra and colleagues [48] on a group of women from Colombia during the first three months of lactation demonstrated that anthropometric indexes (e.g., body mass index, BMI), the gestational weight gain, and the macro and micronutrient intake during both gestation and lactation could impact the content of HBM bacterial genera, as well as the consumption of certain micronutrients, which could contribute to the presence of bacteria with probiotic potential [48]. Indeed, it has been observed that women with adequate BMI reported a higher alpha diversity even without statistical differences, while a lower richness and diversity were observed in those women who had excessive gestational weight gain. Regarding macronutrient intake, the consumption of simple carbohydrates during pregnancy and lactation also positively correlated with *Enterobacter* spp. and negatively correlated with the genus *Bifidobacterium* spp. during lactation [48]. Similarly, the maternal intake during lactation of total fat, saturated fat, and monounsaturated fat showed a positive correlation with the genus *Eubacterium* spp. [48]. Conversely, the maternal consumption during lactation of total protein, total carbohydrates, cholesterol, and dietary fiber negatively correlated with the genus *Aerococcus* spp. [48]. As for the micronutrient intake during lactation, a positive correlation was observed between (i) folic acid intake and *Akkermansia* spp.; (ii) B-group vitamins, including B_1, B_2, B_3 and genus *Gemella* spp.; (iii) vitamin A intake and the genera *Bifidobacterium* spp., *Corynebacterium* spp. and *Ruminococcus UCG.009* spp. [48]. At the same time, the genus *Aerococcus* spp. negatively correlated with vitamin A, vitamin C, folic acid, pantothenic acid, and magnesium [48]. Significant associations were also observed between maternal macro and micronutrient intake during pregnancy and HBM microbiota composition [48]. Indeed, positive correlations were identified between the intake of simple and total carbohydrates, saturated fat, and total protein with the genus *Enterobacter* spp.; saturated fat intake also positively correlated with the genus *Halomonas* spp. [48]. Regarding micronutrient intake, zinc and vitamin C positively correlated with *Pseudomonas* spp. and *Rothia* spp., respectively [48]. On the contrary, protein and saturated fat intake were negatively correlated with *Bifidobacterium* spp., while fiber intake negatively correlated with *Ruminiclostridium 9* spp., *Ruminococcaceae UCG.005*, *Ruminococcaceae UCG.014*, and *Ruminococcus 1* spp. [48].

Others [49], in a prospective longitudinal study conducted on 47 HBM samples from mothers belonging to the Cambridge Baby Growth and Breastfeeding Study (CBGS-BF), have recently reported that adequate intake of non-digestible carbohydrates (e.g., fibers and resistant starch) could also promote the metabolism of the *Clostridiales*-dominant microbiota, resulting in the fermentation of short-chain Fatty Acids (SCFAs) in HBM, including butyrate, which is known to have anti-inflammatory properties and may be protective against increased adiposity in children [49,61]. Indeed, the authors observed cross-sectionally at 6 weeks of infant age that HBM butyrate content was associated with HBM microbiota composition [49]. However, the source of butyrate in HBM is still unclear. Indeed, some researchers consider that it is produced in the intestines and later transferred to breast milk through the enteromammary pathway [62]; others argue that it is a metabolite yielded by the microorganisms that colonize HBM, such as HMO [63,64].

It is important to highlight that the HBM samples in the above-mentioned studies all came from healthy mothers; however, there are also studies in the literature that consider mothers with gestational glucose intolerance [50] and celiac disease (CD) on a gluten-free diet [51]. Thus, LeMay-Nedjelski et al. conducted an explorative analysis to investigate the association between lactating diet and HBM microbiota at 3 months post-partum in 93 mothers with different degrees of gestational glucose intolerance. The results suggested that maternal consumption of fiber and fat were important determinants of the HBM microbiota [50]. Indeed, the authors demonstrated that polyunsaturated fat and fiber from grains were positively correlated with the α-diversity of HBM microbiota and negatively correlated with *Acinetobacter* incidence; trans fats showed a positive association with both *Staphylococcus* and *Gemella* incidence [50]. Additionally, fiber from grain intake was positively correlated with the β-diversity of HBM microbiota and associated with a

reduced incidence of *Streptococcus* [50]. Again, Olshan and colleagues [51] cross-sectionally analyzed the HBM microbiome, with CD on a gluten-free diet and healthy controls ingesting gluten. The authors reported that the HBM composition of subjects with CD on a gluten-free diet appeared quite like the HBM composition of healthy control subjects at 7–14 days post-partum since they did not identify any significant differences in α- or β-diversity between groups at the species or strain levels [51]. However, milk samples of mothers adhering to a gluten-free diet resulted in an association with a significantly higher abundance of *Acinetobacter ursingii*, *Rothia mucilaginosa*, and *Acintobacter* spp. ($p < 0.05$) [51]; *Rothia mucilaginosa* has been detected in the gut microbiota of subjects with autoimmune inflammatory diseases [51].

3.2. Other Lifestyle Variables

3.2.1. Maternal Physical Activity and Physical Exercise

During breastfeeding and lactation, regular physical activity (PA)/physical exercise (PE) may lead to several benefits for maternal health [65,66]; furthermore, moderate to vigorous PA does not impact the volume and quality of HBM if it is supported by adequate maternal nutrition and hydration only [65,66].

To our knowledge, no research has studied the effect of maternal PA or PE on the composition of the HBM microbiota; however, several studies have reported a positive impact of PA on the composition of the human gut microbiota, such as an increase in bacterial diversity and richness [55–57].

For instance, a recent systematic review was conducted by Cataldi et al. [56] to explore the current scientific evidence investigating the relationship between PA/PE and gut microbiota shaping, focusing on the different types/variables of PA/PE and age-related effects, both in healthy and unhealthy individuals. Results highlighted that gut microbiota diversity was associated with aerobic exercise contrary to resistance training; abundance of *Prevotella* genus was associated with training duration; exercising according to the minimum dose recommended by the World Health Organization (WHO) did not significantly change the gut microbiota richness and diversity; intense and prolonged PE can induce a higher abundance of pro-inflammatory bacteria [56]. Another systematic review [57] aimed at evaluating the role that PA played in determining the gut microbiota composition in healthy subjects (without gender or age limitations), trying to distinguish its effects from those of diet. In general, considering the results of the 10 studies included, the authors reported that PA can increase the abundance of health-promoting bacteria, hindering some negative genera [57]. Indeed, a higher variability and abundance of *Firmicutes* (genera *Ruminococcaceae* or *Fecalibacteria*) were reported in active subjects when compared to the inactive ones, especially in athletes; these findings were less robust in the studies performed in the general population because of the different volume of exercise between athletes and non-athletes, suggesting the influence of the PA volume in shaping the gut microbiota [57]. Bressa and colleagues [58] conducted an observational study on Caucasian childbearing-age women and compared the composition of the intestinal microbiota between women who did not practice any PE and women who at least practiced exercise at the minimum dose recommended by the WHO. Results showed PE did not produce significant changes to the microbiota diversity/richness [58]; however, sedentary parameters (i.e., sedentary time and breaks) correlated with microbiota richness (number of species and Shannon and Simpson indices), hypothesizing that physical exercise pattern, such as breaks in sedentary time, the avoidance of long periods of inactivity in daily life, might shape the intestinal microbiota [58]. In general, results reported that PA performed at low doses but continuously could increase the abundance of health-promoting bacteria, such as *Bifidobacterium* spp., *R. hominis*, *A. muciniphila*, and *F. prausnitzii* [58]. Indeed, although at the phylum level, a higher presence of *Firmicutes* and a lower presence of *Bacteroidetes* in active women with a trend toward significance was observed, at the genus level, there were significant differences in the following genera: *Bifidobacterium*; *Barnesiellaceae*; *Odoribacter*;

Paraprevotella; *Turicibacter*; *Clostridiales*; *Coprococcus*; *Ruminococcus*; and two unknown genera of *Ruminococcaceae* family [58].

Therefore, based on the positive influence of PA in shaping gut microbiota and based on the fact that the maternal intestinal tract relates to two seemingly unrelated organs of the mammary gland, suggesting that bacteria in the mother's gut migrate to the mammary gland via an endogenous pathway (active migration theory) [45], the modulation of the maternal gut microbiota through PA or PE could, albeit indirectly, influence the microbiota composition of the HBM [67].

3.2.2. Quality of Sleep

Recently, a current study demonstrated that sleep quality was one of the predictors of breastfeeding self-efficacy since as women's sleep quality increases, their self-efficacy in breastfeeding also increases [68]. Indeed, sleep deprivation (SD) is known to affect a mother's quality of life and her mental health status [69,70], and it has been reported that continuous sleep shortage (four hours of sleep per day) lasting more than a month increased the risk of post-partum depression [71].

To date, several studies [59,72] have shown no association between sleep quality and HBM macro- and micronutrient composition; furthermore, to our knowledge, no study has evaluated the role of SD either on the microbial composition of HMB or on the composition of the maternal intestinal microbiota. However, Sun and colleagues [59], in a recent narrative review, depicted how SD causes gut microbiota dysbiosis, as well as systemic changes such as immune defense reduction, increased energy intake, broken glucose metabolism, and impaired cognitive functions. The results highlighted that SD might affect gut microbiota in both abundances and compositions, leading to a reduction in α-diversity and chao1 indexes [59]. Furthermore, SD results in (i) higher abundances of the families *Coriobacteriaceae* and *Erysipelotrichaceae*, (ii) lower abundance of the *Tenericutes* phylum, and reduction at the genus-level relative abundance of g_*Prevotella*; g_*Sutterella*, g_*Parasutterella*, g_*Alloprevotella*, g_*Anaeroplasma*, g_*Elusimicrobium*. Last, SD increased the ratio *Firmicutes:Bacteroidetes* [59]. Although the study examined in the review by Sun et al. [56] was conducted on a small sample, which sometimes involved only young, healthy men, the results seem promising and of interest for extending the investigation to larger populations, also including breastfeeding women. Indeed, ensuring good sleep hygiene and managing stress are, therefore, critical components of postnatal care where adequate quality of sleep is essential for breastfeeding mothers since sleep supports the balance of the mother's gut microbiota, which directly affects the quality of HBM and, subsequently, the infant's gut health and overall well-being.

3.2.3. Tobacco Exposure

Tobacco exposure, either directly or indirectly, has harmful effects on human health and increases the risk of several diseases [73]. According to data, in the United States, 10.7% of women smoke during pregnancy, while in Europe, this habit involves 1 in 10 women [74]. Even if mothers stop smoking during pregnancy, 50–80% of them start again within six months after giving birth, during breastfeeding [74].

Evidence has highlighted the harmful effects of tobacco smoke, which contains over 5300 compounds and 70 carcinogens, as well as second-hand smoke exposure on the HBM composition, which can reduce the human milk protective properties and, thus, negatively impact infant health and development [75]. Indeed, breastfed infants of smoking mothers are more prone to experience adverse effects, including allergies, sleep disorders, increased colic, upper respiratory tract infections, cardiac rhythm disorders, and sudden infant death syndrome [75]; however, the mechanisms underlying the infant risks mentioned above, which are associated with exposure to smoking, are still under investigation. In addition, mothers who smoke and choose to breastfeed have a shorter lactation period and produce less milk compared to non-smoking mothers, particularly between weeks 2 and 4 after initiation of lactation [74]. These changes can be explained by the effects of nicotine on

hormonal levels [74]. Studies have shown that nicotine in the mother's bloodstream reduces prolactin levels; furthermore, maternal smoking habits during breastfeeding may alter the taste of milk, which may affect the baby's desire to suck [74]. A recent systematic review concluded that milk produced by smoking mothers is associated with a lower macro- and micronutrient content [74], reduced antioxidant properties, and altered immune status [76,77]. Interestingly, mothers exposed to passive smoking showed higher levels of heavy metals such as arsenic, cadmium, mercury, and lead [75].

To our knowledge, only one cohort study by Moossavi et al. investigated the effect of smoking on the HBM microbiota and did not observe any effect on the diversity or composition of HBM. In this contest, researchers analyzed a subset of the CHILD cohort study, investigating the impact of several maternal and infant factors on the microbial composition of HBM [40]. They examined HBM samples produced 3–4 months after birth, considering both mothers who have always smoked and mothers who smoked during the prenatal period [40]. Smoking habit was associated with microbiota diversity in a phylum-specific manner; however, no association was highlighted between overall α-diversity in milk samples and smoking habit [40]. Thus, exposure to tobacco appears not to influence the composition of breast milk in microbial terms, although this relationship is poorly studied to date, and studies are needed to reach definitive conclusions. Similarly, the effect of cigarette smoking exposure on the gut microbiota composition remains unclear, and there is no agreement on how smoking affects the gut microorganisms.

However, others [78] compared the difference in gut microbial composition between current and never smokers in a Chinese cohort, showing that although there were no significant differences in the microbiota α-diversity among the two groups, smoking altered the relative abundance of several specific taxa, where *Phascolarctobacterium* and *Fusobacterium* increased, and *Dialister* decreased. Furthermore, this research revealed that smoking introduced more microbial interactions between taxa and a decrease in the network modularity [78]. Therefore, in this case, considering the maternal active migration theory previously elucidated [45], the habit of smoking during breastfeeding could somehow modify the composition of the HBM; this relationship deserves to be investigated more precisely.

3.2.4. Alcohol Consumption

Alcohol consumption during pregnancy and lactation is generally not recommended due to the potential risks it poses to the developing fetus, such as Fetal Alcohol Spectrum Disorders (FASD), which can lead to physical, behavioral, as well as cognitive impairments, including low birth weight, facial abnormalities, learning disabilities, and behavioral problems [79], miscarriage and stillbirth [80], pre-term births, and births defects [80]. Moreover, alcohol passes into breast milk and can reach the baby's bloodstream; the amount of alcohol in HBM generally peaks 30–60 min after consumption, but this time may be delayed if it is consumed with the meal [81]. Thus, also during lactation, alcohol exerts its harmful effects on the infant since even small amounts of alcohol in HBM can affect a baby's sleep patterns, feeding, and development [82]; at the same time, excessive maternal alcohol consumption may interfere with the milk let-down reflex, potentially reducing milk production as well as lead to delays in infant motor development [82].

Since it has been established that alcohol consumption affects the composition of the gut microbiota, leading to dysbiosis and increased intestinal permeability [83,84], similar effects may be transferred on the microbial composition of the HBM either through the direct impact exerted by alcohol on the HBM or through the indirect effect through the enteromammary pathway [45], as described for the other factors; however, the literature is still scarce. For instance, a population-based birth cohort study conducted by Wang and colleagues [85] on mother–child dyads enrolled in central China investigated the effect of alcohol consumption and maternal diet during pregnancy on maternal and infant's gut microbiota, showing that alcohol consumption during breastfeeding induced changes in the maternal gut microbiota shaping. Indeed, general linear model analysis performed to con-

trol possible confounders revealed that maternal alcohol consumption during pregnancy correlated negatively with *Faecalibacterium* while correlating positively with *Phascolarctobacterium* and *Blautia* [85]. In addition, although the α-diversity of maternal gut microbiota was not associated with alcohol consumption, the PCoA (β-diversity) revealed a significant difference in maternal gut microbiota composition between the alcohol-consumption group and the non-alcohol consumption group [85]. Again, Shenker and colleagues [52] evaluated cross-sectionally and, for the first time, the metabolite (rapid evaporative ionization mass spectrometry—REIMS—for metabolic fingerprinting) and bacterial composition of HBM from mothers of infants aged 3–48 months, also considering alcohol consumption, among other lifestyle factors. The authors showed that the macro-level metabolic and microbial composition of HBM was maintained between 3 and 24 months of nursing age. Following other previous studies, results reported a core genus-level microbiome within HBM, which was dominated by *Streptococcus* and *Rothia*, with other genera in lower-level abundance, including *Actinomyces*, *Acinetobacter*, *Veillonella*, *Granulicatella*, *Burkholderia*, *Sediminibacterium*, and *Corynebacterium* [52]. Lifestyle factors were correlated with microbiome features, but contrary to what was described by Wang and colleagues [82], no significant associations were described for alcohol consumption [52].

3.2.5. Postnatal Psychosocial Distress

In breastfeeding women, postnatal psychosocial distress is a common issue that can lead to anxiety, stress, or depressive symptoms [53]. Although physiological mechanisms remained unclear, findings described a relation between maternal psychological distress (e.g., anxiety, perceived stress, depression) and non-optimal breastfeeding outcomes, including a decreased proportion and duration of exclusive breastfeeding [86].

A high level of maternal psychological distress may influence the HBM microbiota, reducing microbial diversity and altering the abundance of specific bacterial groups through both direct and indirect mechanisms [53,87]. Indeed, since stress can affect both gut microbial composition and intestinal permeability [88–90], the direct pathway could involve the transfer of aberrant maternal gastrointestinal microorganisms via the enteromammary pathway [53]; on the other hand, the indirect mechanism may impact the nutrient content in HBM, which could potentially affect the microorganism content in milk [53].

Browne and colleagues [53], in the longitudinal BINGO (Dutch acronym for Biological Influences on Baby's Health and Development) cohort study, investigated for the first time the association between postnatal maternal stress and HBM microbial composition, analyzing milk samples at 2, 6, and 12 weeks after delivery. Variables related to stress, anxiety, and depressive symptoms were assessed, and subjects were grouped into women with high (H) and low (L) psychosocial distress [53]. Considering HBM microbial composition and psychological distress, no significant differences in the relative abundance of major bacterial genera were revealed [53]. However, progressive changes in the content of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* at the phylum level and *Acinetobacter*, *Flavobacterium*, and *Lactobacillus* at the genera level were found in the HBM of the L-women group [53]. Concerning milk microbial diversity, women with low psychosocial distress showed a significantly increased microbial α-diversity abundance [53].

Perinatal stress experienced by mothers of very premature infants can modulate HBM and the infant's gut microbial composition [54]. Recently, a 2-year prospective study of mothers of very preterm newborns fed with mother's milk was carried out, showing that maternal stress influenced bacterial diversity in the HBM as well as in fecal samples from their premature newborns with clear trends despite non-significant results (due to the study limitations) [54]. Indeed, the researchers described an inverse relationship between stress and HBM production in the first days of life and a progressive decrease and increase in the proportions of *Firmicutes* and *Proteobacteria* species, respectively, over 15 days post-delivery, in HBM of mothers with high stress levels [54]. The results of the studies investigating the influence of other lifestyle variables are summarized in Table 2.

Table 2. Studies evaluating the association between lifestyle factors (e.g., physical activity/physical exercise, quality of sleep, smoking habit, postnatal psychosocial distress) and gut or HBM microbiota composition.

	Author(s) and Year	Type of the Study	Aim of the Study	Samples Collection and Study Population	Microbiome Analysis Method	Results
Regular physical activity (PA)/physical exercise (PE)	Cataldi et al., 2022 [56]	Systematic review	To explore the current scientific evidence investigating the relationship between PA/PE and gut microbiota shaping, both in healthy and unhealthy general population	A total of 25 studies (randomized and non-randomized trials, observational studies) conducted on healthy and unhealthy subjects (no age restrictions, both sexes), following: aerobic PA/PE (n = 11); changes occurring after an endurance protocol (n = 2); changes induced by concurrent training (n = 3) or separate interventions of resistance and aerobic exercises (n = 3); effects produced from the practice of specific sports (n = 6).	• 16S rRNA amplicon sequencing; • Metagenomic whole-genome shotgun sequencing.	• Gut microbiota diversity is associated with aerobic exercise contrary to resistance training; • Abundance of *Prevotella* genus was associated with training duration; • Exercising according to the minimum dose recommended by the WHO did not change significantly the gut microbiota richness and diversity; • Intense and prolonged PE can induce a higher abundance of pro-inflammatory bacteria.
	Dorelli et al., 2021 [57]	Systematic review	To investigate the role that PA plays in determining the gut microbiota composition in healthy humans, trying to distinguish its effects from those of diet.	A total of 10 studies (any study design with a control group) conducted on athletes (n = 5), active people classified based on habitual PA level (n = 3), and sedentary subjects undergoing exercise interventions (n = 2), without gender or age limitations.	16S rRNA amplicon sequencing	• PA increases the abundance of health-promoting bacteria, hindering some negative genera; • Higher variability and abundance of *Firmicutes* (genera *Ruminococcaceae* or *Fecalibacteria*) in athletes than sedentary people; • Influence of the PA volume in shaping the gut microbiota.
	Bressa et al., 2017 [58]	Cross-sectional and case-control study	To compare the composition of the gut microbiota between individuals who did not practice any physical exercise and those who, at the very least, practiced exercise at the minimum dose recommended by WHO.	A total of 40 stool samples from Caucasian women (aged: 18–40; exclusion criteria; any kind of pathology, previous gastrointestinal surgery, antibiotics intake during three months before this study, smoking, prebiotics, probiotics, vegetarian or vegan, nutritional or ergogenic complements, pregnancy or lactation) divided into women who did not practice any PE (n = 21) and women who at least practiced exercise at the minimum dose recommended by the WHO (n = 19).	16S rRNA amplicon sequencing	• PE did not produce significant changes to the microbiota diversity/richness; • Sedentary parameters (i.e., sedentary time and breaks) correlated with microbiota richness (number of species and Shannon and Simpson indices); • At the phylum level, a higher presence of *Firmicutes* (p = 0.085) and a lower presence of *Bacteroidetes* (p = 0.076) in active women, with a trend towards significance; • At the genus level, significant abundance differences ($p < 0.001$) in the following: *Bifidobacterium* (↑active); *Barnesiellaceae* (↑sedentary); *Odoribacter* (↑sedentary); *Paraprevotella* (↑active); *Turicibacter* (↑active); *Clostridiales* (↑active); *Coprococcus* (↑active); *Ruminococcus* (↑sedentary); and two unknown genera of *Ruminococcaceae* family (↑active); • Significant higher abundance of healthy bacteria such as *F. prautznnii* (p = 0.029), *R. hominis* (p = 0.005), and *A. muciniphila* (p = 0.002) in active than in sedentary women.

Table 2. *Cont.*

	Author(s) and Year	Type of the Study	Aim of the Study	Samples Collection and Study Population	Microbiome Analysis Method	Results
Quality of sleep	Sun et al., 2023 [59]	Narrative review, including 4 representative studies conducted on animal models (mice, $n = 2$) and human population ($n = 2$; one randomized within-subject crossover study and one longitudinal clinical trial.	To summarize the gut microbiota dysbiosis caused by SD and the related diseases, elucidating the potential biological mechanisms.	A total of 25 stool samples from healthy participants (13M/12F) enrolled in the clinical trial; 9 healthy young men (age 23.3 ± 0.6 years) enrolled in the randomized within-subject crossover study.	16S rRNA amplicon sequencing	• Only results from studies on humans were considered in the present review. • SD may reduce dramatically diversity, including α-diversity ($p < 0.001$) and chao1 indexes ($p = 0.011$); • SD may affect gut's microbiota in abundance and compositions, leading to a reduction in *Bacteroidetes* and an increase in the ratio *Firmicutes:Bacteroidetes*; • SD results in higher abundances of the families *Coriobacteriaceae* and *Erysipelotrichaceae* ($p = 0.049$); • SD results in lower abundance of *Tenericutes* ($p = 0.03$); • SD results in reduction in genus-level relative abundance (e.g., *g_Prevotella*, $p < 0.001$; *g_Sutterella*, $p = 0.047$; *g_Parasutterella*, $p = 0.030$; *g_Alloprevotella*, $p < 0.001$; *g_Anaeroplasma*, $p = 0.001$; *g_Elusimicrobium*, $p = 0.001$).
Smoking habits	Moossavi et al., 2019 [40]	Cohort study	To profile the HBM microbiota and to examine the association of maternal, infant, early-life, and milk factors with HBM microbiota composition	A total of 393 HBM samples (collected at 3–4 months postpartum) from healthy mother–infant dyads.	16S rRNA amplicon sequencing	• Smoking habit was associated with microbiota diversity in a phylum-specific manner; • No association was highlighted between overall α-diversity in HBM samples and smoking habits.
	Zhu et al., 2024 [78]	Observational, cross-sectional, and case-control study	To compare the difference in gut microbial composition between current and never smokers in the Chinese cohort.	A total of 80 stool samples from participants grouped as current smokers ($n = 33$, with 25 years of smoking experience, on average, and consumption of an average of 15 cigarettes per day) or never smokers ($n = 47$).	16S rRNA amplicon sequencing	• No significant differences in the diversity (e.g., ASV number and Chao1, Shannon, and Simpson indices) of the gut microbiota between the current and non-smokers; • Smoking altered the relative abundance of several specific taxa, where *Phascolarctobacterium* and *Fusobacterium* increased and *Dialister* decreased.

Table 2. *Cont.*

	Author(s) and Year	Type of the Study	Aim of the Study	Samples Collection and Study Population	Microbiome Analysis Method	Results
Alcohol consumption	Wang et al., 2021 [85]	Population-based birth cohort study	To analyze the effect of maternal alcohol consumption and diet during pregnancy on maternal and infant gut microbiota composition	A total of 29 stool samples both from mother and their infants enrolled in central China. Women were divided into the alcohol consumption group (n = 10) and the non-alcohol consumption (n = 19) group.	16S rRNA amplicon sequencing	• Maternal alcohol consumption during pregnancy correlated negatively with *Faecalibacterium* (p = 0.001), while positively with *Phascolarctobacterium* (p = 0.032), and *Blautia* (p = 0.019) (GLM analysis); • α-diversity of maternal gut microbiota was not associated with alcohol consumption; • PCoA (β-diversity) revealed a significant difference in maternal gut microbiota composition between the alcohol-consumption and the non-alcohol-consumption groups (p = 0.006).
	Shenker et al., 2020 [52]	Cross-sectional study	To investigate the metabolite and bacterial composition of HBM, also considering alcohol consumption, among other lifestyle factors.	A total of 62 HBM samples (collected at infants' ages of 3–48 months). Sample collection was carried out as a sub-project of the Breastmilk Epigenetic Cohort Study (Imperial College Healthcare Tissue Bank). Only 46 samples were suitable for the microbic profile analysis.	16S rRNA amplicon sequencing	• Identification of a core genus-level microbiome within HBM, dominated by Streptococcus and Rothia, with other genera in lower-level abundance, including *Actinomyces*, *Acinetobacter*, *Veillonella*, *Granulicatella*, *Burkholderia*, *Sediminibacterium*, and *Corynebacterium* (REF); • No significant association between microbial composition and alcohol consumption
Psychological distress	Browne et al., 2019 [53]	Longitudinal BINGO cohort study	To explore the association between postnatal maternal stress and HBM microbial composition	A total of 77 HBM samples milk samples (n = 51 with complete data) (collected at 2, 6, and 12 weeks after delivery) from women grouped into subjects with high (H, n = 13) and low (L, n = 13) psychosocial distress.	16S rRNA amplicon sequencing	• No significant differences in the relative abundance of major bacterial genera were revealed; • L-women group showed a significantly increased (p = 0.004) microbial α-diversity abundance (Shannon and Simpson index); • Progressive changes in the content of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* at the phylum level and *Acinetobacter*, *Flavobacterium*, and *Lactobacillus* at the genera level were found in the HBM of the L-women group.
	Fernández-Tuñas et al., 2023 [54]	2-year prospective study	To investigate the association between postnatal maternal psychological distress and HBM production and microbial composition through the process of bacterial colonization of the gut of very preterm infants	A total of 45 HBM samples (collected on days 3, 7, and 15 after the birth) from mothers of very preterm newborns (n = 52; GA $\leq$ 32 weeks and/or bodyweight $\leq$ 1500 g) and 52 infant stool samples.	16S rRNA amplicon sequencing	• Inverse relationship between stress and HBM production in the first days of life (p = 0.012); • Progressive decrease and increase in the proportions of *Firmicutes* and *Proteobacteria* species, respectively, over 15 days post-delivery in HBM of mothers with high-stress levels.

Legend. HBM: human breast milk; WHO: World Health Organization; GLM: general linear model analysis.

4. Probiotics: Potential Bridge Between Breastfed and Formula-Fed Infants

Breastfed infants typically harbor a gut microbiota predominantly composed of *Bifidobacterium* and *Lactobacillus*, which are linked to beneficial immunological and metabolic effects [91]; these bacteria are selectively supported by HMOs, which act as prebiotics [91–93]. In contrast, formula-fed infants tend to exhibit a more diverse but less beneficial microbial profile, characterized by increased levels of potentially pathogenic bacteria, including *Clostridium difficile* and *Enterobacteriaceae* [94]. Studies demonstrated that these microbial imbalances in formula-fed infants have been associated with a heightened risk of infections, allergies, and metabolic disorders [95].

Probiotics, particularly strains of *Bifidobacterium* and *Lactobacillus*, have been explored for their ability to modulate the gut microbiota of formula-fed infants, potentially shifting it to resemble the microbiota of breastfed infants [96–98]. The premise is that introducing beneficial bacteria through probiotic supplementation may foster a more favorable gut environment akin to that induced by breastfeeding [97,98]. Studies showed that probiotics could increase the abundance of *Bifidobacterium* and *Lactobacillus* in formula-fed infants, promoting a microbial composition more similar to that of breastfed infants; however, the extent of this modulation depends on factors such as the probiotic strain, dosage, and duration of administration [99].

Currently, it has been reported [100,101] that probiotic supplementation in formula-fed infants has been associated with improved immune outcomes, including reductions in the incidence of diarrhea, necrotizing enterocolitis (NEC), and respiratory infections and suggesting that probiotics might help offset some of the immunological advantages conferred by breast milk. Similarly, from a metabolic perspective, probiotics may reduce inflammation and enhance gut barrier function, mimicking some of the protective effects of breast milk [102].

Nevertheless, there are limited long-term data on the sustained impact of probiotic-induced microbiota changes on the health outcomes of formula-fed infants, and several challenges and limitations must be acknowledged. In the first place, not all probiotics exert the same effect, and the beneficial outcomes observed with specific *Bifidobacterium* and *Lactobacillus* strains are not consistent across all strains, emphasizing the need for careful selection of probiotic species and strains to achieve the desired microbiota modulation [97].

Additionally, probiotics tend to provide only temporary colonization of the gut, and once supplementation ceases, the introduced strains may decline, and the infant's microbiota may revert to its pre-supplementation state [97,99]. Therefore, continuous administration or long-term dietary changes may be necessary to maintain the benefits.

Last, probiotics alone, however, may not fully replicate the effects of HBM, particularly due to the absence of HMOs in most infant formulas. The inclusion of HMOs in the formula, along with probiotics, offers a more comprehensive strategy to emulate HBM microbiota-shaping effects since they serve as selective substrates for beneficial bacteria [91,103]. Studies indicate that formulas combining HMOs and probiotics, also named synbiotics, can produce a microbiota profile more similar to that of breastfed infants than probiotics alone [98]. However, further research is required to optimize synbiotic formulations and assess their long-term health impacts.

5. Discussion

The WHO recommends exclusive breastfeeding for the first six months, followed by continued breastfeeding alongside safe complementary foods until at least two years of age [104]. Breastfeeding offers numerous benefits, including improved infant health and development, strengthened maternal–infant bonding, and a lower risk of chronic conditions [105,106]. Human breast milk is increasingly recognized not only as a biological fluid supporting neonatal growth but also as a complex medium with functions that extend beyond its nutrient content [4,107]. The microbial composition of HBM is crucial for establishing the infant gut microbiota, and any alterations may pose health risks, with early dysbiosis linked to a higher risk of non-communicable diseases like obesity and type

2 diabetes in adulthood [108]. Maternal environmental factors, particularly lifestyle habits during pregnancy and lactation, significantly influence the microbiota of HBM [43,109]. Therefore, maintaining a healthy lifestyle—including proper nutrition, physical activity, quality sleep, and the avoidance of harmful behaviors—is essential for promoting immediate maternal and infant health and preventing long-term disease risk in offspring, potentially through the modulation of HBM components, especially its microbiota [13].

This narrative review summarizes for the first time the influence of these specific maternal lifestyle habits on the modulation of the HBM microbiota composition. Although other authors [110] have investigated the influence of maternal diet on HBM microbiota composition, the novelty of this review lies in the fact that, to date, maternal lifestyle as a whole has not been considered as a factor influencing microbial composition. This aspect is particularly significant as it aligns with the principles of Lifestyle Medicine, an evidence-based medical specialty that focuses on the prevention, treatment, and management of diseases through lifestyle changes [111]. It emphasizes the importance of making informed choices about diet, physical activity, sleep, stress management, and other lifestyle factors to improve overall health and well-being [111]. Indeed, lifestyle medicine is essential for promoting the health of both mothers and children by encouraging sustainable lifestyle changes that prevent disease and enhance well-being throughout the maternal–child relationship [112,113].

The scientific literature predominantly examines the role that the maternal diet (defined both as dietary patterns and as macro-and micronutrient intake) during pregnancy and breastfeeding has in modulating the microbial composition of HBM. Dietary patterns (e.g., vegan, vegetarian, and omnivore) may influence HBM microbiota composition at the species level [46], and the HBM of mothers with a high intake of plant-based proteins and complex carbohydrates have shown a different microbial composition of HBM [47,48] when compared to patterns characterized by a high content of simple sugars and animal proteins [47,48]. At the same time, the type of fats (SF, UF, and TF) significantly influenced the HBM microbiota uniformity [46]. To date, there are no studies analyzing the direct role that maternal PA/PE and quality of sleep during pregnancy and breastfeeding have in modulating the microbial composition of the HBM. However, because bacteria in the mother's intestine migrate to the mammary gland via an endogenous pathway [45], the influence that reduced PA/PE and SD potentially exert on the maternal intestinal microbiota [57–59], although indirectly, could negatively modulate the composition of the HBM microbiota [45]. On the contrary, the positive influence of PE patterns and good sleep hygiene in increasing the abundance of beneficial bacteria for the health of the intestinal microbiota has been previously described [55,59]. Concerning the effects of cigarette smoking and alcohol consumption in the modulation of HBM composition, in the present narrative review, it was highlighted that, even only in two studies, a significant association has been reported between the consumption of these substances and the composition of the intestinal microbiota [78,85]; in contrast, no significant associations have been described regarding the direct effect of alcohol consumption on the composition of the HBM microbiota [52], as well as smoking habits and microbial diversity of the HBM [40].

Last, even if it is still poorly explored, researchers [53,54] reported how the reduction in maternal psychological distress progressively influenced the content of certain microorganisms both at the phylum at the genera level of the HBM, as well as the increase in its microbial α-diversity abundance, also in mothers of very premature infants.

Currently, probiotics can help bridge some microbiota differences between breastfed and formula-fed infants by promoting beneficial bacterial populations, particularly *Bifidobacterium* and *Lactobacillus* [96,97,99]. However, probiotics alone are unlikely to fully replicate the complex microbiota and immune-modulating effects conferred by HBM, especially in the absence of HMOs and other bioactive components of human milk [98]. A more holistic approach that combines probiotics with HMOs or other prebiotics may offer a more effective strategy for modulating the gut microbiota of formula-fed infants toward a breastfed-like profile [98].

It is, finally, important to mention that currently, bacterial analysis of HBM microbes, while essential for understanding the maternal–infant microbiome, faces several scientific and methodological limitations introducing heterogeneity in results and making it challenging to compare across studies. Sampling variability remains a major challenge; inconsistent collection methods (e.g., manual expression vs. pump extraction) can lead to contamination and variation in microbial load, while improper handling or delays in processing affect bacterial viability and diversity [30,114]. Additionally, contamination from breast skin and nipple microbiota complicates the distinction between native and external microbes, requiring sterile protocols that are not always rigorously applied [31,114].

The low-biomass nature of HBM further complicates analysis [114]. Indeed, compared to other biological fluids, HBM contains fewer bacterial cells, increasing the signal-to-noise ratio and making it difficult to distinguish native bacteria from environmental or reagent contaminants [30,31,114]. While common, techniques like 16S rRNA sequencing often struggle with sparse bacterial DNA, providing only taxonomic data at the genus level and lacking functional or strain-specific insights [27]. Whole-genome metagenomics, though more informative, is costlier and requires greater computational resources [115]. Data processing in bacterial studies adds another layer of complexity where high-throughput sequencing generates large datasets that require advanced bioinformatics tools, and factors such as sequencing errors, data normalization, and statistical models for low microbial abundance can influence results [115]. Moreover, most studies focus on bacterial identification, neglecting the functional roles of these microbes and techniques like metatranscriptomics, metabolomics, and proteomics are needed to assess microbial activity and interactions, but they are underutilized due to their complexity and expense [115]. The study design also presents limitations; for instance, cross-sectional studies, which analyze samples at a one-time point, offer limited insight into the dynamic nature of HBM microbiota [44,46–48,50–52,58,72]. On the contrary, longitudinal studies are more informative but are logistically demanding and costly [40,49,53,54,85]. Furthermore, associations between HBM microbiota and infant health outcomes, while frequently observed, remain correlative, as experimental studies that manipulate the microbiota to establish causality are ethically and practically challenging [30]. Finally, rare taxa with potentially significant roles often go undetected due to limitations in sequencing depth, with most methodologies focusing on more abundant species [30,114].

6. Conclusions

Human breast milk (HBM) is widely recognized as the optimal source of nutrition for infants, supporting both immediate growth and long-term health outcomes. This narrative review underscores the significant influence of maternal lifestyle habits—such as diet, physical activity, sleep quality, smoking, and alcohol consumption—on the composition of HBM microbiota. The findings suggest that maintaining a healthy lifestyle during pregnancy and lactation is essential not only for the mother's well-being but also for shaping the infant's gut microbiota and immune system development.

While the influence of diet on HBM microbiota is increasingly well-documented, other factors such as physical activity, sleep, and psychological stress remain underexplored. Current evidence indicates that lifestyle modifications during pregnancy and lactation could positively affect breast milk microbiota and, in turn, the infant's health. However, more research, particularly longitudinal and interventional studies, is needed to fully understand the extent of these influences and to develop tailored recommendations.

Health professionals should integrate these findings into prenatal and postnatal care, advising mothers on the importance of maintaining a balanced diet, engaging in regular physical activity, and managing stress to promote optimal HBM composition. Additionally, public health policies must account for these factors when developing breastfeeding support programs, ensuring that maternal well-being is prioritized for the benefit of both mother and child.

A deeper understanding of how maternal lifestyle shapes HBM microbiota is crucial for designing effective interventions that promote infant health. Future research should focus on filling the gaps in our knowledge, particularly in areas like physical activity and stress, to develop comprehensive guidelines that will enhance breastfeeding practices and optimize long-term health outcomes for both mothers and their children.

At the same time, it is crucial to highlight that methodological and scientific limitations in studying the HBM microbiota, including inconsistencies in sampling, low microbial biomass, biases in detection technologies, and lack of functional insights, need to be addressed to gain more in-depth, accurate, and complete understanding. Standardizing protocols, using multi-omics approaches, and adopting longitudinal designs can help mitigate some of these limitations.

Author Contributions: I.B., C.F. and F.R., writing—the original draft preparation; F.L., F.S., D.E.M. and A.V., review and editing; R.D.G., review and editing, supervision; H.C., review, editing, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Funding: Project funded under the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.3-Call for proposal No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU. Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security-Working ON Foods".

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The research group acknowledges the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.4-Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP 13C22000720007, Project title "National Biodiversity Future Center-NBFC"; for supporting the research grants of Federica Loperfido and Rachele De Giuseppe.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Selma-Royo, M.; Calvo Lerma, J.; Cortés-Macías, E.; Collado, M.C. Human milk microbiome: From actual knowledge to future perspective. *Semin. Perinatol.* **2021**, *45*, 151450. [CrossRef] [PubMed]
2. Kumari, P.; Raval, A.; Rana, P.; Mahto, S.K. Regenerative Potential of Human Breast Milk: A Natural Reservoir of Nutrients, Bioactive Components and Stem cells. *Stem Cell Rev. Rep.* **2023**, *19*, 1307–1327. [CrossRef] [PubMed]
3. Krebs, N.F.; Belfort, M.B.; Meier, P.P.; Mennella, J.A.; O'Connor, D.L.; Taylor, S.N.; Raiten, D.J. Infant factors that impact the ecology of human milk secretion and composition-a report from "Breastmilk Ecology: Genesis of Infant Nutrition (BEGIN)" Working Group. *Am. J. Clin. Nutr.* **2023**, *117* (Suppl. S1), S43–S60. [CrossRef] [PubMed]
4. Boudry, G.; Charton, E.; Le Huerou-Luron, I.; Ferret-Bernard, S.; Le Gall, S.; Even, S.; Blat, S. The Relationship Between Breast Milk Components and the Infant Gut Microbiota. *Front. Nutr.* **2021**, *8*, 629740. [CrossRef]
5. Ip, S.; Chung, M.; Raman, G.; Chew, P.; Magula, N.; DeVine, D.; Trikalinos, T.; Lau, J. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid. Rep./Technol. Assess.* **2007**, *153*, 1–186.
6. ESPGHAN Committee on Nutrition; Agostoni, C.; Braegger, C.; Decsi, T.; Kolacek, S.; Koletzko, B.; Michaelsen, K.F.; Mihatsch, W.; Moreno, L.A.; Puntis, J.; et al. Breast-feeding: A commentary by the ESPGHAN Committee on Nutrition. *J. Pediatr. Gastroenterol. Nutr.* **2007**, *49*, 112–125. [CrossRef]
7. Cabrera-Rubio, R.; Collado, M.C.; Laitinen, K.; Salminen, S.; Isolauri, E.; Mira, A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am. J. Clin. Nutr.* **2012**, *96*, 544–551. [CrossRef]
8. Johnson, K.E.; Heisel, T.; Allert, M.; Fürst, A.; Yerabandi, N.; Knights, D.; Jacobs, K.M.; Lock, E.F.; Bode, L.; Fields, D.A.; et al. Human milk variation is shaped by maternal genetics and impacts the infant gut microbiome. *bioRxiv* **2023**. [CrossRef]
9. Yi, D.Y.; Kim, S.Y. Human Breast Milk Composition and Function in Human Health: From Nutritional Components to Microbiome and MicroRNAs. *Nutrients* **2021**, *13*, 3094. [CrossRef]
10. Section on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics* **2021**, *129*, e827–e841. [CrossRef]
11. Wang, L.; Collins, C.; Ratliff, M.; Xie, B.; Wang, Y. Breastfeeding Reduces Childhood Obesity Risks. *Child. Obes.* **2017**, *13*, 197–204. [CrossRef] [PubMed]

12. Horta, B.L.; Loret de Mola, C.; Victora, C.G. Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: A systematic review and meta-analysis. *Acta Paediatr.* **2015**, *104*, 30–37. [CrossRef] [PubMed]
13. Le Doare, K.; Holder, B.; Bassett, A.; Pannaraj, P.S. Mother's milk: A purposeful contribution to the development of the infant microbiota and immunity. *Front. Immunol.* **2015**, *9*, 361. [CrossRef]
14. Mackie, R.I.; Sghir, A.; Gaskins, H.R. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am. J. Clin. Nutr.* **1999**, *69*, 1035S–1045S. [CrossRef]
15. Mulligan, C.M.; Friedman, J.E. Maternal modifiers of the infant gut microbiota: Metabolic consequences. *J. Endocrinol.* **2017**, *235*, R1–R12. [CrossRef]
16. Olshan, K.L.; Leonard, M.M.; Serena, G.; Zomorrodi, A.R.; Fasano, A. Gut microbiota in Celiac Disease: Microbes, metabolites, pathways and therapeutics. *Expert Rev. Clin. Immunol.* **2020**, *16*, 1075–1092. [CrossRef]
17. Olivares, M.; Albrecht, S.; De Palma, G.; Ferrer, M.D.; Castillejo, G.; Schols, H.A.; Sanz, Y. Human milk composition differs in healthy mothers and mothers with celiac disease. *Eur. J. Nutr.* **2015**, *54*, 119–128. [CrossRef] [PubMed]
18. Cicalini, I.; Moffa, S.; Tommolini, M.L.; Valentinuzzi, S.; Zucchelli, M.; Bucci, I.; Chiacchiaretta, P.; Fontana, A.; Federici, L.; De Laurenzi, V.; et al. Impact of Maternal Lifestyle and Dietary Habits during Pregnancy on Newborn Metabolic Profile. *Nutrients* **2023**, *15*, 2297. [CrossRef] [PubMed]
19. Gidrewicz, D.A.; Fenton, T.R. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatr.* **2014**, *14*, 216. [CrossRef]
20. Ballard, O.; Morrow, A.L. Human milk composition: Nutrients and bioactive factors. *Pediatr. Clin. N. Am.* **2013**, *60*, 49–74. [CrossRef]
21. Juan, J.; Yang, H. Early life 1000 days: Opportunities for preventing adult diseases. *Chin. Med. J.* **2021**, *135*, 516–518. [CrossRef] [PubMed]
22. Belkaid, Y.; Hand, T.W. Role of the microbiota in immunity and inflammation. *Cell* **2014**, *157*, 121–141. [CrossRef] [PubMed]
23. Martin, C.R.; Ling, P.R.; Blackburn, G.L. Review of Infant Feeding: Key Features of Breast Milk and Infant Formula. *Nutrients* **2016**, *8*, 279. [CrossRef] [PubMed]
24. Smilowitz, J.T.; O'Sullivan, A.; Barile, D.; German, J.B.; Lönnerdal, B.; Slupsky, C.M. The human milk metabolome reveals diverse oligosaccharide profiles. *J. Nutr.* **2013**, *143*, 1709–1718. [CrossRef] [PubMed]
25. Walsh, C.; Lane, J.A.; van Sinderen, D.; Hickey, R.M. Human milk oligosaccharides: Shaping the infant gut microbiota and supporting health. *J. Funct. Foods* **2020**, *72*, 104074. [CrossRef]
26. Pannaraj, P.S.; Li, F.; Cerini, C.; Bender, J.M.; Yang, S.; Rollie, A.; Adisetiyo, H.; Zabih, S.; Lincez, P.J.; Bittinger, K.; et al. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatr.* **2017**, *171*, 647–654. [CrossRef]
27. Lopez Leyva, L.; Brereton, N.J.B.; Koski, K.G. Emerging frontiers in human milk microbiome research and suggested primers for 16S rRNA gene analysis. *Comput. Struct. Biotechnol. J.* **2020**, *19*, 121–133. [CrossRef]
28. Notarbartolo, V.; Giuffrè, M.; Montante, C.; Corsello, G.; Carta, M. Composition of Human Breast Milk Microbiota and Its Role in Children's Health. *Pediatr. Gastroenterol. Hepatol. Nutr.* **2022**, *25*, 194–210. [CrossRef]
29. Singh, P.; Al Mohannadi, N.; Murugesan, S.; Almarzooqi, F.; Kabeer, B.S.A.; Marr, A.K.; Kino, T.; Brummaier, T.; Terranegra, A.; McGready, R.; et al. Unveiling the dynamics of the breast milk microbiome: Impact of lactation stage and gestational age. *J. Transl. Med.* **2023**, *21*, 784. [CrossRef]
30. Groer, M.W.; Morgan, K.H.; Louis-Jacques, A.; Miller, E.M. A Scoping Review of Research on the Human Milk Microbiome. *J. Hum. Lact. Off. J. Int. Lact. Consult. Assoc.* **2020**, *36*, 628–643. [CrossRef]
31. Biagi, E.; Quercia, S.; Aceti, A.; Beghetti, I.; Rampelli, S.; Turroni, S.; Faldella, G.; Candela, M.; Brigidi, P.; Corvaglia, L. The Bacterial Ecosystem of Mother's Milk and Infant's Mouth and Gut. *Front. Microbiol.* **2017**, *8*, 1214. [CrossRef] [PubMed]
32. Selvamani, S.; Dailin, D.J.; Gupta, V.K.; Wahid, M.; Keat, H.C.; Natasya, K.H.; Malek, R.A.; Haque, S.; Sayyed, R.Z.; Abomoelak, B.; et al. An Insight into Probiotics Bio-Route: Translocation from the Mother's Gut to the Mammary Gland. *Appl. Sci.* **2021**, *11*, 7247. [CrossRef]
33. Vélez-Ixta, J.M.; Juárez-Castelán, C.J.; Ramírez-Sánchez, D.; Lázaro-Pérez, N.d.S.; Castro-Arellano, J.J.; Romero-Maldonado, S.; Rico-Arzate, E.; Hoyo-Vadillo, C.; Salgado-Mancilla, M.; Gómez-Cruz, C.Y.; et al. Post Natal Microbial and Metabolite Transmission: The Path from Mother to Infant. *Nutrients* **2024**, *16*, 1990. [CrossRef] [PubMed]
34. Juárez-Castelán, C.J.; Vélez-Ixta, J.M.; Corona-Cervantes, K.; Piña-Escobedo, A.; Cruz-Narváez, Y.; Hinojosa-Velasco, A.; Landero-Montes-de-Oca, M.E.; Davila-Gonzalez, E.; González-Del-Olmo, E.; Bastida-Gonzalez, F.; et al. Entero-Mammary Pathway and Perinatal Transmission of Gut Microbiota and SARS-CoV-2. *Int. J. Mol. Sci.* **2022**, *23*, 10306. [CrossRef]
35. Soto, A.; Martín, V.; Jiménez, E.; Mader, I.; Rodríguez, J.M.; Fernández, L. Lactobacilli and bifidobacteria in human breast milk: Influence of antibiotherapy and other host and clinical factors. *J. Pediatr. Gastroenterol. Nutr.* **2014**, *59*, 78–88. [CrossRef]
36. Greiner, L.L.; Humphrey, D.C.; Holland, S.N.; Anderson, C.J.; Schmitz-Esser, S. The validation of the existence of the entero-mammary pathway and the assessment of the differences of the pathway between first and third parity sows. *Transl. Anim. Sci.* **2022**, *6*, txac047. [CrossRef]
37. Van den Elsen, L.W.J.; Garssen, J.; Burcelin, R.; Verhasselt, V. Shaping the Gut Microbiota by Breastfeeding: The Gateway to Allergy Prevention. *Front. Pediatr.* **2019**, *7*, 47. [CrossRef]

38. Fehr, K.; Moossavi, S.; Sbihi, H.; Boutin, R.C.T.; Bode, L.; Robertson, B.; Yonemitsu, C.; Field, C.J.; Becker, A.B.; Mandhane, P.J.; et al. Breastmilk Feeding Practices Are Associated with the Co-Occurrence of Bacteria in Mothers' Milk and the Infant Gut: The CHILD Cohort Study. *Cell Host Microbe* **2020**, *28*, 285–297.e4. [CrossRef] [PubMed]
39. Spreckels, J.E.; Fernández-Pato, A.; Kruk, M.; Kurilshikov, A.; Garmaeva, S.; Sinha, T.; Ghosh, H.; Harmsen, H.; Fu, J.; Gacesa, R. Analysis of microbial composition and sharing in low-biomass human milk samples: A comparison of DNA isolation and sequencing techniques. *ISME Commun.* **2023**, *3*, 116. [CrossRef]
40. Moossavi, S.; Sepehri, S.; Robertson, B.; Bode, L.; Goruk, S.; Field, C.J.; Lix, L.M.; de Souza, R.J.; Becker, A.B.; Mandhane, P.J.; et al. Composition and Variation of the Human Milk Microbiota Are Influenced by Maternal and Early-Life Factors. *Cell Host Microbe* **2019**, *25*, 324–335.e4. [CrossRef]
41. Gopalakrishna, K.P.; Hand, T.W. Influence of Maternal Milk on the Neonatal Intestinal Microbiome. *Nutrients* **2020**, *12*, 823. [CrossRef] [PubMed]
42. Stewart, C.J.; Ajami, N.J.; O'Brien, J.L.; Hutchinson, D.S.; Smith, D.P.; Wong, M.C.; Ross, M.C.; Lloyd, R.E.; Doddapaneni, H.; Metcalf, G.A.; et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* **2018**, *562*, 583–588. [CrossRef] [PubMed]
43. El Masri, D.; Alemayohu, M.A.; Loperfido, F.; Bianco, I.; Ferrara, C.; Cerbo, R.M.; Ghirardello, S.; Monti, M.C.; Maccarini, B.; Sottotetti, F.; et al. Associations of maternal lifestyle factors with inadequate pregnancy weight gain: Findings from the baseline data of the LIMIT prospective cohort study. *Eur. J. Nutr.* **2024**. *Advance online publication*. [CrossRef]
44. Padilha, M.; Danneskiold-Samsøe, N.B.; Brejnrod, A.; Hoffmann, C.; Cabral, V.P.; Iaucci, J.M.; Sales, C.H.; Fisberg, R.M.; Cortez, R.V.; Brix, S.; et al. The Human Milk Microbiota is Modulated by Maternal Diet. *Microorganisms* **2019**, *7*, 502. [CrossRef]
45. Sun, Q.; Zhou, Q.; Ge, S.; Liu, L.; Li, P.; Gu, Q. Effects of Maternal Diet on Infant Health: A Review Based on Entero-Mammary Pathway of Intestinal Microbiota. *Mol. Nutr. Food Res.* **2024**, *68*, e2400077. [CrossRef]
46. Marsh, A.J.; Azcarate-Peril, M.A.; Aljumaah, M.R.; Neville, J.; Perrin, M.T.; Dean, L.L.; Wheeler, M.D.; Hines, I.N.; Pawlak, R. Fatty acid profile driven by maternal diet is associated with the composition of human milk microbiota. *Front. Microbiom.* **2022**, *1*, 1041752. [CrossRef]
47. Cortes-Macías, E.; Selma-Royo, M.; García-Mantrana, I.; Calatayud, M.; González, S.; Martínez-Costa, C.; Collado, M.C. Maternal Diet Shapes the Breast Milk Microbiota Composition and Diversity: Impact of Mode of Delivery and Antibiotic Exposure. *J. Nutr.* **2021**, *151*, 330–340. [CrossRef] [PubMed]
48. Londoño-Sierra, D.C.; Mesa, V.; Guzmán, N.C.; Bolívar Parra, L.; Montoya-Campuzano, O.I.; Restrepo-Mesa, S.L. Maternal Diet May Modulate Breast Milk Microbiota-A Case Study in a Group of Colombian Women. *Microorganisms* **2023**, *11*, 1812. [CrossRef]
49. Olga, L.; Van Diepen, J.A.; Chichlowski, M.; Petry, C.J.; Vervoort, J.; Dunger, D.B.; Kortman, G.A.M.; Gross, G.; Ong, K.K. Butyrate in Human Milk: Associations with Milk Microbiota, Milk Intake Volume, and Infant Growth. *Nutrients* **2023**, *15*, 916. [CrossRef]
50. LeMay-Nedjelski, L.; Asbury, M.R.; Butcher, J.; Ley, S.H.; Hanley, A.J.; Kiss, A.; Unger, S.; Copeland, J.K.; Wang, P.W.; Stintzi, A.; et al. Maternal Diet and Infant Feeding Practices Are Associated with Variation in the Human Milk Microbiota at 3 Months Postpartum in a Cohort of Women with High Rates of Gestational Glucose Intolerance. *J. Nutr.* **2021**, *151*, 320–329. [CrossRef]
51. Olshan, K.L.; Zomorrodi, A.R.; Pujolassos, M.; Troisi, J.; Khan, N.; Fanelli, B.; Kenyon, V.; Fasano, A.; Leonard, M.M. Microbiota and Metabolomic Patterns in the Breast Milk of Subjects with Celiac Disease on a Gluten-Free Diet. *Nutrients* **2021**, *13*, 2243. [CrossRef] [PubMed]
52. Shenker, N.S.; Perdones-Montero, A.; Burke, A.; Stickland, S.; McDonald, J.A.K.; Alexander-Hardiman, K.; Flanagan, J.; Takats, Z.; Cameron, S.J.S. Metabolomic and Metataxonomic Fingerprinting of Human Milk Suggests Compositional Stability over a Natural Term of Breastfeeding to 24 Months. *Nutrients* **2020**, *12*, 3450. [CrossRef] [PubMed]
53. Browne, P.D.; Aparicio, M.; Alba, C.; Hechler, C.; Beijers, R.; Rodríguez, J.M.; Fernández, L.; de Weerth, C. Human Milk Microbiome and Maternal Postnatal Psychosocial Distress. *Front. Microbiol.* **2019**, *10*, 2333. [CrossRef] [PubMed]
54. Fernández-Tuñas, M.D.C.; Pérez-Muñuzuri, A.; Trastoy-Pena, R.; Pérez Del Molino, M.L.; Couce, M.L. Effects of Maternal Stress on Breast Milk Production and the Microbiota of Very Premature Infants. *Nutrients* **2023**, *15*, 4006. [CrossRef]
55. Aya, V.; Flórez, A.; Perez, L.; Ramírez, J.D. Association between physical activity and changes in intestinal microbiota composition: A systematic review. *PLoS ONE* **2021**, *16*, e0247039. [CrossRef]
56. Cataldi, S.; Bonavolontà, V.; Poli, L.; Clemente, F.M.; De Candia, M.; Carvutto, R.; Silva, A.F.; Badicu, G.; Greco, G.; Fischetti, F. The Relationship between Physical Activity, Physical Exercise, and Human Gut Microbiota in Healthy and Unhealthy Subjects: A Systematic Review. *Biology* **2022**, *11*, 479. [CrossRef]
57. Dorelli, B.; Gallè, F.; De Vito, C.; Duranti, G.; Iachini, M.; Zaccarin, M.; Preziosi Standoli, J.; Ceci, R.; Romano, F.; Liguori, G.; et al. Can Physical Activity Influence Human Gut Microbiota Composition Independently of Diet? A Systematic Review. *Nutrients* **2021**, *13*, 1890. [CrossRef]
58. Bressa, C.; Bailén-Andrino, M.; Pérez-Santiago, J.; González-Soltero, R.; Pérez, M.; Montalvo-Lominchar, M.G.; Maté-Muñoz, J.L.; Domínguez, R.; Moreno, D.; Larrosa, M. Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLoS ONE* **2017**, *12*, e0171352. [CrossRef] [PubMed]
59. Sun, J.; Fang, D.; Wang, Z.; Liu, Y. Sleep Deprivation and Gut Microbiota Dysbiosis: Current Understandings and Implications. *Int. J. Mol. Sci.* **2023**, *24*, 9603. [CrossRef]

60. Gonzalez, E.; Brereton, N.J.B.; Li, C.; Lopez Leyva, L.; Solomons, N.W.; Agellon, L.B.; Scott, M.E.; Koski, K.G. Distinct changes occur in the human breast milk microbiome between early and established lactation in breastfeeding Guatemalan mothers. *Front. Microbiol.* **2021**, *12*, 557180. [CrossRef]
61. Brahe, L.K.; Astrup, A.; Larsen, L.H. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? *Obes. Rev.* **2013**, *14*, 950–959. [PubMed]
62. Paparo, L.; Nocerino, R.; Ciaglia, E.; Di Scala, C.; De Caro, C.; Russo, R.; Trinchese, G.; Aitoro, R.; Amoroso, A.; Bruno, C.; et al. Butyrate as a bioactive human milk protective component against food allergy. *Allergy* **2021**, *76*, 1398–1415. [CrossRef]
63. McGuire, M.K.; McGuire, M.A. Got bacteria? The astounding, yet not-so-surprising, microbiome of human milk. *Curr. Opin. Biotechnol.* **2017**, *44*, 63–68. [CrossRef] [PubMed]
64. Coppola, S.; Avagliano, C.; Calignano, A.; Berni Canani, R. The Protective Role of Butyrate against Obesity and Obesity-Related Diseases. *Molecules* **2021**, *26*, 682. [CrossRef]
65. Harrison, C.L.; Brown, W.J.; Hayman, M.; Moran, L.J.; Redman, L.M. The Role of Physical Activity in Preconception, Pregnancy and Postpartum Health. *Semin. Reprod. Med.* **2016**, *34*, e28–e37. [CrossRef] [PubMed]
66. Be'er, M.; Mandel, D.; Yelak, A.; Gal, D.L.; Mangel, L.; Lubetzky, R. The Effect of Physical Activity on Human Milk Macronutrient Content and Its Volume. *Breastfeed. Med. Off. J. Acad. Breastfeed. Med.* **2020**, *15*, 357–361. [CrossRef]
67. Rodríguez, J.M.; Fernández, L.; Verhasselt, V. The Gut–Breast Axis: Programming Health for Life. *Nutrients* **2021**, *13*, 606. [CrossRef]
68. Konukbay, D.; Öksüz, E.; Guvenc, G. Breastfeeding self-efficacy in terms of sleep quality, perceived social support, depression and certain variables: A cross-sectional study of postpartum women in Turkey. *BMC Pregnancy Childbirth* **2024**, *24*, 231. [CrossRef]
69. Cabrera-Domínguez, G.; De la Calle, M.; Herranz Carrillo, G.; Ruvira, S.; Rodríguez-Rodríguez, P.; Arribas, S.M.; Ramiro-Cortijo, D. Women during Lactation Reduce Their Physical Activity and Sleep Duration Compared to Pregnancy. *Int. J. Environ. Res. Public Health* **2022**, *19*, 11199. [CrossRef]
70. Mutic, A.D.; Jordan, S.; Edwards, S.M.; Ferranti, E.P.; Thul, T.A.; Yang, I. The Postpartum Maternal and Newborn Microbiomes. *MCN Am. J. Matern. Child. Nurs.* **2017**, *42*, 326–331. [CrossRef]
71. Kawashima, A.; Detsuka, N.; Yano, R. Sleep deprivation and fatigue in early postpartum and their association with postpartum depression in primiparas intending to establish breastfeeding. *J. Rural. Med.* **2022**, *17*, 40–49. [CrossRef] [PubMed]
72. Han, M.; Yuan, S.; Zhang, J. The interplay between sleep and gut microbiota. *Brain Res. Bull.* **2022**, *180*, 131–146. [CrossRef]
73. Cicchinelli, S.; Rosa, F.; Manca, F.; Zanza, C.; Ojetti, V.; Covino, M.; Candelli, M.; Gasbarrini, A.; Franceschi, F.; Piccioni, A. The Impact of Smoking on Microbiota: A Narrative Review. *Biomedicines* **2023**, *11*, 1144. [CrossRef] [PubMed]
74. Napierala, M.; Mazela, J.; Merritt, T.A.; Florek, E. Tobacco smoking and breastfeeding: Effect on the lactation process, breast milk composition and infant development. A critical review. *Environ. Res.* **2016**, *151*, 321–338. [CrossRef] [PubMed]
75. Mansouri, B.; Azadi, N.A.; Sharafi, K.; Nakhaee, S. The effects of active and passive smoking on selected trace element levels in human milk. *Sci. Rep.* **2023**, *13*, 20756. [CrossRef]
76. Macchi, M.; Bambini, L.; Franceschini, S.; Alexa, I.D.; Agostoni, C. The effect of tobacco smoking during pregnancy and breastfeeding on human milk composition-a systematic review. *Eur. J. Clin. Nutr.* **2021**, *75*, 736–747. [CrossRef]
77. Zimmermann, P.; Curtis, N. Breast milk microbiota: A review of the factors that influence composition. *J. Infect.* **2021**, *81*, 17–47. [CrossRef]
78. Zhu, Z.; Wang, M.; Guan, Y.; Li, M.; Peng, Q.; Zheng, N.; Ma, W. Altered interaction network in the gut microbiota of current cigarette smokers. *Eng. Microbiol.* **2024**, *4*, 100138. [CrossRef]
79. Mattson, S.N.; Bernes, G.A.; Doyle, L.R. Fetal Alcohol Spectrum Disorders: A Review of the Neurobehavioral Deficits Associated With Prenatal Alcohol Exposure. *Alcohol. Clin. Exp. Res.* **2019**, *43*, 1046–1062. [CrossRef]
80. Bailey, B.A.; Sokol, R.J. Prenatal alcohol exposure and miscarriage, stillbirth, preterm delivery, and sudden infant death syndrome. *Alcohol. Res. Health* **2011**, *34*, 86–91.
81. Drugs and Lactation Database (LactMed®) [Internet]. Bethesda (MD): National Institute of Child Health and Human Development; Alcohol. 2006. Available online: https://www.ncbi.nlm.nih.gov/books/NBK501469/ (accessed on 15 September 2024).
82. Amezcua López, J.A.; García Morales, E.; Pérez-Rulfo Ibarra, D.; Solís Pacheco, J.R.; Aguilar Uscanga, B.R. Effects of maternal toxic substance consumption during breastfeeding on lactic acid bacteria abundance and nutritional content. *Int. J. Med. Sci.* **2023**, *20*, 1513–1526. [CrossRef] [PubMed]
83. Chen, G.; Shi, F.; Yin, W.; Guo, Y.; Liu, A.; Shuai, J.; Sun, J. Gut microbiota dysbiosis: The potential mechanisms by which alcohol disrupts gut and brain functions. *Front. Microbiol.* **2022**, *13*, 916765. [CrossRef]
84. Leclercq, S.; Matamoros, S.; Cani, P.D.; Neyrinck, A.M.; Jamar, F.; Stärkel, P.; Windey, K.; Tremaroli, V.; Bäckhed, F.; Verbeke, K.; et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E4485–E4493. [CrossRef]
85. Wang, Y.; Xie, T.; Wu, Y.; Liu, Y.; Zou, Z.; Bai, J. Impacts of Maternal Diet and Alcohol Consumption during Pregnancy on Maternal and Infant Gut Microbiota. *Biomolecules* **2021**, *11*, 369. [CrossRef] [PubMed]
86. Nagel, E.M.; Howland, M.A.; Pando, C.; Stang, J.; Mason, S.M.; Fields, D.A.; Demerath, E.W. Maternal Psychological Distress and Lactation and Breastfeeding Outcomes: A Narrative Review. *Clin. Ther.* **2022**, *44*, 215–227. [CrossRef]

87. Moubareck, C.A. Human Milk Microbiota and Oligosaccharides: A Glimpse into Benefits, Diversity, and Correlations. *Nutrients* **2021**, *13*, 1123. [CrossRef] [PubMed]
88. Vanuytsel, T.; Van Wanrooy, S.; Vanheel, H.; Vanormelingen, C.; Verschueren, S.; Houben, E.; Salim Rasoel, S.; Tóth, J.; Holvoet, L.; Farré, R.; et al. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* **2014**, *63*, 1293–1299. [CrossRef]
89. Hechler, C.; Borewicz, K.; Beijers, R.; Saccenti, E.; Riksen-Walraven, M.; Smidt, H.; de Weerth, C. Association between Psychosocial Stress and Fecal Microbiota in Pregnant Women. *Sci. Rep.* **2019**, *9*, 4463. [CrossRef]
90. Bailey, M.T.; Dowd, S.E.; Galley, J.D.; Hufnagle, A.R.; Allen, R.G.; Lyte, M. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain Behav. Immun.* **2011**, *25*, 397–407. [CrossRef]
91. Sanchez-Gallardo, R.; Bottacini, F.; O'Neill, I.J.; Esteban-Torres, M.; Moore, R.; McAuliffe, F.M.; Cotter, P.D.; van Sinderen, D. Selective human milk oligosaccharide utilization by members of the *Bifidobacterium pseudocatenulatum* taxon. *Appl. Environ. Microbiol.* **2024**, *Epub ahead of print*. [CrossRef]
92. Okburan, G.; Kızıler, S. Human milk oligosaccharides as prebiotics. *Pediatr. Neonatol.* **2023**, *64*, 231–238. [CrossRef] [PubMed]
93. Lordan, C.; Roche, A.K.; Delsing, D.; Nauta, A.; Groeneveld, A.; MacSharry, J.; Cotter, P.D.; van Sinderen, D. Linking human milk oligosaccharide metabolism and early life gut microbiota: Bifidobacteria and beyond. *Microbiol. Mol. Biol. Rev.* **2024**, *88*, e0009423. [CrossRef] [PubMed]
94. Inchingolo, F.; Inchingolo, A.M.; Latini, G.; Ferrante, L.; de Ruvo, E.; Campanelli, M.; Longo, M.; Palermo, A.; Inchingolo, A.D.; Dipalma, G. Difference in the Intestinal Microbiota between Breastfeed Infants and Infants Fed with Artificial Milk: A Systematic Review. *Pathogens* **2024**, *13*, 533. [CrossRef] [PubMed]
95. Herrera-Quintana, L.; Vázquez-Lorente, H.; Hinojosa-Nogueira, D.; Plaza-Diaz, J. Relationship between Infant Feeding and the Microbiome: Implications for Allergies and Food Intolerances. *Children* **2024**, *11*, 1030. [CrossRef]
96. Eor, J.Y.; Lee, C.S.; Moon, S.H.; Cheon, J.Y.; Pathiraja, D.; Park, B.; Shin, M.J.; Kim, J.Y.; Kim, S.; Noh, Y.; et al. Effect of Probiotic-Fortified Infant Formula on Infant Gut Health and Microbiota Modulation. *Food Sci. Anim. Resour.* **2023**, *43*, 659–673. [CrossRef]
97. Navarro-Tapia, E.; Sebastiani, G.; Sailer, S.; Toledano, L.A.; Serra-Delgado, M.; García-Algar, Ó.; Andreu-Fernández, V. Probiotic Supplementation During the Perinatal and Infant Period: Effects on Gut Dysbiosis and Disease. *Nutrients* **2020**, *12*, 2243. [CrossRef]
98. Ferro, L.E.; Crowley, L.N.; Bittinger, K.; Friedman, E.S.; Decker, J.E.; Russel, K.; Katz, S.; Kim, J.K.; Trabulsi, J.C. Effects of prebiotics, probiotics, and synbiotics on the infant gut microbiota and other health outcomes: A systematic review. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 5620–5642. [CrossRef]
99. Srinivasjois, R.; Gebremedhin, A.; Silva, D.; Rao, S.; Pereira, G. Probiotic supplementation in neonates and long-term gut colonisation: A systematic review of randomised controlled trials. *J. Paediatr. Child Health* **2023**, *59*, 212–217. [CrossRef]
100. Patel, R.M.; Underwood, M.A. Probiotics and necrotizing enterocolitis. *Semin. Pediatr. Surg.* **2018**, *27*, 39–46. [CrossRef]
101. Sharif, S.; Meader, N.; Oddie, S.J.; Rojas-Reyes, M.X.; McGuire, W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database Syst. Rev.* **2020**, *10*, CD005496. [CrossRef]
102. Mazziotta, C.; Tognon, M.; Martini, F.; Torreggiani, E.; Rotondo, J.C. Probiotics Mechanism of Action on Immune Cells and Beneficial Effects on Human Health. *Cells* **2023**, *12*, 184. [CrossRef] [PubMed] [PubMed Central]
103. Chong, H.Y.; Tan, L.T.; Law, J.W.; Hong, K.W.; Ratnasingam, V.; Ab Mutalib, N.S.; Lee, L.H.; Letchumanan, V. Exploring the Potential of Human Milk and Formula Milk on Infants' Gut and Health. *Nutrients* **2022**, *14*, 3554. [CrossRef]
104. World Health Organization. WHO Infant and Young Child Feeding. 2021. Available online: https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding (accessed on 25 February 2023).
105. Dieterich, C.M.; Felice, J.P.; O'Sullivan, E.; Rasmussen, K.M. Breastfeeding and health outcomes for the mother-infant dyad. *Pediatr. Clin. N. Am.* **2013**, *60*, 31–48. [CrossRef] [PubMed]
106. Kwon, E.J.; Kim, Y.J. What is fetal programming? a lifetime health is under the control of in utero health. *Obstet. Gynecol. Sci.* **2017**, *60*, 506–519. [CrossRef]
107. Van Best, N.; Hornef, M.W.; Savelkoul, P.H.; Penders, J. On the origin of species: Factors shaping the establishment of infant's gut microbiota. *Birth Defects Res. Part C Embryo Today Rev.* **2015**, *105*, 240–251. [CrossRef]
108. Verduci, E.; Giannì, M.L.; Vizzari, G.; Vizzuso, S.; Cerasani, J.; Mosca, F.; Zuccotti, G.V. The Triad Mother-Breast Milk-Infant as Predictor of Future Health: A Narrative Review. *Nutrients* **2021**, *13*, 486. [CrossRef] [PubMed]
109. Kim, S.Y.; Yi, D.Y. Analysis of the human breast milk microbiome and bacterial extracellular vesicles in healthy mothers. *Exp. Mol. Med.* **2020**, *52*, 1288–1297. [CrossRef]
110. Taylor, R.; Keane, D.; Borrego, P.; Arcaro, K. Effect of Maternal Diet on Maternal Milk and Breastfed Infant Gut Microbiomes: A Scoping Review. *Nutrients* **2023**, *15*, 1420. [CrossRef]
111. American College of Lifestyle Medicine. Available online: https://lifestylemedicine.org/ (accessed on 13 October 2024).
112. Krishnaswami, J.; Del C Colon-Gonzalez, M. Reforming Women's Health Care: A Call to Action for Lifestyle Medicine Practitioners to Save Lives of Mothers and Infants. *Am. J. Lifestyle Med.* **2019**, *13*, 495–504. [CrossRef]
113. McHugh, J.; Dalal, M.; Agarwal, N. From Preconception Care to the First Day of School: Transforming the Health of New Families With Lifestyle Medicine. *Am. J. Lifestyle Med.* **2020**, *14*, 532–540. [CrossRef]

114. Stinson, L.F.; Ma, J.; Sindi, A.S.; Geddes, D.T. Methodological approaches for studying the human milk microbiome. *Nutr. Rev.* **2023**, *81*, 705–715. [CrossRef] [PubMed]
115. Purushothaman, S.; Meola, M.; Egli, A. Combination of Whole Genome Sequencing and Metagenomics for Microbiological Diagnostics. *Int. J. Mol. Sci.* **2022**, *23*, 9834. [CrossRef] [PubMed]

Appendix B

Folic Acid Supplementation in European Women of Reproductive Age and During Pregnancy with Excessive Weight

Loperfido *et al. Reproductive Health (2025) 22:13*
https://doi.org/10.1186/s12978-025-01953-y

Reproductive Health

REVIEW

Open Access

Folic acid supplementation in European women of reproductive age and during pregnancy with excessive weight: a systematic review

Federica Loperfido[1*†], Francesca Sottotetti[1†], Irene Bianco[1], Dana El Masri[1], Beatrice Maccarini[1], Chiara Ferrara[1], Antonio Limitone[3], Hellas Cena[1,2†] and Rachele De Giuseppe[1†]

Abstract

Objective Neural tube defects (NTDs), well-known consequences of folate deficiency, are the second most common cause of serious birth defects, affecting approximately one in a thousand pregnancies in Europe. Maternal folate deficiency before conception and during early pregnancy has been suggested as the most important preventable risk factor for NTDs; thus women should be supplemented before conception with 0.4 mg of folic acid (FA) until the first trimester of gestation. Findings have described a positive association between elevated Body Mass Index (BMI) and birth defect risk; data on plasma folate levels in pregnant women with obesity have shown values lower than recommended because of a state of chronic low-grade inflammation, resulting in increased metabolic demands. Nowadays, disparities exist regarding the recommended dose of FA in women at risk, including women of childbearing age with excessive weight. Therefore, this systematic review aimed to investigate if European childbearing age/pregnant women with overweight/obesity are supplemented according to the current country-specific FA recommendations and whether the dosage of 5 mg recommended for pregnant women with obesity is effective in preventing NTDs.

Methods The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed. An electronic database search of manuscripts was carried out in Web of Sciences, PubMed and Medline. The quality of the included studies was assessed by using the Quality Assessment for Diverse Studies statement.

Results Out of 1718 records identified, 8 manuscripts met all the inclusion criteria. Overall, the results showed that pregnant women with obesity adherent to FA recommendations ranged between 4% and 9.5%. Furthermore, the majority (61%) started the supplementation after conception, highlighting that European pregnant women are not particularly adherent to recommendations during the period of greatest need.

Conclusions The scarce adherence to the current guidelines shows an urgent need to standardize the recommendations across European countries. Particularly, women of childbearing age with excess weight should be monitored

†Federica Loperfido and Francesca Sottotetti contributed equally to this work.

†Hellas Cena and Rachele De Giuseppe contributed equally to this work.

*Correspondence:
Federica Loperfido
federica.loperfido@unipv.it
Full list of author information is available at the end of the article

assessing serum folate, RBC folate, and homocysteine levels developing tailored supplementation protocols, to counteract the occurrence of NTDs.

Keywords Neural tube defects, Folic acid, Childbearing age, Pregnancy, Women with excessive weight, Supplementation

Plain English Summary

- Neural tube defects (NTDs) are severe congenital abnormalities connected to maternal folate deficiency. Current international guidelines recommend a daily supplementation of 0.4 mg of folic acid (FA) starting before conception and during the first trimester of pregnancy, to prevent NTDs.
- Women with excessive weight need higher folic acid doses due to their altered metabolic demands. Women with obesity encounter a greater risk of NTDs, as they often have lower plasma folate levels. This deficiency may result from chronic low-grade inflammation and increased nutrient requirements. For these vulnerable women, a higher dose of 5 mg of FA is recommended by the World Health Organization guidelines.
- However, only 4–9.5% of pregnant women with obesity adhere to FA supplementation recommendations, taking FA supplementation after conception, and missing the critical preconception period necessary for effective NTDs prevention.
- There is an urgent need to standardize FA supplementation recommendations across European countries and to monitor the folate status of women with excess weight. This will help develop personalized supplementation strategies aimed at effectively reducing the risk of NTDs.

Introduction

Neural tube defects (NTDs), are the second most common cause of serious congenital disorders and affect 0.2 –10 per 1000 established pregnancies worldwide, including about 1 in 1000 pregnancies in Europe [1, 2]. NTDs result from a failure of the neural tube to close properly within 4 weeks following conception. There are different types of NTDs, among which spina bifida, anencephaly, and encephalocele, are the most prevalent forms, while iniencephaly and craniorachischisis, are considered rare. The clinical characteristics and outcomes differ depending on the type of NTD [3]. In recent decades, maternal folate deficiency before conception and during early pregnancy has been suggested as the most preventable risk factor for NTDs. As reported by the World Health Organization (WHO), Red Blood Cell (RBC) folate concentrations should be above 400 ng/mL (906 nmol/L) in women of reproductive age to achieve the greatest reduction in NTDs [4]. Thus, women should i) supplement with 0.4 mg of folic acid (FA) before conception until the first trimester of pregnancy, ii) regularly include foods naturally rich in folate into their diet (e.g. leafy green vegetables such as spinach, asparagus, beets, broccoli, and artichokes), iii) consume fortified foods [5–7]. Indeed, mandatory fortification of staple foods, such as wheat flour, maize flour, and rice with folic acid, has become an important public health strategy for the primary prevention of NTDs. This safe and cost-effective initiative is currently implemented in nearly 60 countries worldwide and has successfully prevented a substantial number of NTD cases [3].

Data reveal that folate deficiency (defined as < 7 nmol/L) [8] is rare (0–5%) in developed countries; however, insufficient levels of folate (defined as < 25.5 nmol/L) [8] are more common (40–50%), suggesting a higher risk of NTDs even though folate storage may be adequate [9]. To date, despite the WHO's recommendation to start FA supplementation during childbearing age, many women begin later, often during the first trimester of pregnancy, which reduces its protective effect [10]. In this regard, existing literature reports a notable prevalence of unplanned pregnancies, potentially leading to delays in supplementation [11, 12].

Concurrently, the rising incidence of overweight and obesity among adolescents, particularly during the childbearing years, become one of the most significant challenges in obstetric care, due to its potential implications for maternal and fetal health [13]. Notably, in Europe, the prevalence of pre-pregnancy overweight and obesity ranges between 26.8% and 54.0% [14]. Research has indicated markedly decreased plasma folate levels in pregnant women with obesity, showing much lower values than those recommended, exposing them to higher risks of developing NTDs [15, 16]. Specifically, the literature indicates that during the first trimester, women with excessive weight exhibit lower serum folate levels when compared to those of normal weight ($\beta = -2.3$, $p < 0.01$),

showing an association with a higher likelihood of folate deficiency (OR = 2.0, p < 0.01) [17].

Findings from a recent meta-analysis registered a positive association between women with excessive pre-gravid Body Mass Index (BMI) and congenital abnormalities such as spina bifida and other NTDs when compared to those with normal pre-gravid BMI [18].

Women with obesity may have a lower folate level caused by a state of chronic low-grade inflammation, which results in an increased metabolic requirement [19]. This phenomenon could be explained by what is commonly defined as the "obesity paradox" [20]. According to this theory, individuals with excessive weight exhibit higher activity of the enzyme cytochrome P450 (CYP) 2E1, which can use FA as a substrate [21]. Indeed, women with overweight or obesity have been found to have higher levels of plasma folate oxidation products (specifically, 5-methyltetrahydrofolate oxidation product; MeFox) compared to those without obesity [19]. Overall, current global guidelines agree that women with a history of NTDs should take a higher dose of folic acid (5 mg) [5]. Although the same dosage is also recommended for women of childbearing age who are planning for pregnancy and pregnant women with obesity, the supporting scientific evidence remains limited [22, 23]. Therefore, further research and systematic reviews are essential to enable clinicians to provide an appropriate dosage.

Furthermore, because NTDs and obesity continue to have a significant health and economic impact at the European level, it is crucial to identify women at risk, to develop tailored recommendations and specific guidelines for FA supplementation and NTDs prevention. Considering the substantial evidence highlighting the benefits of folate fortification, this strategy should be considered alongside supplementation to achieve equitable primary prevention of NTDs globally.

Based on these considerations, the main research question of the present systematic review aimed at investigating if European childbearing age/pregnant women with overweight/obesity are supplemented according to the current country-specific FA recommendations.

Additionally, the authors will examine whether the dosage of 5 mg recommended for pregnant women with obesity is effective in preventing NTDs. The potential role of folate food fortification, when addressed in the studies included, will also be discussed.

Methods

The present systematic review has been registered in the PROSPERO International prospective register of systematic reviews (www.crd.york.ac.uk/PROSPERO; register no. CRD42024469780), and has been reported following the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) guidelines [24].

Search strategy

A systematic literature review was initially performed in October 2023, then repeated in December 2023, and finally in February 2024. PubMed (http://www.ncbi.nlm.nih.gov/pubmed), and Web of Science have been searched, without time range restriction. A literature search was performed using structured search strings considering the following combined search terms for pregnancy and childbearing age, weight status, and FA supplementation. Therefore, "pregnan*" OR "gestation*" OR "preconception" OR "peri-conception" OR "prepregnancy" OR "pre-pregnancy" OR "childbearing" combined to "obes*" OR "overweight" OR "body mass index" OR "BMI", as well as "folic acid" OR "folin*" OR "vitamin B9" OR "folat*". A search sample has been added in the Additional File section (Additional file 1): "Query used for the search in the different databases".

Types of studies

Studies referred to humans were considered. Clinical Study, Clinical Trial, Clinical Trial, Phase IV, Clinical Trial Protocol, Clinical Trial, Phase I, Clinical Trial, Phase II, Clinical Trial, Phase III, Comparative Study, Controlled Clinical Trial, Multicenter Study, Observational Study, Randomized Controlled Trial in English language investigating both childbearing/pregnant women with overweight/obesity related to FA supplementation and completed in European countries were included. Research articles have considered referring to European women of childbearing age/European pregnant women with overweight and obesity as one of the populations at greater risk of giving birth to newborns with NTDs.

Types of participants

Eligible participants were women of childbearing age or pregnant (age range ≥ 18 years) with overweight or obesity defined by the WHO (overweight, 25.0–29.9; grade 1 obesity, 30.0–34.9; grade 2 obesity, 35.0–39.9; and grade 3 obesity, ≥ 40.0.) [25].

Outcome

The present systematic review aimed to explore if women of childbearing age/pregnant women with overweight/obesity across European countries supplement with FA according to the recommendations. Since childbearing age/pregnant women with obesity require a higher dosage of FA, the secondary objective of our systematic review was to determine whether the current recommended dosage is adequate to achieve blood folate levels within the reference range. Finally, the

authors evaluated whether the higher FA dose (5 mg) recommended for pregnant women with obesity, is effective in preventing NTDs in the offspring.

Inclusion and exclusion criteria

The inclusion criteria for exposed women were: aged ≥ 18 years; not diagnosed with type 2 Diabetes Mellitus (T2DM) and diseases related to malabsorption (*e.g.* celiac disease; Inflammatory Bowel Disease (IBD); bariatric surgery); no alcohol abuse; no enzyme defects related to folate metabolism (MTHFR mutation).

The exclusion criteria were as follows: aged < 18 years; diagnosed with T2DM and diseases related to malabsorption (e.g. celiac disease; IBD; bariatric surgery); alcohol abuse; defects of enzymes related to folate metabolism.

Study selection, data collection, and extraction

The flowchart of the study selection process is presented in Fig. 1, according to the PRISMA guidelines [24]. Three coauthors (FL, FS, and IB) determined whether the studies met the criteria previously established by undertaking the initial duplicates, title screening, and abstract review independently. In brief, after applying the search filters, the studies were equally divided among the three coauthors for the data extraction. A fourth co-author (DEM) randomly checked a sample of about 20% of the studies. Before the inclusion in the manuscript, each full-text article selected for retrieval has been reviewed independently by the three coauthors, checking the eligibility. Any difference in the selection process has been determined by discussion. Whenever there is no full consensus, a fourth co-author has been consulted. The three co-authors independently extracted relevant information from all the included studies on an Office Excel datasheet, as follows: *i)* authors and publication year; *ii)* type of the study; *iv)* country; *v)* sample size; *vi)* characteristics of participants (*e.g.* age, childbearing age or pregnant, demographic and socioeconomic characteristics, BMI expressed as Kg/m^2; *vii)* outcome assessment (FA supplementation evaluation—expressed as Y/N – related to BMI; FA supplementation dosage—expressed as mg/die—related to BMI; blood folate levels – expressed as ng/mL – related to BMI); *viii)* timing of supplementation (preconceptionally/during the first trimester); *ix)* protocol of supplementation (type of supplementation e.g. exclusively FA, multi-vitamins supplementation, formulation of supplement, frequency of supplementation); *x)* summary of findings; *xi)* European country-specific FA policy fortification. Whenever there was no consensus, a fourth co-author was consulted.

Data synthesis

Data extracted from this systematic research are presented as a summary of findings and the quality assessment of the eligible studies are shown in Tables 1 and 2.

Moreover, the extracted data are summarized according to the following columns: *i)* authors; *ii)* type of the study; *iv)* country; *vi)* characteristics of participants (*e.g.* age, childbearing age or pregnancy, demographic and socioeconomic characteristics, BMI expressed as Kg/m^2; *vii)* outcome assessment (FA supplementation evaluation—expressed as Y/N – related to BMI; FA supplementation dosage—expressed as mg/die—related to BMI; blood folate levels – expressed as ng/mL – related to BMI), *viii)* timing of supplementation (preconceptionally/during the first trimester); *ix)* protocol of supplementation (type of supplementation e.g. (exclusively FA, multi-vitamins supplementation, formulation of supplement, frequency of supplementation); *xi)* European country-specific FA policy fortification.

The blood folate levels have been taken into account about the FA-supplemented dosage, considering different classes of BMI (normal weight: 18.5 ≤ BMI < 24.99 kg/m^2; overweight: 25.00 ≤ BMI < 29.99 kg/m^2; obesity: BMI ≥ 30.00 kg/m^2) and countries.

In general, all continuous variables are reported/converted into means and SDs. Data regarding frequencies will be presented as percentages and the absolute number.

Study quality assessment

Regarding the quality assessment of the human-included studies, three co-authors (FL, FS, IB) independently assessed the quality of each study that met inclusion criteria. Following a discussion, the final score was determined; any differences were discussed with a fourth reviewer (DEM).

The authors adopted the criteria for quality appraisal from the *Quality Assessment for Diverse Studies (QuADS)* [26]. QuADS consists of 16 quality criteria, with 14 applying to qualitative studies, 14 to quantitative studies, and all 16 to any mixed methods study. Each criterion is rated on a scale from 0 to 3. The scores have been converted into percentages. No specific score was set as a cut-off for determining whether a study is of high or low quality. For instance, the authors then discuss the quality assessment considering the studies included.

Results

Overview of the studies

Searches from PubMed and Web of Science (WoS) returned 1718 records: 759 were excluded based on the applied filters (language, species, study design) (Fig. 1).

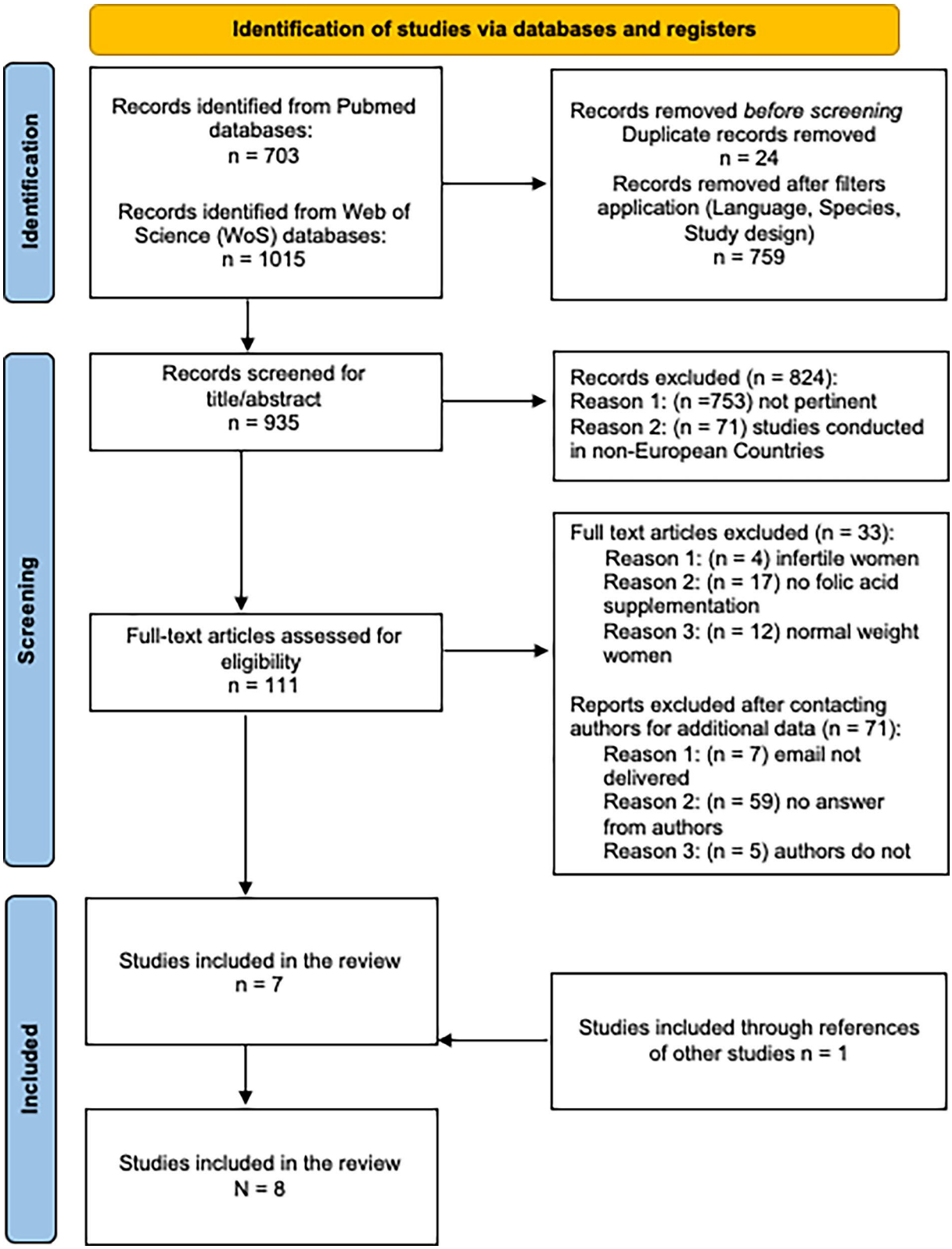


Fig. 1 Flowchart of the study selection process, based on the PRISMA guidelines

Table 1 Description of the studies selected according to the inclusion and exclusion criteria and included in the systematic review

Authors	Study Design	Country	Sample Size	Study Participants	Aim of the Study	FA supplementation evaluation Y/N	Dietary folate intake Y/N	FA dosage (mg)	FA supplementation protocol	FA supplementation related to BMI	FA timing of supplementation	FA Recommendation	Folate Blood Level (if available)	Fortification Policy
Cawley et al. [28]	Prospective Cohort Study	Ireland	n = 587	Pregnant women in the 1rst trimester: - BMI < 18.5 kg/m2: 1.9% (n = 11) - 18.5 < BMI < 25 kg/m2: 50.7% (n = 296) - 25 < BMI < 30 kg/m2: n = 171 (29,3%) - BMI > 30 kg/m2: n = 106 (18,1%)	To analyse folic acid supplementation among women seeking antenatal care at a large maternity hospital in Ireland	Y	N	- 0,4 mg of FA; - 5 mg as high-dose	na	- 0.4 mg FA (n = 549) - 5 mg (2 of them with obesity) (n = 15)	-Preconceptionally; - > 12 weeks preconceptionally; - Post conception	- 12 weeks of 400 mg folic acid (FA) supplementation is needed to reach the target RCF level of 906 nmols[38]; - International guidelines suggest that women with obesity should take high-dose (5 mg) FA around the time of conception to lower their risk of neural tube defects (NTDs)[38]	na	Northern Ireland: Mandatory fortification of wheat flour [35]. The NCFAFF recommends the mandatory fortification with FA of most white, brown and wholemeal breads on sale in Ireland [36]
Linell et al. [29]	Prospective Cohort Study	Ireland	n = 328	Pregnant women at ≤ 18 weeks' gestation: - BMI < 18.5 kg/m2: 1,2% (n = 4) - 18.5 < BMI < 25 kg/m2: 41,2% (n = 135) - 25 < BMI < 30 kg/m2: 33,8% (n = 111) - BMI > 30 kg/m2: 23,8% (n = 78)	To assess the use of folic acid supplementation among pregnant women, based on their obesity status	Y	N	- 0,4 mg of FA; - 5 mg as high-dose	na	- 0.4 mg FA; n = 216 (65,9%) - 5 mg; n = 22 (6,7%) out of the 78 women with obesity	- 30.2% (n = 99) commenced FA < 12 weeks prior to conception; - 30.5% (n = 100) commenced FA > 12 weeks prior to conception; - 61% (n = 199) commenced FA when found out they were pregnant	-Women of childbearing age should take 400 µg of folic acid daily, alongside dietary sources, for at least 12 weeks before conception - Women with obesity are recommended a higher dose of 5 mg of folic acid to reduce the risk of neural tube defects (NTDs) - The 5 mg dosage of FA should be taken before conception and continued through the first trimester of pregnancy[39]	na	Northern Ireland: Mandatory fortification of wheat flour [35]. The NCFAFF recommends the mandatory fortification with FA of most white, brown and wholemeal breads on sale in Ireland [36]

Table 1 (continued)

Authors	Study Design	Country	Sample Size	Study Participants	Aim of the Study	FA supplementation evaluation Y/N	Dietary folate intake Y/N	FA dosage (mg)	FA supplementation protocol	FA supplementation related to BMI	FA timing of supplementation	FA Recommendation	Folate Blood Level (if available)	Fortification Policy
Malvasi A. et al. [30]	Prospective, randomized, double-blind, placebo controlled clinical trial, pilot study	Italy, Greece, Russia	n = 48	- Uniparous healthy pregnant women between 13 and 24th week of pregnancy - 25 < BMI < 30 kg/m2	To assess biochemical parameters during the second trimester of pregnancy in women who received Inositol supplementation	N (preconception not mentioned)	N	- 0,4 mg of FA	Formulation of Supplement (MDFN)—Daily	- n = 24 (treated group): 0,4 mg - n = 24 (control group): NA	Between 13 and 24th week of pregnancy	na	na	No official food fortification policy [35]
Mohd-Shukri N. et al. [31]	Prospective case–control study	UK	n = 241	- BMI > 40 kg/m2: n = 148 - BMI < 25 kg/m2: n = 93	To compare dietary habits and physical activity levels during pregnancy between women with very severe obesity and those of normal weight	Y	Y	- 0,4 mg of FA; - 5 mg as high-dose	na	BMI < 25 kg/m2: - 0.4 mg FA; n = 84 -5 mg of FA; n = 2 BMI > 40 kg/m2: - 0.4 mg FA; n = 129 (96%) -5 mg of FA; n = 5 (4%)	- Early pregnancy (16 weeks of gestation); - Late pregnancy (28 weeks of gestation)	na	Early pregnancy measurements (16 weeks), (mcg/mL)(SD) - Women with BMI > 40 kg/m2, (n = 25): 7.9 mcg/mL (4.2) - Women with BMI < 25 kg/m2, (n = 25): 15.0 mcg/mL (2.7) Late pregnancy (28 weeks), (mcg/mL)(SD) - Women with BMI > 40 kg/m2, (n = 25): 3.9 mcg/mL (2.8) - Women with BMI < 25 kg/m2, (n = 25): 10.6 mcg/mL (5.7)	Mandatory fortification of wheat flour [35] New legislation (effective November 2024) mandates FA fortification of non-whole-meal wheat flour by late 2026 [37]

Table 1 (continued)

Authors	Study Design	Country	Sample Size	Study Participants	Aim of the Study	FA supplementation evaluation Y/N	Dietary folate intake Y/N	FA dosage (mg)	FA supplementation protocol	FA supplementation related to BMI	FA timing of supplementation	FA Recommendation	Folate Blood Level (if available)	Fortification Policy
Redfern K. et al. [32]	Prospective observational study	UK	n = 66	Pregnant women with obesity: a BMI ≥ 30 kg/m2 and < 40 kg/m2, at 12 and 14 weeks of gestation	To examine the intake of key micronutrients (iodine, vitamin D, folate) among pregnant women with obesity in the UK, considering relevant demographic characteristics	Y	Y	- 0,4 mg of FA; - 5 mg as high-dose	- Within pregnancy multivitamin; - FA	5 mg of FA, n = 17 (26%) - n = 5 women supplement 5 mg of FA (2/5 with GDM) - n = 12 women supplement 5 mg + 0,4 mg (6/12 with GDM) 0.4 mg FA, n = 24 (36%)	1st trimester	- Women should supplement with 400 µg of folic acid daily from pre-conception until 12 weeks of gestation [40]; - The RCOG, advises that women with obesity who are planning to become pregnant or are already pregnant should take a higher dose of 5 mg of folic acid daily until the end of the first trimester [40]	na	Mandatory fortification of wheat flour [35] New legislation (effective November 2024) mandates FA fortification of non-whole-meal wheat flour by late 2026 [37]
Santamaria A. et al. [33]	Randomized Controlled Trial	Italy	n = 220	- 25 < BMI < 30 kg/m2	To assess whether myo-inositol supplementation can reduce the rate of gestational diabetes mellitus (GDM) in overweight women	N (preconception not mentioned)	N	0,4 mg of FA	- Treated group received: 2 g myo-inositol + 0.2 mg of FA twice a day, - Placebo group received: 0.2 mg of FA twice a day	n = 220 supplemented with 0.2 mg of FA twice a day	12th–13th week of gestation	na	na	No official food fortification policy [35]
Vitale S. et al. [34]	Randomized and Open-Label, placebo Controlled clinical Trial	Italy	n = 223	- 25 < BMI < 30 kg/m2	To examine the effects of myo-inositol supplementation on GDM rates and body water distribution in women with overweight	N (preconception not mentioned)	N	0,4 mg of FA	- Treated group received: 2 g myo-inositol + 0.2 mg of FA twice a day, - Placebo group received: 0.2 mg of FA twice a day	n = 223 supplemented with 0.2 mg of FA twice a day	12th–13th week of gestation The treatment lasted until 3 weeks after delivery	na	na	No official food fortification policy [35]

Table 1 (continued)

Authors	Study Design	Country	Sample Size	Study Participants	Aim of the Study	FA supplementation evaluation Y/N	Dietary folate intake Y/N	FA dosage (mg)	FA supplementation protocol	FA supplementation related to BMI	FA timing of supplementation	FA Recommendation	Folate Blood Level (if available)	Fortification Policy
O'Malley E. et al. [27]	Prospective observational study	Ireland	n = 496	- 18.5 < BMI < 25 kg/m2: n = 269 - BMI > 30 kg/m2: n = 97	To investigate the association between maternal BMI in early pregnancy and serum and RBC folate and plasma vitamin B12 levels	Y	Y	- 0.4 mg of FA; - 5 mg as high-dose	na	Higher dose of FA (5 mg): - BMI > 30 kg/m2: 9.5% (n = 8); - 18.5 < BMI < 25 kg/m2: 7.1% (n = 19) 0.4 mg of FA: - BMI > 30 kg/m2: 90.4% (n = 76);	Women were asked about their FA supplementation - at enrollment (first visit: 12,1 weeks) - pre-pregnancy (retrospectively)	The RCOG [39], RANZCOG[41] and Irish guideline[42] recommendations for obese women advise a dose of 5 mg daily	There was no statistical difference in the mean values of RBC folate between BMI categories: - BMI < 25.0 kg/m2: 139.8 nmol/L (SD 413.5) - BMI > 30 kg/m2: 1184.0 nmol/L (SD 476.7) (p = 0.18)	Northern Ireland: Mandatory fortification of wheat flour [35]. The NCFAFF recommends the mandatory fortification with FA of most white, brown and wholemeal breads on sale in Ireland [36]

Data are presented as percentage and absolute number of pregnant women with overweight or obesity that are supplemented with normal (0.4 mg) or higher (5 mg) of folic acid. Authors also reported the normal weight and underweight status data other than folate blood level (mcg/mL) (if applicable)

FA: Folic Acid; RCF: Red Cell Folate; GDM: Gestational Diabetes Mellitus; RCOG: Royal College of Obstetricians and Gynaecologists; RANZCOG: Royal Australian and New Zealand College of Obstetricians and Gynecologists; BMI: Body Mass Index; na: not applicable; NCFAFF: National Committee for Folic Acid Food Fortification

Table 2 Quality assessment

Items	Cawley et al. [28]	Linell et al. [29]	Malvasi et al. [30]	Nor Moh-d-Shukri et al. [31]	Redfern K et al. [32]	Santamaria et al. [33]	Vitale S et al. [34]	O'Malley et al. [27]
1. Theoretical or conceptual underpinning to the research	3	2	3	2	3	3	2	3
2. Statement of research aim/s	3	3	3	2	3	3	3	3
3. Clear description of research setting and target population	3	3	3	2	3	3	1	2
4. The study design is appropriate to address the stated research aim/s	3	3	2	3	3	3	2	3
5. Appropriate sampling to address the research aim/s	3	3	1	2	2	2	0	2
6. Rationale for choice of data collection tool/s	2	3	2	2	2	2	2	3
7. The format and content of data collection tool is appropriate to address the stated research aim/s	3	2	2	3	3	2	2	3
8. Description of data collection procedure	2	2	3	3	3	3	2	3
9. Recruitment data provided	3	2	3	2	3	2	2	3
10. Justification for analytic method selected	2	3	2	2	2	2	2	2
11. The method of analysis was appropriate to answer the research aim/s	2	3	2	2	2	3	3	3
12. Evidence that the research stakeholders have been considered in research design or conduct	2	1	3	2	2	2	2	2
13. Strengths and limitations critically discussed	3	1	3	1	3	3	3	3
Total score (sum and % of 13 items' score)	34 (80,90%)	31 (73,80%)	32 (76,20%)	28 (66,60%)	34 (80,90%)	33 (78,50%)	26 (66,66%)	35 (83,30%)

The study quality for the eligible studies is presented. A value of between 0 and 3 was assigned to each item

Duplicates (n = 24) were removed. Thus, 824 studies were excluded as their titles/abstracts were not relevant, and 111 studies were assessed for eligibility and full-text screening. Of these, 33 studies were excluded since they did not meet the inclusion criteria, and 71 studies were excluded after contacting the corresponding authors to obtain the datasets and extrapolate the required additional data. Moreover, one other study was selected during the full-text screening since it reported outcomes of relevant interest [27]. In total, 8 studies [27–34] were included in this systematic review. Overall, the studies were conducted in Ireland, Italy, the United Kingdom, Greece, and Russia. Data extracted from this systematic research, including those related to food fortification policies [35–37], are summarized in Table 1.

Overview of the study quality

The assessment of the study's quality for the eligible studies in this systematic review is presented in Table 2. Out of the total studies, the majority (n = 6) [27–30, 32, 33] achieved a total score higher than 70%; the others (n = 2) scored slightly lower, with a percentage above 66% [31, 34].

Characteristics of studies included in the systematic review

The 8 included studies in this systematic review [27–34] are summarized below. All the studies take into account pregnant women with excessive weight and consider FA supplementation in the preconception period and/or during pregnancy. Considering BMI, the studies focused on women with both overweight and/or obesity based on WHO classification (BMI ≥ 25.0 kg/m^2: overweight; BMI ≥ 30.0 kg/m^2: obesity) [43]. As far as studies included in this review, three of them involved women who were taking FA supplements preconceptionally [28, 29], while the rest focused on FA supplementation starting from the 1st trimester [27, 30–34]. Briefly, Cawley and colleagues [28] conducted a prospective cohort study to assess the impact of FA supplementation in n = 587 pregnant women. Questionnaires were used to gather information on FA supplementation during either pre- and periconceptional periods, including details on dosage and specific brand names of the supplements. The authors stratified the population according to BMI, reporting 29.3% (n = 171) women with overweight and 18.1% (n = 106) with obesity. Approximately 75% of the participants did not meet FA supplementation recommendations, which increased the risk of having low Red Cell Folate (RCF) levels. Specifically, only 5.7% (n = 6) of women with obesity were taking the recommended higher dosage of FA (5 mg). Similarly, in their prospective cohort study, Linell et al. [29], stated that out of n = 78 women with a BMI ≥ 30.0 kg/m^2, only 6.7% were supplemented with the recommended higher dosage of FA (p = < 0,001). Furthermore, data revealed that 61% (n = 199) of women started FA supplementation upon becoming aware of their pregnancy, which increased the risk of NTDs in the offspring. These findings are consistent with the results of a case–control study conducted by Mohd-Shukri et al. [31] which examined the role of dietary habits and physical activity in a sample of pregnant women with severe obesity (BMI > 40 kg/m^2) compared to the control group. Notably, only 31% of the former started taking FA supplementations before conception. The study also encompassed the assessment of serum folate levels at 16 weeks (early pregnancy) and 28 weeks (late pregnancy) of gestation in a subset of both groups (n = 25). Results revealed that at the 28th week, women with severe obesity exhibited an average serum folate level of 3.9 ng/mL (± 2.8 SD), while the control group registered 10.6 ng/mL (± 5.7 SD) (p < 0.0001). In this context, the authors highlighted that pregnant woman with obesity exhibited significantly lower circulating folate levels compared to the control group, with early pregnancy (16 weeks) levels averaging 7.9 ng/mL (± 4.2 SD) in women with obesity, versus 15.0 ng/mL (± 2.7 SD) in the control group. Noteworthy, 96% of women with obesity supplemented with 0.4 mg of FA, and did not adhere to the recommendations. While 2% (n = 5) of them did not supplement either before or during pregnancy. Focusing on pregnant women with obesity, Redfern et al.[32], in a cohort of English women, recorded differences in the FA dosage. Out of the 66 recruited women, 26% (n = 17) took 5 mg of FA during the first trimester; n = 12 of these added an extra dose of 0.4 mg. The remaining 36% did not adhere to the recommendations and only supplemented with 0.4 mg. Out of 24 women who were not taking FA by the end of the first trimester, only one achieved the Recommended Nutritional Intake (RNI) of 300 ug of folate through diet. Notably, the study did not assess serum folate levels. In contrast, the prospective-observational study by O'Malley et al. [27], aimed to analyze the association between maternal BMI in early pregnancy and serum folate levels, considering both dietary intake and FA supplementation protocol. Among the 84 women with obesity who were aware of the supplemented dosage of FA, only 9,5% (n = 8) reported taking 5.0 mg, as recommended. These data were consistent with serum folate levels being lower in women with BMI > 30 kg/m^2 compared to the control group (9.23 ng/mL vs 10.44 ng/mL, P = 0.02). However, no significant difference was observed in the mean RCF between the two groups.

Two randomized controlled trials (RCT) were included in this systematic review [30, 33]. Their objective was to evaluate the effectiveness of a supplement containing Myo-inositol (4 g/day) and FA (0.4 mg/day) in preventing

gestational diabetes compared to the placebo group, which received only 0.4 mg of FA per day. Both studies involved overweight pregnant women who started the supplementation protocol at 12–13 weeks of gestation. Overall, the studies demonstrated that the group of women undergoing treatment with Myo-inositol and FA exhibited improved lipid (cholesterol, $p=0.0001$; LDL, $p=0.0001$; HDL, $p=0.047$; TG, $p=0.0001$) and glycemic profiles (glycemia, $p=0.019$) compared with the control group [30]. Furthermore, a reduction in the incidence of GDM was recognised within the same group (11.6% versus 27.4%, respectively, $p=0.004$) [33].

Discussion

Although a decline in the prevalence of NTDs was observed between 2001 and 2015, the prevailing data remains alarming, counting two cases per 1000 births, amounting to an estimated 214.000–322.000 affected pregnancies worldwide annually [3]. Furthermore, while historically NTDs were predominant in low-income countries, nowadays their occurrence increased in high-middle-income countries, notably in Europe. From 1998 to 2017, an estimated 95.213 NTD-affected pregnancies were recorded among 104 million births across 28 countries in the European Union, reflecting a prevalence rate of 0.92 per 1.000 births [44].

Studies reported an association between maternal high BMI (≥ 30 kg/m^2) and the severity of NTDs, identifying this population as more vulnerable [45]. Indeed, women with excessive weight exhibit lower folate levels due to several factors, such as chronic low-grade inflammation, poor-quality diet adherence, and non-compliance to supplementation recommendations [46]. Moreover, reduced intake of FA is often attributed to unplanned pregnancies and ineffective contraceptive methods [47]. For these reasons, scientific literature related to FA supplementation in women of childbearing age with excessive weight has been systematically reviewed. Studies conducted in European countries were evaluated to assess FA supplementation practices. Although this review primarily focuses on FA supplementation, the contribution of folate fortification in staple foods as an additional preventive strategy for NTDs is also recognized and discussed, due to its demonstrated effectiveness in reducing folate deficiency at the population level.

As shown in Table 2, most of the studies included were of good quality showing a percentage higher than 66% according to the QUADs criteria.

Overall, most of the studies analyzed in this review reported a high number of women non-compliant with the FA recommendations during the periconceptional period [27–29, 31, 32, 34]. In particular, the study conducted by Cawley and colleagues exhibits that only 5.7% ($n=6$) of pregnant women with obesity were taking the recommended higher dosage (5 mg) of FA supplementation [28]. These data were in line with the results by Linell et al., showing that only 6.7% of women with obesity were supplemented with the recommended higher dose, emphasizing the low adherence to FA recommendations in this vulnerable group [29, 48]. Mohd-Shukri and colleagues [31] also reported lower folate levels in women with obesity, emphasizing the need for improved supplementation practices. These results might be due to inadequate adherence to the FA protocol and chronic low-grade inflammatory state, typical of obesity [49]. Regarding diet, a poor-quality and unbalanced dietary pattern can lead to micronutrient deficiencies, including vitamin B12 and folate, which are often observed in individuals with a high BMI [50]. Specifically, folate deficiency can reduce methyl group availability, resulting in higher homocysteine levels. This condition is known as a risk factor for different adverse health outcomes, including neurological disorders, vascular diseases, and reproductive health [51]. High maternal homocysteine levels are also associated with pregnancy complications, posing a threat to the health of the maternal–fetal dyad in the short and long term [52]. For instance, pregnant women with obesity have increased risks related to pregnancy, delivery, and postpartum, such as pregnancy-induced hypertension and GDM, preeclampsia, and cesarean section [53]. Still, cardiometabolic and neurodevelopment impairment has been detected in the offspring of women with obesity, other than higher incidence of large for gestational age (LGA) babies, perinatal mortality, and congenital anomalies, including NTDs [54]. The existing body of literature reveals a paucity of studies delving into the effect of FA supplementation on NTDs incidence. Notably, out of the studies included in this systematic review, only two investigated health outcomes in newborns [33, 34]. However, they evaluated anthropometric measurements at birth, such as weight, height, and head circumference, as well as the incidence of macrosomia, without addressing the occurrence of NTDs [33, 34].

Two randomized controlled trials included in the present review evaluated the efficacy of FA supplementation combined with Myo-inositol on the incidence of GDM [33, 34], indicating a positive impact. However, it was unclear whether the effect was due to one molecule or both, or their potential synergistic effect. In the last decades, research has emphasized the role of inositol in different forms, such as Myo-inositol and D-chiro-inositol, in improving insulin sensitivity and related conditions such as diabetes mellitus and reproductive disorders [55]. One study not included in this systematic review, revealed that administering Myo-inositol during the first trimester of pregnancy to

women with a BMI > 30 kg/m^2 and pre-gestational diabetes, decreased the occurrence of GDM in the treated group (Myo-inositol 2 g and 200 μg FA twice a day) compared to the control (200 μg FA twice a day), by enhancing insulin sensitivity (P = 0,001; OR = 0,34, 95% CI: 0,17–0,68) [55]. Conversely, despite being affected by obesity, women in this study did not adhere to the recommended higher dosage of 5 mg [56]. Similar to FA, Myo-inositol has emerged as a contributing factor in reducing the prevalence of NTDs. Studies in mice have demonstrated that mothers with significantly lower blood inositol concentration levels had a 2.6-fold increased risk of having NTDs affected offspring [57]. Further research is needed to deepen understanding of the role of inositol in preventing NTDs and its interaction with folate metabolism.

During the study selection process, the authors recorded that most of the included studies focused on supplementation practice once pregnancy has already begun, investigating the pre-gestational FA protocol, retrospectively [27–34]. The authors highlighted the lack of comprehensive data on women of reproductive age, despite the well-established importance of raising serum folate levels before pregnancy. Concerning dietary habits, Mohd-Shukri et al. [31] reported that pregnant women with very severe obesity exhibited significantly lower dietary folate intake; this was also confirmed by Redfern and colleagues [32]. Notably, these studies referred to different RNI values regarding folate (600 μg/day *vs.* 300 μg/day), even though they were both conducted in the UK [31, 32]. Folate is essential for synthesizing nucleic acids and amino acids, and it plays a crucial role in cell growth and differentiation during the periconceptional period [58]. Daily intakes of 800 μg to 5 mg of folic acid from supplementations have been linked to an increased risk of perinatal mortality and cancer development [59]. Therefore, it is crucial to establish precise and consistent guidelines to better provide healthcare professionals addressing the dietary needs of pregnant women.

This systematic review included studies from European countries that adhered to a policy of voluntary fortification. Mandatory fortification has been implemented in different non-European countries, such as the USA in 1998, followed by Canada, Israel, Chile, and others. In Europe, the proposal for mandatory fortification has been placed forward by the UK and Ireland, which registered a high incidence of NTDs [60]. Fortification aims to contrast folate deficiency, particularly in unplanned pregnancies, without replacing periconceptional supplementation. Notably, fortification in the USA has demonstrated a reduction in NTDs incidence by 25–30%, approximately 50% of the preventable fraction with folic acid [61].

Voluntary fortification is an alternative approach, where fortified foods are available on the market and promoted by public and private initiatives. However, this method requires well-informed citizens and faces challenges in effectively controlling and monitoring intake levels over an extended period [62]. Despite the widespread use of dietary supplements, even among non-fertile women, concerns have been raised about potential excessive FA intake [63]. However, as noted in the present systematic review, European pregnant women are not particularly adherent to recommendations during the period of greatest need [27–34]. All the included studies highlighted the influence of different factors on the poor adherence of women with obesity to the recommended higher dose of FA. These include age, level of education, smoking, alcohol consumption, and pregnancy planning. Notably, the latter emerges as a pivotal determinant in compliance with FA supplementation, particularly during the preconception period [29]. From the studies conducted so far, it is not possible to make definitive considerations about the pre-pregnancy period due to insufficient comprehensive data. There is a need to conduct longitudinal cohort studies, rather than retrospective ones, on a larger sample size using validated and targeted questionnaires, to collect a greater quantity of data that can be used in clinical practice.

Conclusion

The literature analysis demonstrates that pregnant women with overweight/obesity frequently do not adhere to current FA supplementation recommendations. Furthermore, there is an urgent need to standardize the recommendations across European countries. To date, the scientific community should: i) conduct higher quality clinical trials to ascertain if the highest recommended dose (5 mg) is the most suitable and safe for both women with obesity and their offspring; ii) educate women of childbearing age, particularly those with excessive weight, on the significance of commencing FA supplementation before pregnancy, as neural tube closure occurs around the 28th day of gestation; iii) encourage women of childbearing age with obesity to embrace a healthy lifestyle and argument the consumption of folate-rich foods before conception; iv) promote the implementation of effective food fortification policies with folic acid through the active engagement of healthcare providers, to achieve equitable primary prevention of NTDs across countries.

In conclusion, women of childbearing age with excess weight should be monitored assessing serum folate, RBC folate, and homocysteine levels to gain a better understanding of one-carbon metabolism and devise tailored supplementation protocols. These improvements should

be supported by educational policies involving cooperation among healthcare professionals, medical doctors, policymakers, and citizens, to enhance awareness among women of childbearing age about their pivotal role in supporting health across generations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12978-025-01953-y.

Supplementary material 1.

Acknowledgements
The research group acknowledges the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.4-Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP F13C22000720007, Project title "National Biodiversity Future Center-NBFC"; for supporting the research grants of Federica Loperfido, Rachele De Giuseppe and Beatrice Maccarini.

Author contributions
Author Contributions: F.L., F.S. writing—the original draft preparation; F.L., F.S., I.B. D.E.M., B.M., and C.F., review and editing; A.L., review; H.C., review and editing, supervision; R.D.G., review, editing, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Funding
Project funded under the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.3-Call for proposal No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU. Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP F13C22001210007, Project title "ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security-Working ON Foods".

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interest
The authors declare no competing interests.

Author details
[1]Laboratory of Dietetics and Clinical Nutrition, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, 27100 Pavia, Italy. [2]Clinical Nutrition Unit, General Medicine, ICS Maugeri IRCCS, 27100 Pavia, Italy. [3]Haleon Italy S.R.L, SocietàUnipersonale, Via Monte Rosa 91, 20149 Milan, MI, Italy.

Received: 24 October 2024 Accepted: 14 January 2025
Published: 31 January 2025

References

1. Salih MA, Murshid WR, Seidahmed MZ. Epidemiology, prenatal management, and prevention of neural tube defects. Saudi Med J. 2014;35(Suppl 1):15.
2. European Commission. Folic Acid and Neural Tube Defects | EU RD Platform. [cited 2024 Oct 16]. https://eu-rd-platform.jrc.ec.europa.eu/eurocat/prevention-and-risk-factors/folic-acid-neural-tube-defects_en
3. Kancherla V. Neural tube defects: a review of global prevalence, causes, and primary prevention. Childs Nerv Syst. 2023;39(7):1703–10.
4. World Health Organization (WHO). Guideline: Optimal Serum and Red Blood Cell Folate Concentrations in Women of Reproductive Age for Prevention of Neural Tube Defects - PubMed.
5. World Health Organization (WHO). Periconceptional folic acid supplementation to prevent neural tube defects. 2023. https://www.who.int/tools/elena/interventions/folate-periconceptional.
6. Food and Nutrition - CREA. Linee guida per una sana alimentazione 2018 - Linee guida per una sana alimentazione 2018 - Alimenti e Nutrizione - CREA. [cited 2024 Oct 16]. https://www.crea.gov.it/web/alimenti-e-nutrizione/-/linee-guida-per-una-sana-alimentazione-2018
7. Dunlap B, Shelke K, Salem SA, Keith LG. Folic acid and human reproduction—ten important issues for clinicians. J Exp Clin Assist Reprod. 2011;8:1.
8. Martinez H, Benavides-Lara A, Arynchyna-Smith A, Ghotme KA, Arabi M, Arynchyn A. Global strategies for the prevention of neural tube defects through the improvement of folate status in women of reproductive age. Child's Nerv Syst. 2023;39(7):1719–36. https://doi.org/10.1007/s00381-023-05913-4.
9. Vandevijvere S, Amsalkhir S, Van Oyen H, Moreno-Reyes R. Determinants of folate status in pregnant women: results from a national cross-sectional survey in Belgium. Eur J Clin Nutr. 2012;66(10):1172–7.
10. Nilsen RM, Leoncini E, Gastaldi P, Allegri V, Agostino R, Faravelli F, et al. Prevalence and determinants of preconception folic acid use: an Italian multicenter survey. Ital J Pediatr. 2016;42:1.
11. Cheng D, Schwarz EB, Douglas E, Horon I. Unintended pregnancy and associated maternal preconception, prenatal and postpartum behaviors. Contraception. 2009;79(3):194–8.
12. Blanc-Petitjean P, Jézequel M, Manunta A, Olivari-Philiponnet C, Samson E, Bajeux E. Folic acid supplementation use during the peri-conceptional period among professionals of a hospital in France. Prev Med reports. 2023;38:1.
13. Hoffman DJ, Reynolds RM, Hardy DB. Developmental origins of health and disease: current knowledge and potential mechanisms. Nutr Rev. 2017;75(12):951–70.
14. Devlieger R, Benhalima K, Damm P, Van Assche A, Mathieu C, Mahmood T, et al. Maternal obesity in Europe: Where do we stand and how to move forward?: A scientific paper commissioned by the European Board and College of Obstetrics and Gynaecology (EBCOG). Eur J Obstet Gynecol Reprod Biol. 2016;201:203–8.
15. Mojtabai R. Body mass index and serum folate in childbearing age women. Eur J Epidemiol. 2004;19(11):1029–36.
16. Knight BA, Shields BM, Brook A, Hill A, Bhat DS, Hattersley AT, et al. Lower Circulating B12 Is Associated with Higher Obesity and Insulin Resistance during Pregnancy in a Non-Diabetic White British Population. PLoS One. 2015;10:8.
17. Hojeij B, Rousian M, Sinclair KD, Dinnyes A, Steegers-Theunissen RPM, Schoenmakers S. Periconceptional biomarkers for maternal obesity: a systematic review. Rev Endocr Metab Disord. 2023;24(2):139–75.
18. Vena F, D'Ambrosio V, Paladini V, Saluzzi E, Di Mascio D, Boccherini C, et al. Risk of neural tube defects according to maternal body mass index: a systematic review and meta-analysis. J Matern Neonatal Med. 2022;35(25):7296–305. https://doi.org/10.1080/14767058.2021.1946789.
19. Bird JK, Ronnenberg AG, Choi SW, Du F, Mason JB, Liu Z. Obesity is associated with increased red blood cell folate despite lower dietary intakes and serum concentrations. J Nutr. 2015;145(1):79–86.
20. Zhou SS, Li D. Vitamin paradox in obesity: Deficiency or excess? World J Diabetes. 2015;6(10):1158.
21. Emery MG, Fisher JM, Chien JY, Kharasch ED, Dellinger EP, Kowdley KV, et al. CYP2E1 activity before and after weight loss in morbidly obese subjects with nonalcoholic fatty liver disease. Hepatology. 2003;38(2):428–35.
22. Mm W, Louik C. Prepregnant weight in relation to risk of neural tube defects. JAMA. 1996;275(14):1089.

23. Parker SE, Yazdy MM, Tinker SC, Mitchell AA, Werler MM. The impact of folic acid intake on the association among diabetes mellitus, obesity, and spina bifida. Am J Obstet Gynecol. 2013;209:3.
24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev. 2021;10:1.
25. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation - PubMed.
26. Harrison R, Jones B, Gardener P, Lawton R. Quality assessment with diverse studies (QuADS): an appraisal tool for methodological and reporting quality in systematic reviews of mixed- or multi-method studies. BMC Health Serv Res. 2021;21:1.
27. O' Malley EG, Reynolds CME, Cawley S, Woodside JV, Molloy AM, Turner MJ. Folate and vitamin B12 levels in early pregnancy and maternal obesity. Eur J Obstet Gynecol Reprod Biol. 2018;231:80–4.
28. Cawley S, Mullaney L, McKeating A, Farren M, McCartney D, Turner MJ. An analysis of folic acid supplementation in women presenting for antenatal care. J Public Health. 2016;38(1):122–9.
29. Linnell A, Murphy N, Godwin J, Cremona A. An evaluation of adherence to folic acid supplementation in pregnant women during early gestation for the prevention of neural tube defects. Public Health Nutr. 2022;25(11):3025–35.
30. A. Malvasi, F. Casciaro, M.M. Minervini, I. Kosmas, O.A. Mynbaev, E. Pacella, V. Monti Condesnitt, A. Creanza, G.C. Di Renzo AT. Myo-inositol, D-chiro-inositol, folic acid and manganese in second trimester of pregnancy: a preliminary investigation. 2014. https://www.europeanreview.org/article/6605
31. Mohd-Shukri NA, Duncan A, Denison FC, Forbes S, Walker BR, Norman JE, et al. Health Behaviours during Pregnancy in Women with Very Severe Obesity. Nutr 2015, 7, 8431–8443. 2015;7(10):8431–43. https://www.mdpi.com/2072-6643/7/10/5403/htm
32. Redfern KM, Hollands HJ, Welch CR, Pinkney JH, Rees GA. Dietary Intakes of Folate, Vitamin D and Iodine during the First Trimester of Pregnancy and the Association between Supplement Use and Demographic Characteristics amongst White Caucasian Women Living with Obesity in the UK. Nutrients. 2022;14:23.
33. Santamaria A, Di Benedetto A, Petrella E, Pintaudi B, Corrado F, D'Anna R, et al. Myo-inositol may prevent gestational diabetes onset in overweight women: a randomized, controlled trial. J Matern Fetal Neonatal Med. 2016;29(19):3234–7.
34. Vitale SG, Corrado F, Caruso S, Di Benedetto A, Giunta L, Cianci A, et al. Myo-inositol supplementation to prevent gestational diabetes in overweight non-obese women: bioelectrical impedance analysis, metabolic aspects, obstetric and neonatal outcomes – a randomized and open-label, placebo-controlled clinical trial. Int J Food Sci Nutr. 2021;72(5):670–9. https://doi.org/10.1080/09637486.2020.1852191.
35. Food Fortification Initiative. Europe Food Fortification Initiative enhancing grains for healthier life. https://www.ffinetwork.org/europe
36. Food Safety Authority of Ireland (FSAI). Report of the National Committee on Folic Acid Food Fortification. 2006. http://hdl.handle.net/10147/44809
37. Department of Health and Social Care, Department for Environment, Food & Rural Affairs, Andrew Gwynne MP, Baroness Merron, Daniel Zeichner MP. Birth defects prevented by fortifying flour with folic acid - GOV.UK. 2024 [cited 2025 Jan 6]. https://www.gov.uk/government/news/birth-defects-prevented-by-fortifying-flour-with-folic-acid
38. Berti C, Biesalski HK, Gärtner R, Lapillonne A, Pietrzik K, Poston L, et al. Micronutrients in pregnancy: current knowledge and unresolved questions. Clin Nutr. 2011;30(6):689–701.
39. Institute of Obstetricians and Gynaecologists RC of P of I. CLINICAL PRACTICE GUIDELINE NUTRITION DURING PREGNANCY. 2022 [cited 2024 Oct 22]; Available from: https://www.hse.ie/eng/about/who/acute-hospitals-division/woman-infants/clinical-guidelines/national-clinical-guidelines-nutrition-in-pregnancy-guideline.pdf
40. Denison FC, Aedla NR, Keag O, Hor K, Reynolds RM, Milne A, et al. Care of Women with Obesity in Pregnancy. BJOG An Int J Obstet Gynaecol. 2019;126(3):e62-106. https://doi.org/10.1111/1471-0528.15386.
41. The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG). Vitamin and Mineral Supplementation and Pregnancy. 2022. https://ranzcog.edu.au/wp-content/uploads/Vitamin-Mineral-Supplementation-Pregnancy.pdf
42. Aline Allain-ron, Andrée Gruslin, Sheila M. Innis, Kristine G. Koski, Michel Lucas, Ann Montgomery, Deborah L. O'Connor KY. Prenatal nutrition guidelines for health professionals : folate. 2009;10. https://publications.gc.ca/collections/collection_2009/sc-hc/H164-109-2-2009E.pdf
43. WHO. Malnutrition in women. [cited 2024 Oct 16]. https://www.who.int/data/nutrition/nlis/info/malnutrition-in-women
44. Morris JK, Addor MC, Ballardini E, Barisic I, Barrachina-Bonet L, Braz P, et al. Prevention of neural tube defects in Europe: a public health failure. Front Pediatr. 2021;9:647038.
45. Pace ND, Siega-Riz AM, Olshan AF, Chescheir NC, Cole SR, Desrosiers TA, et al. Survival of infants with spina bifida and the role of maternal prepregnancy body mass index. Birth defects Res. 2019;111(16):1205–16.
46. van der Windt M, Schoenmakers S, van Rijn B, Galjaard S, Steegers-Theunissen R, van Rossem L. Epidemiology and (Patho)Physiology of Folic Acid Supplement Use in Obese Women before and during Pregnancy. Nutrients. 2021;13(2):1–16.
47. Camier A, Kadawathagedara M, Lioret S, Bois C, Cheminat M, Dufourg MN, et al. Social Inequalities in Prenatal Folic Acid Supplementation: Results from the ELFE Cohort. Nutrients. 2019;11:5.
48. Scholing JM, Olthof MR, Jonker FAM, Vrijkotte TGM. Association between pre-pregnancy weight status and maternal micronutrient status in early pregnancy. Public Health Nutr. 2018;21(11):2046–55.
49. Khanna D, Khanna S, Khanna P, Kahar P, Patel BM. Obesity: A Chronic Low-Grade Inflammation and Its Markers. Cureus. 2022;14:2.
50. Yang Y, Cai Z, Zhang J. The effect of prepregnancy body mass index on maternal micronutrient status: a meta-analysis. Sci Rep. 2021;11(1):18100.
51. Shukla S, Shrivastava D. Unraveling the link between serum homocysteine levels and nutrient deficiency in subfertility: a comprehensive review. Cureus. 2023;15:11.
52. Rubini E, Schenkelaars N, Rousian M, Sinclair KD, Wekema L, Faas MM, et al. Maternal obesity during pregnancy leads to derangements in one-carbon metabolism and the gut microbiota: implications for fetal development and offspring wellbeing. Am J Obstet Gynecol. 2022;227(3):392–400.
53. Zehravi M, Maqbool M, Ara I. Correlation between obesity, gestational diabetes mellitus, and pregnancy outcomes: an overview. Int J Adolesc Med Health. 2021;33(6):339–45. https://doi.org/10.1515/ijamh-2021-0058/html.
54. Alves FCR, Moreira A, Moutinho O. Maternal and long-term offspring outcomes of obesity during pregnancy. Arch Gynecol Obstet. 2024;309(6):2315–21.
55. D'Anna R, Di Benedetto A, Scilipoti A, Santamaria A, Interdonato ML, Petrella E, et al. Myo-inositol supplementation for prevention of gestational diabetes in obese pregnant women: a randomized controlled trial. Obstet Gynecol. 2015;126(2):310–5.
56. SIGO. Nutrizione in Gravidanza e Durante l'Allattamento - SIGO. https://www.sigo.it/blog/linee_guida/nutrizione-in-gravidanza-e-durante-lallattamento/
57. Groenen PM, Peer PG, Wevers RA, Swinkels DW, Franke B, Mariman EC, et al. Maternal myo-inositol, glucose, and zinc status is associated with the risk of offspring with spina bifida. Am J Obstet Gynecol. 2003;189(6):1713–9.
58. Argyridis S. Folic acid in pregnancy. Obstet Gynaecol Reprod Med. 2019;29(4):118–20. http://www.obstetrics-gynaecology-journal.com/article/S1751721419300260/fulltext
59. Valera-Gran D, Hera M, Navarrete-Muñoz EM, Fernandez-Somoano A, Tardón A, Julvez J, et al. Folic acid supplements during pregnancy and child psychomotor development after the first year of life. JAMA Pediatr. 2014;168:11.
60. EFSA. ESCO Report on Analysis of Risks and Benefits of Fortification of Food with Folic Acid. EFSA Support Publ. 2017;6:8.
61. Canfield MA, Collins JS, Botto LD, Williams LJ, Mai CT, Kirby RS, et al. Changes in the birth prevalence of selected birth defects after grain

fortification with folic acid in the United States: findings from a multi-state population-based study. Birth Defects Res A Clin Mol Teratol. 2005;73(10):679–89.
62. Fletcher RJ, Bell IP, Lambert JP. Public health aspects of food fortification: a question of balance. Proc Nutr Soc. 2004;63(4):605–14.
63. Turck D, Bohn T, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Knutsen HK, et al. Scientific opinion on the tolerable upper intake level for folate. EFSA J Eur Food Saf Auth. 2023;21:11.

Publisher's Note

Appendix C

Offspring's exposome: a narrative review on the influence of early-life factors on childhood obesity risk

frontiers | Frontiers in Nutrition

TYPE Review
PUBLISHED 06 October 2025
DOI 10.3389/fnut.2025.1597746

OPEN ACCESS

EDITED BY
Xinran Liu,
Peking University People's Hospital, China

REVIEWED BY
Lijun Chen,
Beijing Sanyuan Foods Co., Ltd., China
Juraj Stanik,
Comenius University, Slovakia

*CORRESPONDENCE
Federica Loperfido
federica.loperfido@unipv.it

†These authors have contributed equally to this work

RECEIVED 31 March 2025
ACCEPTED 15 September 2025
PUBLISHED 06 October 2025

CITATION
Maccarini B, Loperfido F, Bianco I, Sottotetti F, El Masri D, Ferrara C, Verme F, Cangelosi E, Meriggi N, De Filippo C, Cena H and De Giuseppe R (2025) Offspring's exposome: a narrative review on the influence of early-life factors on childhood obesity risk. *Front. Nutr.* 12:1597746. doi: 10.3389/fnut.2025.1597746

Offspring's exposome: a narrative review on the influence of early-life factors on childhood obesity risk

Beatrice Maccarini[1], Federica Loperfido[1]*, Irene Bianco[1], Francesca Sottotetti[1], Dana El Masri[1], Chiara Ferrara[1], Federica Verme[1], Erika Cangelosi[1], Niccolò Meriggi[2], Carlotta De Filippo[2], Hellas Cena[1,3†] and Rachele De Giuseppe[1†]

[1]Laboratory of Dietetics and Clinical Nutrition, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy, [2]Institute of Agricultural Biology and Biotechnology (IBBA), National Research Council (CNR), Pisa, Italy, [3]Clinical Nutrition and Dietetics Service, Unit of Internal Medicine and Endocrinology, ICS Maugeri IRCCS, Pavia, Italy

Childhood obesity has emerged as a global health challenge, with significant long-term health consequences, including an increased risk of non-communicable diseases. The "first 1,000 days" period of life is a critical window for shaping long-term health outcomes. This narrative review aims to explore the role of environmental exposures, categorized within the exposome framework, in developing childhood obesity. The exposome encompasses three domains: general external exposures (e.g., air pollution, urbanization), specific external exposures [e.g., nutrition, physical activity, socioeconomic status (SES)], and internal exposures (e.g., metabolic responses, oxidative stress). Evidence identifies risk factors such as maternal smoking during pregnancy, early-life exposure to endocrine-disrupting chemicals, and air pollution, which contribute to obesogenic processes. In contrast, protective factors include access to green and blue spaces, exclusive breastfeeding, adequate complementary feeding, regular physical activity, limited screen time, and sufficient sleep, which support healthy growth trajectories. Findings regarding SES, perfluoroalkyl and polyfluoroalkyl substances exposure, and human breast milk macronutrient composition remain heterogeneous and context-dependent. The findings highlight the need to integrate public health strategies addressing modifiable environmental and lifestyle factors. Identifying a "healthy exposome" that protects against obesity risk can steer the development of personalized prevention strategies, ultimately reducing the burden of obesity and associated diseases.

KEYWORDS

childhood obesity, infant nutrition, exposome, gut microbiota, maternal lifestyle, human breast milk

1 Introduction

The prevalence of childhood obesity has risen alarmingly worldwide. In 2022, 159 million children and adolescents were affected by obesity, a rate four times higher than in 1990 (1). Childhood obesity is strongly associated with an increased risk of early onset of other non-communicable diseases (NCDs), including type 2 diabetes, cardiovascular

diseases, and certain cancers, leading to adverse health, economic, and social consequences (2, 3).

The developmental origins of health and disease (DOHaD) hypothesis suggests that environmental exposures during critical periods of early life influence disease risk by altering biological pathways related to metabolism, inflammation, and energy homeostasis (4). Preventing childhood obesity has become a global public health challenge, giving attention to modifiable exposure factors during critical developmental periods, such as the prenatal and early childhood stages (5). In particular, the "first 1,000 days" period, from conception to the child's second year of age, represents a crucial window for shaping long-term development and health outcomes (6, 7).

Particularly, maternal factors, including nutrition, stress, and exposure to environmental pollutants, can influence fetal programming, shaping the development of the child's immune system, metabolic pathways, and brain function (8). These exposures may induce epigenetic changes and alter gene expression, impacting long-term health outcomes. The interplay between maternal exposures and early-life environment highlights the pivotal role of the "first 1,000 days" in determining lifelong health trajectories (7, 9).

Consequently, there is an increasing need to investigate the complex totality of external and internal exposures that affect the risk of obesity and NCDs from conception onward (10). Measuring the interaction of different exposures throughout life is highly complicated and challenging. In this context, the exposome concept was introduced in 2005, defined as the totality of environmental factors that potentially influence human health across the lifespan (11). The "exposome approach" represents a novel perspective aimed at moving beyond the study of the relationship between individual environmental factors and health outcomes, by integrating multiple risk factors and examining their interactions and potential causal mechanisms related to various health outcomes (11).

The exposome is divided into three different domains: (i) general external exposome, encompassing factors such as social capital, urbanization, air pollution, and climate; (ii) specific external exposome, which includes aspects such as nutrition, physical activity (PA), and other lifestyle habits, as well as social-economic determinants; and (iii) internal exposome, which involves endogenous biological responses unique to everyone, including metabolic factors, oxidative stress, inflammation, circulating blood biomarkers, hormones, and microbiome (12).

In the context of the "first 1,000 days" of life, the present review will provide an in-depth analysis of the infant exposome, categorized according to the domains previously described and adapted to the following research question.

Specifically, the review aims to identify exposure determinants associated with an increased risk of obesity during childhood and to define the characteristics and components of a "healthy exposome" in early life. The identification of protective factors may contribute to the development of effective preventive strategies for childhood obesity.

2 General external exposome and childhood obesity

Environmental pollutants, metals, chemicals, such as endocrine-disrupting chemicals (EDCs), and urbanization are components of the general external exposome (12). Recently, research has analyzed both prenatal and postnatal exposure to environmental factors, focusing on their influence on a higher risk of developing chronic diseases, including obesity.

2.1 Endocrine disrupting chemicals

Exposure to EDCs during early life seems to have an impact on the development of obesity, as shown in several original studies as well as systematic reviews (13).

EDCs include compounds such as bisphenol A (BPA), phthalates, and perfluoroalkyl and polyfluoroalkyl substances (PFAS). EDCs primarily originate from industrial processes and can be found in everyday settings, other than pesticides, clothing additives, toys, food items (e.g., beverages, cereals, canned food, and drinks, or labeled fruit), and packaging materials, including ultra-processed food (13, 14). These compounds can disrupt the endocrine system's function, affecting organs such as the liver, pancreas, and reproductive system, potentially leading to various health issues, including neurodevelopmental and metabolic disorders like obesity (13). Particularly, phthalates and BPA have potential obesogenic effects, especially in vulnerable populations, including infants (15). Gutiérrez-Torres and colleagues investigated whether exposure to EDCs during the prenatal period may affect anthropometric variables and biochemical parameters in preschool-age children (ages 3-5) (16). Positive associations have been found in (i) percentage of fat mass, (ii) body mass index (BMI), (iii) waist circumference, and (iv) skinfolds. Furthermore, the risk of being overweight persisted after adjustment for key confounding variables (e.g., maternal BMI, birth weight, breastfeeding, sex of the child, smoking, and other environmental exposures). No association was detected between prenatal exposure and lipid profile or glucose levels in childhood (16).

In contrast, a recent systematic review and meta-analysis of 13 studies found no statistical association between prenatal exposure to four different PFAS compounds and BMI fluctuations or waist circumference in children aged 18 months to 11 years. Notably, the authors highlight that these results may be influenced by the timing of exposure and individual vulnerability (17).

These findings were in line with the results of Lin and colleagues, which reported no significant associations between prenatal BPA exposure and birth weight, birth length, or head circumference (18).

Symeonides and colleagues (19) conducted an umbrella review on both prenatal and postnatal exposures to various chemicals and their adverse effects on children's health. They found that exposure to BPA was linked to insulin resistance, obesity, and hypertension. Specifically, phthalate compounds were associated with insulin resistance, elevated blood pressure, and precocious puberty in girls.

Furthermore, exposure to PFAS was related to an increased BMI and overweight status (19).

Research on postnatal exposure primarily focuses on children aged 3–19 years. Notably, Ribeiro et al. (20) found positive associations between EDCs exposure and several indicators of overweight or obesity, including BMI and waist circumference, in children (aged between 6 and 19 years). In particular, the meta-analysis highlighted a correlation between child exposure to 2,5-dichlorophenol (2,5-DCP) and obesity (OR = 1.8; CI: 1.1018, 3.184) (20). However, since most of the results are from observational studies, causality couldn't be definitively established (20).

Recent evidence (21) has also identified a positive correlation between exposure to several phthalate acid ester compounds (PAEs) and childhood obesity. Studies included were conducted on children and adolescents aged 3-19 worldwide, including the United States, China, Iran, South Korea, and Sweden. Strong associations were found throughout subgroup analysis between several phthalate metabolites in urinary samples and childhood obesity, especially in Asia (21). However, the heterogeneity of the upper tolerable values established by government authorities worldwide must be considered.

2.2 Air pollution

Current literature on air pollution has examined the risk of developing obesity, with a focus on exposures during early childhood and adulthood (22–24). Air pollutants mainly originate from combustion processes, including vehicular emissions, industrial activities, and the burning of fossil fuels (23). Although the mechanism connecting air pollution to a higher risk of obesity is not completely understood, biochemical processes are widely recognized as primary contributors. When air pollutants enter the body through the respiratory system, they can enhance oxidative stress levels in several tissues. Consequently, the inflammatory response may result in vascular damage and insulin resistance, affecting body weight (25). Prenatal exposure to air pollution, notably fine particulate matter (PM) with a diameter of $\leq$10 mm (PM_{10}) and PM with a diameter of $\leq$2,5 mm ($PM_{2.5}$), has been related to fetal growth restriction (26, 27). Young adults born with fetal growth restriction (FGR) are at increased risk of experiencing high blood pressure, reduced kidney function, hypertension, and cardiovascular complications later in life (28). However, the effects related to PM exposure on postnatal growth and childhood obesity remain unclear (29). Shao and colleagues investigated the impact of PM prenatal exposure on fetal development and its potential long-term health consequences, showing that prenatal exposure to $PM_{2,5}$, PM_{10}, sulfur dioxide (SO_2), and ozone (O_3) was significantly associated with reduced fetal biometry at 24 weeks of gestation (GW), with SO_2 having the most pronounced effect (26). At GW36, exposure to air pollution continued to negatively affect fetal size, although the effects were less significant compared to the earlier stage of pregnancy. Fetuses in the highest exposure quartile registered intrauterine weights that were 6.3% lower at GW24 and 2.1% lower at GW36 than those in the lowest quartile (26). However, no significant difference in birth weight was observed, suggesting that rapid growth occurred during the third trimester to offset earlier growth restrictions (30). Mergetaki et al. (29) examined the association between prenatal air pollution exposure and obesity-related parameters in children by analyzing data from 633 mother-child pairs. Prenatal exposure to PM was not associated with adiposity at 4 years of age. However, increased prenatal exposure to $PM_{2.5}$ and PM_{10} was linked to a higher risk of obesity (OR = 1.15; 95% CI: 1.01–1.31, $p = 0.04$) and abdominal obesity (OR = 1.18; 95% CI: 1.03–1.35, $p = 0.03$) at 6 years, respectively (29).

Recently, Zheng et al. confirmed findings from previous research in their systematic review, emphasizing several key factors (e.g., duration of exposure, geographic region, country's level of development) that influence the relationship between exposure to air pollution and childhood excessive weight. The study found that short-term exposure (<1 year) and long-term exposure (1 year or more) to $PM_{2.5}$ had different effects on the risk of being overweight and obesity, with an OR of 1.18 (95% CI: 1.09, 1.27) (24).

Considering geographic differences, $PM_{2.5}$ exposure significantly increased the risk of being overweight and obese in Asia, with an OR of 1.19 (95% CI: 1.10, 1.28). However, studies conducted in America and Europe did not find significant results. Moreover, when assessing the risk of overweight or obesity, developing countries exhibited a higher risk than developed countries for all pollutants considered (24).

Among the various pollutants examined, PM_1 showed a significant negative impact on the development of overweight/obesity and BMI increase (24).

2.3 Urbanization

A further significant component of the general external exposome is urbanization, a global phenomenon that involves population growth and densification in urban areas (31). The extension of developed areas leads to greater environmental challenges, such as increased traffic congestion, higher levels of air and noise pollution, exacerbation of the urban heat island effect, and the depletion of accessible green and blue spaces (31). In this context, several systematic reviews investigated the role of Nature-based Solutions (NbS) on human health (32).

Currently, scientific literature lacks systematic reviews and meta-analyses that specifically address the role of prenatal exposure to green spaces. Consequently, our analysis draws upon alternative study designs. Heo et al. conducted a prospective cohort study in New York City, examining the effect of residential green space exposure on birth outcomes such as preterm birth (PTB), birth weight, and estimated fetal weight (EFW) (33). They found that although green space exposure did not significantly affect birth weight or EFW, greater exposure was associated with a reduced risk of PTB, suggesting potential benefits for fetal maturity and neonatal health beginning from the gestational period (33). Similarly, Toda et al., in an analysis of 11 European birth cohorts, reported that increased residential green space exposure was linked to higher birth weight and lower odds of being small for gestational age (SGA), with increased effects observed in more deprived populations (34). Furthermore, Marteines and colleagues explored

the consequences of prenatal environmental exposures on BMI from birth to 24 months, finding that access to green spaces during pregnancy was associated with lower BMI *z*-scores at 24 months in a cohort of predominantly lower socioeconomic status (SES) participants (35).

Concerning postnatal exposure, a systematic review and meta-analysis showed that living in rural areas is associated with a higher prevalence of childhood obesity compared to children residing in urban areas of the United States. The meta-analysis ($n = 74{,}168$ participants aged 2–19) found that rural children have 26% higher odds of living with obesity compared to their urban counterparts (OR = 1.26; 95% CI: 1.21-1.32) (36).

The authors highlighted the obesity disparity between urban and rural children; however, the mechanisms driving these differences remain unclear (36). Previously Dunton and colleagues have documented comparable results, emphasizing the association between neighborhood characteristics, urban sprawl, and obesity outcomes among adolescents. Specifically, adolescents residing in rural, exurban, and mixed urban areas exhibited a higher likelihood of being overweight compared to their counterparts living in newer suburban, older suburban, and inner-city regions (37).

In contrast, recent evidence suggests that residing in urban areas is a significant risk factor for developing obesity. Specifically, children living in urban regions, particularly in the southern and northern areas of Mexico, registered higher rates of overweight and obesity compared to their counterparts in rural areas, as well as those in Mexico City and the central regions (38). Street connectivity, residential density, access to green spaces, public transportation, sidewalks, fast-food restaurants, and fresh markets are factors within urban areas that may have a significant role in the onset of childhood obesity (39, 40).

A recent meta-analysis comprising 457 studies revealed that most built environmental factors were inversely associated with childhood obesity. Specifically, access to green spaces was associated with increased PA and reduced screen time (38). Furthermore, access to food outlets, excluding convenience stores and fast food, was also correlated with healthier dietary behaviors. In contrast, greater proximity to fast-food restaurants was linked to higher consumption of ultra-processed foods, contributing to the creation of an obesogenic environment (41).

The built environment, such as transportation infrastructure and recreational facilities, may influence individual behaviors, potentially leading to reduced PA and increased obesity rates among children and adolescents (40). According to recent results, green and blue spaces play a significant role in promoting PA and influencing eating behaviors among children under 18 years old from various countries, including New Zealand, the UK, the USA, the Netherlands, Canada, Turkey, and Germany. Green spaces encompass parks, sports fields, playgrounds, nature reserves, and open picnic areas, while blue spaces include lakes, rivers, canals, and waterfronts. Green and Blue Spaces (GABS) provide safe environments for children to socialize and engage in lengthy and enjoyable PA. Additionally, the creation of school and home gardens has a positive impact on children's attitudes toward eating vegetables, thus promoting healthier dietary habits (39).

2.4 Climate change

The decline in the nutritional quality of food due to rising CO_2 levels and increasing global temperatures has significant implications for human health, particularly through the exacerbation of hidden hunger. The reduction of essential micronutrients such as iron, zinc, and protein in staple crops like wheat, rice, and maize (42, 43) contributes to widespread deficiencies, potentially affecting hundreds of millions of people by 2050 (44–46). Hidden hunger, a condition in which individuals consume sufficient calories but lack essential nutrients, is expected to increase by 10% due to climate-driven decreases in nutrient bioavailability (47). Additionally, extreme weather events and high ambient temperatures further compromise agricultural productivity, reducing protein content and overall crop yields (48–50).

These nutritional deficits are compounded by environmental contaminants, as climate change enhances the bioaccumulation of heavy metals like arsenic in food crops, particularly rice, leading to toxic effects on the gut microbiota (51, 52). Warmer waters similarly diminish omega-3 fatty acid concentrations in marine food webs, weakening their beneficial effects on gut health and immune function (53). Beyond direct dietary impacts, prolonged exposure to heat stress increases gut permeability, disrupts microbial balance, and promotes systemic inflammation, heightening susceptibility to gastrointestinal disorders such as inflammatory bowel disease (54–56). Furthermore, extreme temperatures exacerbate malnutrition in vulnerable populations, particularly children in low- and middle-income countries, where dysbiotic gut microbiota may persist despite nutritional interventions (57).

Breastfeeding patterns may be altered under high heat stress, influencing early-life gut microbiome development and potentially reinforcing health disparities (58–60). The interplay of these factors underscores the urgent need for climate-resilient agricultural strategies, biofortification efforts, and microbiota-targeted interventions to mitigate the long-term health consequences of climate change on human nutrition and gut health.

3 Specific external exposome and childhood obesity

During the "first 1,000 days" feeding practices, maternal substance consumption, and infant lifestyle factors are key components of the specific external exposome and may affect the risk of childhood obesity (61–63).

3.1 Infant feeding practices

Concerning the role of breastfeeding, several studies support the evidence that exclusively breastfed infants are at lower risk of accumulating excessive fat mass and experiencing overweight and obesity, in comparison with formula-fed infants (64, 65).

This protective effect can be attributed to the unique composition of human breast milk (HBM), which contains

metabolic hormones, bioactive molecules, and essential nutrients (66, 67). For example, leptin, ghrelin, growth factors, and hormones play a crucial role in regulating children's food intake and energy balance, controlling appetite, glucose, and lipid levels (68).

Human milk oligosaccharides (HMOs), which are highly concentrated in HBM, promote the growth of beneficial gut bacteria and may influence infant development, including weight gain and body composition (69). A recent systematic review conducted by Zheng et al. (68) summarized findings from 27 studies to assess the association between breastfeeding and BMI trajectory changes over time between childhood and adulthood. Findings revealed that breastfeeding, whether exclusive or combined with formula feeding, is associated with lower BMI trajectories compared to exclusively formula-fed infants (68). Moreover, the WHO conducted a study on 16 European countries to examine the relationship between different feeding practices, and children's body weight. The results showed a clear protective effect of exclusively breastfeeding, reducing the risk of obesity by 25%. On the other hand, an increased risk of 22% was observed among exclusively formula-fed infants (70, 71). The study also highlighted the influence of breastfeeding duration on the risk of obesity, showing that children breastfed for <6 months had a 12% increased risk (70, 71). However, a systematic review published in 2023 revealed conflicting evidence regarding the composition of HBM and the subsequent risk of obesity (72). The review investigated the role of hormones present in HBM, such as leptin, adiponectin, and insulin, in relation to the risk of later obesity, finding heterogeneous results. Additionally, the association between the macronutrient composition of HBM and the risk of subsequent obesity or body composition was examined, with only one study identifying that a higher fat percentage in HBM was associated with lower adiposity at 12 months, while a higher carbohydrate percentage was linked to increased adiposity at the same age, independent of other factors (72).

These findings emphasize the need for a better understanding of the mechanisms supporting the protective effects of breastfeeding against later obesity and highlight the necessity for further research in this area (72).

3.1.1 Human breast milk as a vehicle of EDCs and nicotine

Although HBM represents the primary and healthiest nutritional source, it may also serve as a significant vehicle for environmental pollutants and various harmful substances, such as EDCs, and nicotine (73, 74). Vulnerable groups including fetuses, infants, and children, may face greater susceptibility to environmental chemicals due to differences in toxicokinetics, resulting in an elevated risk of childhood diseases (75). In 2022, Iribarne-Durán et al. published the first results about the concentrations of some EDCs in HBM (73), suggesting that the entero-mammary circulation facilitates the transfer of these chemicals, as certain EDCs can cross the gut barrier, enter the bloodstream, and reach the mammary glands, subsequently appearing in HBM (76) and potentially exposing the infant to harmful effects (77). A recent study conducted by Vacca et al. investigated the association between maternal urinary concentration of EDCs and the gut microbiota composition of 20 breastfed infants, at four time points. The authors identified that maternal EDCs exposure impacts the infant's gut microbiota and potentially influences the risk of metabolic and inflammatory diseases including obesity (78).

Early-life exposure to smoking was also found to be associated with childhood obesity (79, 80). Specifically, nicotine quickly transfers into HBM, potentially harming an infant's development and health (79, 80).

Exposure to tobacco smoke during pregnancy and lactation has been linked to changes in the macronutrient composition of HBM. This may be due to the accumulation of toxic substances in the adipose tissue of the mammary glands, potentially disrupting lactogenesis and lipid synthesis (81).

In this regard, a systematic review conducted by Macchi et al. revealed that breastfed children of smoking mothers have reduced lean body mass and an increased risk of developing obesity within their first year of life (81). Moreover, tobacco exposure leads to a significant rise in thiobarbituric acid reactive substances (TBARS) levels, a marker of lipid peroxidation, and a reduction in trolox equivalent antioxidant capacity (TEAC), a measurement of total antioxidant capacity, within both colostrum and mature milk. In response to the increased reactive oxygen species (ROS) induced by tobacco smoke, there is a corresponding upregulation in the activity of antioxidant enzymes (82). These oxidative changes may contribute to the alterations observed in breast milk composition and potentially impact infant health (82). Several studies have also shown that nicotine not only passes through HBM but also crosses the placenta (83).

As a result, exposure to maternal or paternal smoking during pregnancy has been identified as a risk factor for the development of early overweight (83). A meta-analysis of 229,000 births showed that children from mothers who smoked during pregnancy had an increased risk of developing overweight [OR 1.42 (1.35–1.48), *P*-value <0.001] compared to children from no-smoking mothers. Noteworthy, paternal tobacco consumption was also assessed, revealing an association with a higher risk of childhood overweight, independently of maternal smoking habits (83). A study conducted by Srivastava et al. (79) revealed high rates of obesity in children exposed to parental smoking with the association being stronger with maternal smoking than with paternal smoking (79). This was confirmed in another study conducted by Cummings et al. (80), where the authors identified that a family history of nicotine use, and alcohol consumption was accompanied by an increased reward-driven eating in their children. Such behaviors may later lead to overweight and obesity, since children may eat more for pleasure rather than for satiety (80).

3.1.2 Complementary feeding

The transition from exclusive breast milk feeding to solid foods also plays a significant role in children's weight change, based on the timing of its introduction as well as its composition (71, 84, 85). The complementary feeding period is essential for providing children with safe and nutrient-dense foods and preventing overweight and obesity, as well as influencing their future dietary preferences (85). Early introduction of complementary feeding, before 6 months

of age, was shown to be associated with an increased risk of obesity (84). According to WHO and UNICEF recommendations, exclusive breastfeeding for the first 6 months of life must be followed by continued frequent or on-demand breastfeeding, combined with complementary feeding up to 2 years of age (71, 85). Caregivers play an essential role in protecting their infants and toddlers from the risk of excessive weight gain by providing them with an age-appropriate complementary diet, that is rich in nutritive value and includes a variety of foods from all food groups. This diet should be low in saturated fats, and trans fats and totally free from added sugars and salt (85). In this regard, a systematic review and meta-analysis was conducted by Rousham et al. to assess the impact of consuming unhealthy foods and beverages on the risk of overweight and obesity in children aged 10.9 years or below, in comparison with no or lower consumption levels (86). This review found a positive association between the consumption of sugar-sweetened beverages and both BMI level and body fat percentage, whereas artificially sweetened beverages or 100% fruit juice, had a low or no impact on BMI levels. On the other hand, unhealthy foods including ultra-processed items were found to increase BMI levels and obesity risk (86).

3.2 Children's lifestyle

Children's lifestyle is a significant component of early-life exposome that can impact body weight (87). Regarding daily screen time, the systematic review and meta-analysis conducted by Fang et al. (63) revealed that screen time $\geq$2 h per day was more strongly associated with an increased risk of overweight/obesity compared to screen time of $<$2 h per day. Additionally, the analysis showed that specific types of screen time, such as TV viewing and computer use, were more strongly linked to overweight and obesity than total screen time. Many existing studies have focused on the impact of a single type of screen time, which may not fully capture the overall effects of screen time on childhood obesity. Therefore, it is important to differentiate between the effects of different types of screen time when assessing their influence on childhood obesity. As recommended by WHO, children under 5 years old should limit sedentary behaviors, including screen time and prolonged periods spent in their strollers or chairs, ensure adequate sleep duration, and engage in active play to achieve healthy growth from the beginning of their lives and prevent childhood obesity and its related consequences (87). PA level must be incorporated in the child's daily routine, whether at home, in the nursery, or at school (88). Together with the quality of complementary feeding, PA can maintain energy balance and reduce the risk of overweight and obesity (88). It could be influenced by different factors including weather conditions (hot or cold degrees, wind speed, and precipitation (89, 90). A recent systematic review conducted by Jia et al. (89) showed that high and low temperatures were significantly linked to reduced daily PA levels among children (89). Another longitudinal prospective cohort study conducted on 372 children aged 3 years and followed for 5 years, showed that precipitation, wind speed, higher heating and cooling degrees than the average temperature, were associated with a decreased PA level (90). Apart from PA, sleep duration was also found to have a link with the risk of overweight and obesity. As demonstrated in the systematic review conducted by Morrissey et al. (91) a strong negative association was detected between insufficient sleep duration and increased weight status in primary school-aged children (91).

3.3 Socioeconomic status

Another important determinant of the specific external exposome is represented by the SES, recognized as a significant determinant of numerous adverse health outcomes, including obesity as obesity prevalence is inextricably related to the degree of relative social inequality (92). Although children do not have their own SES, recognized contextual factors include the parental sociodemographic characteristics (e.g., age and sex, race or ethnicity, SES) as a proxy for a child's SES level (93). Recent research categorized contextual factors to the child, parents, or family (e.g., sex, age, race, or ethnicity) and various SES metrics (e.g., annual family income, education level and/or employment of parents, health insurance coverage, and eligibility for free school lunch program) to investigate whether these factors serve as moderators in the relationship between parental stress and childhood obesity (93). Results revealed that parenting role stress may be associated with unhealthy practices such as children's unhealthy food intake, including consumption of fast foods, emotional overeating, screen time, and low PA levels (93). In this regard, recent studies have shown that dietary practices, sleep time, and level of PA in children aged 6-12 years living in the Pacific Region play a significant role in the development of overweight and obesity. Moreover, SES and food availability, parenting practices, and education level contribute to children's weight status (37, 94). Concerning the educational level of the family, while current literature does not directly address its relationship with childhood obesity, findings suggest that socioeconomic factors and parenting practices, which may be influenced by educational attainment, may play a significant role (94, 95). Bertrand and colleagues identified that the education level of caregivers was a key determinant of children's weight status, with higher caregiver education associated with a greater likelihood of childhood obesity in certain contexts. However, these findings are in contrast with other data, where an inverse association was registered between fathers' educational attainment and daughters' adiposity (94). Recently, Alruwaii conducted a systematic review examining the impact of both parents' educational levels on childhood obesity and overweight in Middle Eastern and North African countries, emphasizing the interaction between SES and metabolic health outcomes (95).

Although family-based SES indicators include several parameters, such as parental education level, occupation, living conditions, size of family, family income, and type of medical insurance, SES emerged as one of the most investigated parental factors. McGillivray and colleagues have reported a positive association between SES and BMI in children with intellectual or developmental disability; however, other research did not find a significant statistical association (96).

SES may also influence dietary habits among children. Notably, findings by Avery and colleagues examined the associations

between TV viewing while eating and children's diet quality (97). Four studies in their systematic review identified an association between low SES and increased likelihood of eating while watching TV ($p \leq 0.01$), highlighting the need for educational programs targeting parents, especially those with low-socioeconomic backgrounds (97).

In this context, nutritional education intervention may play a significant role in the prevention and treatment of obesity, mostly during early life. Recently, Spiga and colleagues collected data to explore the effectiveness of educational interventions focused on dietary and/or PA aimed at preventing childhood obesity, depending on factors related to health disparities, such as SES (98). Exploratory analyses of 55 studies targeting low-SES populations found no evidence suggesting that obesity prevention interventions are less effective in children from lower socioeconomic backgrounds (98).

Current literature also explores the role of the neighborhood environment on childhood obesity onset. A systematic review examined the neighborhood environment and obesity risk among urban, low SES Black and Hispanic children (99). Among the 24 included studies, 16 reported an association between neighborhood SES and BMI for the overall study population. These primarily investigated the relationship between neighborhood SES and BMI as measured by neighborhood income or a composite SES measure. These composite indicators integrate various SES-related factors, such as educational attainment, employment status, household income, and financial wellbeing, to generate a single score (100). While four studies found no association between composite SES and BMI, one study identified an inverse relationship. Similarly, three studies showed no association between neighborhood income and BMI, while five studies reported an inverse relationship (99).

4 Internal exposome and childhood obesity

The internal exposome comprises a plethora of biological responses occurring within the human body due to exposure to external stimuli (101). Metabolic processes that begin in childhood can increase the risk of obesity and other long-term health complications (102).

4.1 Exposure to chemical compounds

Considering exposure factors related to the general external exposome, the activation of the PPARs pathway has been implicated in the metabolic effects of phthalates and BPA exposures, which can enhance the risk of obesity by interfering with several pathways. In particular, these chemicals may (i) disrupt adipogenesis by inducing ROS species production, which can interfere with the normal differentiation of adipocytes; (ii) increase the number and size of adipocytes by regulating genes involved in adipogenesis; (iii) alter epigenetic pathways during development, which increases susceptibility to obesity; (iv) disrupt neuroendocrine signals involved in appetite and satiety pathways; (v) foster a proinflammatory environment in adipose tissue, leading to chronic low-grade inflammation; (vi) disrupting the gut microbiome and immune system balance; and (vii) impair the function of thermogenic adipose tissue (103–105). Recent evidence found that phthalates and BPA can pass through HBM, potentially affecting infant health (106, 107). In particular, high molecular weight phthalates and di(2-ethylhexyl) phthalate (DEHP) metabolites have been also linked to increased visceral adipose tissue (VAT) mass and higher Android-to-Gynoid (A/G) ratio in adolescents (108).

A significant association has been observed, with 5-fold increases in phthalate metabolites correlating to 21.7% and 18.0% greater VAT mass, respectively (106). Additionally, recent studies have investigated the role of per- and polyfluoroalkyl substances (PFAS) in childhood obesity. A systematic review and meta-analysis of 13 studies found no strong evidence of a direct association; however, an inverse relationship was suggested between postnatal PFAS exposure and BMI z-score. The limited number of studies available on this topic warrants the need for further investigation (17). In summary, exposure to environmental factors during both the prenatal period and early life can disrupt key metabolic pathways, affecting adipogenesis, lipid and glucose metabolism, gut microbiota homeostasis, and growth trajectories, with long-term implications for a child's health, including a higher risk of developing obesity. The interaction between genetic susceptibility, environmental exposures, and early-life exposure factors, such as breastfeeding, offers new insights into the complex mechanisms driving obesity risk.

4.2 Infant feeding practices

Referring to the specific external exposome, the type of infant feeding is an important factor influencing the risk of childhood obesity. Exclusive breastfeeding until the age of 2 is strongly recommended as a protective factor for the risk of obesity in children (109, 110). Research indicates that this practice may affect obesity-related gene expression, including fat mass and obesity-associated gene (FTO), Nuclear Respiratory Factor 1 gene (NRF1), and Leptin Receptor gene (LEPR), through epigenetic processes such as DNA methylation and regulation of CpG island loci (111). Studies have demonstrated that breastfeeding delays the onset of adiposity peaks and rebounds, helping to prevent excessive weight gain, particularly in children with a genetic predisposition (112, 113).

The FTO gene is essential for cell proliferation and differentiation through the PI3K/Akt signaling pathway; it also interacts with AMP-activated protein kinase (AMPK) and the PI3K/AKT/mTOR pathways, which are key regulators of energy metabolism (113). Recent research has emphasized the contribution of the FTO gene, especially its polymorphism, in promoting increased BMI and adiposity in children (114, 115). A study conducted by Wu and colleagues revealed that exclusive breastfeeding up to 5 months significantly reduces the risk of obesity in children carrying the FTO rs9939609 risk allele. The study further indicated that breastfeeding postpones the age at which peak fat mass and fat accumulation occur. Specifically, breastfed children experienced a delay of 2–3 months in reaching their peak fat compared to non-breastfed children, with girls

showing a delay of up to 6 months. At age 15, the adolescents exhibited a predicted BMI reduction of 0.56 kg/m² for boys [CI 95%:0.11–1.01; $P = 0.003$] and 1.14 kg/m² for girls [CI 95%:0.67–1.62; $P < 0.0001$] (116). These findings highlighted the role of exclusive breastfeeding in mitigating up to 39–70% of genetic obesity risk, particularly in children characterized by high genetic risk scores (116). Also, Verier et al. highlighted the significant interaction between breastfeeding and polymorphisms of the peroxisome proliferator-activated receptor γ (PPAR-γ) gene concerning childhood obesity. Prolonged breastfeeding in children with the high-risk variant Pro12Ala phenotype led to reduced BMI, waist circumference, and skinfold thickness compared to formula-fed children (117). However, for children with the non-high-risk Pro12Pro phenotype, breastfeeding duration had no significant effect on obesity-related indicators (117). The human PPAR genes are involved in regulating lipid and glucose metabolism, lipid storage, and insulin sensitization. Given the gene's role in macronutrient metabolism, the PPAR signaling pathway has become a focal point in obesity research, particularly in elucidating the interactions between environmental exposure and gene expression (118).

4.2.1 Complementary feeding

The introduction of solid foods represents a significant milestone in the development of various infant physiological systems, including the gastrointestinal tract, gut microbiota, and immune system (119). Evidence suggests that initiating solid food consumption at approximately 5–6 months of age may correlate with a reduced risk of greater BMI. However, systematic reviews have emphasized the need for additional prospective studies to better assess the differences between the introduction of solid food and exclusively breastfed, formula-fed, or mixed-fed infants (120, 121). Additionally, the dietary pattern adopted during the introduction of solid foods is particularly relevant, as it can influence behavioral outcomes and molecular pathways that may affect the risk of obesity (122). In fact, in their Randomized Controlled Trial (RCT) including healthy, full-term formula-fed infants, Tang et al. (123) demonstrated that the *z*-score for length-for-age significantly increased in the group of children with higher consumption of meat ($+0.33 \pm 0.09$; $P = 0.001$ over time), whereas it decreased in the dairy group (-0.30 ± 0.10; $P = 0.0002$ over time). Moreover, the *z*-score for weight-for-length increased significantly in the dairy group (0.76 ± 0.21; $P = 0.000002$ over time) compared to the meat group (0.30 ± 0.17; $P = 0.55$ over time) (123). Although the WHO guidelines recommend daily or frequent consumption of animal-source foods such as meat, poultry, fish, or eggs due to their high nutrient density, providing easily digestible proteins, several studies and systematic reviews have shown that higher protein intake before the age of 2 is related to accelerated growth trajectories and increased risk of higher BMI later in childhood (124, 125). According to the early protein hypothesis, high protein intake during lactation and complementary feeding is thought to stimulate insulin and insulin-like growth factor (IGF) secretion, which can promote fat accumulation by enhancing adipogenesis and adipocyte differentiation (126, 127). However, other research has found no significant differences in IGF-I levels among infants consuming varying amounts or sources of protein, indicating that other mechanisms may occur (123). The development of gut microbiota is a complex process, beginning at birth and influencing long-term health. A first factor implicated in the development of the intestinal microbiota of a newborn is the mode of delivery, i.e., natural birth or c-section, followed by the immediate feeding method, i.e., breastfeeding or formula feeding. This sequence of events and future feeding habits is crucial for the long-term development of the intestinal microbiota. The infant gut is initially colonized by facultative anaerobes, such as Staphylococcus, Streptococcus, Enterobacteriaceae, and Lactobacillus, which create an environment suitable for obligate anaerobes like *Bifidobacterium*, *Clostridium*, and *Bacteroides* to thrive (128, 129). Maternal milk, with its nutritional and bioactive components, fosters the optimal microbial growth in the infant gut, influencing both the microbiota's composition and immune system development.

Therefore, the introduction of semi-solid and solid food significantly alters the composition of the gut microbiota, contributing to its maturation and diversification (130). During this transition, the abundance of milk-related bacteria, such as Bifidobacterium and Enterobacteriaceae, decreases, while bacteria such as *Bacteroides* and *Firmicutes*, which preferentially digest fibers and complex carbohydrates, increase (131). Comparisons of different dietary patterns revealed that children following a Mediterranean Diet (MD) exhibited greater intestinal microbial diversity and a higher abundance of beneficial taxa, such as *Coriobacteriaceae*, which can metabolize polyphenols, particularly abundant in the MD (130). Recent research has increasingly highlighted the role of the gut microbiota as a key mediator in the development of obesity, particularly during early life (132). Bacteria from the *Firmicutes* and *Bacteroidetes* phyla are closely linked to the regulation of energy metabolism. A dysbiotic microbiota can lead to reduced production of short-chain fatty acids (SCFAs), which may promote systemic inflammation and, consequently, insulin resistance and visceral fat accumulation (130, 133).

A fiber-based diet favors SCFA-producing bacteria implicated in obesity and in the regulation of intestinal endocrine signals, influencing glucose and lipid metabolism. Furthermore, SCFAs are implicated in the regulation of oxidative metabolism and insulin sensitivity in the liver and adipose tissue, thus managing to improve obesity, determining the reduction of metabolic endotoxemia and inflammation (134, 135). SCFAs play a crucial role in preserving the integrity of the barrier, and *Bacteroides thetaiotaomicron*, through the production of acetate and propionate, regulates the production of mucin; therefore, the balance of the intestinal mucosa (136).

4.3 Maternal smoking habits

Moreover, the maternal smoking habit during pregnancy can affect the risk of excessive weight gain during early life. Peng and colleagues demonstrated that infants exposed to maternal smoking during gestation had higher BMI *z*-scores at 3 years of age than those who were not exposed (Model 3: $\beta = 0.28$, CI: 95%; 0.06–0.49). They were significantly more likely to be affected by obesity at 3 years of age (Model 3: OR 1.78, CI: 95%;

TABLE 1 Summary of the main results of the studies included.

Infant exposome domains	Exposure factors	Study design	References	Main results of the studies
General external exposome	Air quality	Analysis from a longitudinal cohort study (29)	Margetaki et al. (29)	• Exposure to PMs *in utero* was not associated with measures of adiposity at 4 or 6 y (29); • Higher exposure to PM_{10} during pregnancy, combined with maternal consumption of <5 servings of FV/day was associated with increased BMI (beta 0.41 kg/m^2, 95% CI: −0.06, 0.88, p-interaction = 0.037) and increased WC (beta 0.83 cm, 95% CI: −0.38, 2.05, p-interaction = 0.043) in children at 6 years (29); • Higher exposure to $PM_{2.5}$ during pregnancy, combined with maternal consumption of <5 servings of FV/day were associated with increased fat mass (beta 0.5 kg, 95% CI: 0.0, 0.99, p-interaction = 0.039) and percentage of body fat (beta 1.06%, 95% CI: −0.06, 2.17, p-interaction = 0.035) in children at 6 years (29).
		Systematic review and meta-analysis (25)	Huang et al. (25)	• Higher exposure to air pollutants was significantly associated with higher obesity risk [OR = 1.12 (95% CI: 1.06-1.18) for PM_{10}, OR = 1.28 (95% CI: 1.13-1.45) for $PM_{2.5}$, OR = 1.41 (95% CI: 1.30-1.53) for PM_1, and OR = 1.11 (95% CI: 1.06-1.18) for NO_2] (25); • Each 10 μg/m^3 increment in pollutant concentration was associated with BMI increases of +0.08 kg/m^2 (95% CI: 0.03-0.12) for PM_{10}, +0.11 kg/m^2 (95% CI: 0.05-0.17) for $PM_{2.5}$, and +0.03 kg/m^2 (95% CI: 0.01-0.04) for NO_2 (25).
		Systematic review and meta-analysis (17)	Frangione et al. (17)	• No evidence of a positive association was found between prenatal PFAS exposure and pediatric obesity (17); • Prenatal PFAS exposure was not statistically associated with BMI or WC, whereas postnatal exposure showed inverse associations (17).
		Analysis from a longitudinal cohort study (26)	Shao et al. (26)	• Prenatal exposure to $PM_{2.5}$, PM_{10}, SO_2, and O_3 was associated with reduced fetal biometry, specifically at GW24 (SO_2 strongest effect: +10 μg/m^3 femur length −2.20 mm, ≈ −5.3%). Effects persisted but were attenuated at GW36 (26); • No differences in birth weight were registered, indicating rapid catch-up growth in the 3rd trimester (26).
	EDCs	Analysis from a longitudinal cohort study (108)	Webb et al. (108)	• 5-fold increase in HMW (%BF: +2.86 units, 95% CI: 0.69–5.03; % of VAT+21.7%, 95% CI: 10.5–33.9) and DEHP (% of BF: +2.69 units, 95% CI: 0.66–4.72; VAT:) exposure was associated with increased %BF and VAT (+18.0%, 95% CI: 7.72–29.2) in adolescent males (108) • 5-fold increase in LMW exposure was associated with increased %BF (+2.01 units, 95% CI: 0.05–3.98), VAT (+9.38%, 95% CI: 0.01–19.6), VAT (+9.38%, 95% CI: 0.01–19.6) and A/G ratio (+0.03, 95% CI: 0.00–0.07) in adolescent males (105); • No significant associations in adolescent females (108).
		In vitro study (104)	Longo et al. (104)	• BPA induces hypomethylation of the Pparγ promoter and transient gene activation ($p < 0.05$) (104); • BPA stimulates transient lipid accumulation and inflammation, both reversible ($p < 0.01$; proinflammatory cytokines Il6, Ifnγ, Tnfα, Mcp1, Il1β increased during exposure, normalized after BPA removal) (104);
		Systematic review (105)	Naomi et al. (105)	• BPA exposure was associated with lower abundance of *Bifidobacterium* spp. (4.2% vs. 7.8%, $p < 0.01$) and *Clostridium* Cluster XIVa (6.5% vs. 10.3%, $p < 0.05$), lower SCFAs production (1.8 vs. 3.5 μmol/g, $p < 0.01$), and a higher abundance of *Proteobacteria* (9.2% vs. 3.7%, $p < 0.01$) (105); • Sex-dependent differences were observed: males had higher *Bacteroides*, *Mollicutes*, *Prevotellaceae,* and *Akkermansia,* while females had higher *Lachnobacterium* and *Prevotella (105).*
	Urbanization	Systematic review and meta-analysis (36);	Johnson et al. (36)	• Across 10 studies (five in meta-analysis, $n = 74,168$ children, 2–19 years), rural residence was consistently associated with higher obesity prevalence. Pooled data showed 26% greater odds of obesity in rural vs. urban children (OR = 1.26; 95% CI: 1.21–1.32) (36);

(Continued)

TABLE 1 (Continued)

Infant exposome domains	Exposure factors	Study design	References	Main results of the studies
	Nbs	Cross-sectional analysis from a longitudinal cohort study (33)	Heo et al. (33)	• Prenatal greenspace exposure (EVI, park number) was linked to a reduced risk of preterm birth (Q2 OR = 0.65; Q3 OR = 0.51; Q4 OR = 0.56 vs. Q1) (33). • No significant associations were found with the terms low birthweight, birthweight, or estimated fetal weight (33).
		Cross-sectional analysis from 11 cohort studies (34)	Torres Toda et al. (34)	• An IQR increase in residential surrounding greenspace (100 m, 300 m, 500 m) was associated with lower odds of SGA (OR = 0.87, 95% CI: 0.83–0.92; OR = 0.87, 95% CI: 0.82–0.91; OR = 0.86, 95% CI: 0.81–0.90) (34); • Greater residential distance to greenspace increased SGA risk (OR = 1.07, 95% CI: 1.02–1.12). Associations for accessibility to GABS exposure were close to null (34).
	Climate	Meta-analysis (42)	Myers et al. (42)	• CO_2 (546–586 ppm) significantly decreased nutrient concentrations: wheat grains showed −9.3% zinc (95% CI: −12.7 to −5.9), −5.1% iron (95% CI: −6.5 to −3.7), and −6.3% protein (95% CI: −7.5 to −5.2), while rice showed −7.8% protein (95% CI: −8.9 to −6.8) (42); • Reductions in zinc and iron were observed across other C3 legumes and grasses, whereas C4 crops were minimally affected (42).
		Analysis from a longitudinal cohort study (58)	Part et al. (58)	• Each +1 °C increase in daily mean temperature was associated with −2.3 min/day BF (95% CI: −4.6 to 0.04) and +0.6 min/day childcare (95% CI: 0.06–1.2). During the hottest vs. coolest periods, women breastfed ∼25 min/day less. Odds of exclusive BF in very young infants (0–3 months) decreased with higher temperature (OR = 0.88; 95% CI: 0.75–1.02) (58). • No associations were found in exclusively breastfed infants at 3–6 months or for supplementary feeding at 6–12 months (58).
Specific external exposome	Breastfeeding	Systematic review (110)	Kumari et al. (110)	• Protective effect of prolonged BF on excess child body weight (110).
		Analysis from a longitudinal cohort study (111)	Lin et al (111)	• Lower risk of EAR in children breastfed for > 4 months (adjusted RR = 0.80, 95% CI: 0.73-0.87, p <0.001) (111).
		Analysis from a longitudinal cohort study (112)	Wu et al. (112)	• Delayed AR in girls: 5 months EBF associated with +0.64 years (GRS 2.5), +0.53 years (GRS 5.0), +0.44 years (GRS 7.5); $p < 0.05$ (112); • Delayed AP: 5 months EBF associated with +0.21 years (GRS 5.0), +0.25 years (GRS 7.5) in boys and +0.14 years (GRS 2.5), +0.24 years (GRS 7.5) in girls; $p < 0.05$ (112); • BMI reduction at 18 y with 5 months EBF: boys −0.81 to −1.14 kg/m² and girls −0.86 to −1.53 kg/m² (depending on GRS); $p < 0.05$ (112); • Non-exclusive BF associated with lower BMI reduction compared to EBF: −0.31 to −0.37 kg/m² in boys, −0.34 to −0.54 kg/m² in girls ($p < 0.05$) (112); • Shorter EBF (3 months) was associated with smaller effects on delaying AP/AR and reducing BMI (boys −0.49 to −0.68 kg/m², girls −0.52 to −0.92 kg/m²; depending on GRS; $p <$ 0.05) (112).
		Analysis from a longitudinal cohort study (114)	Kanders et al. (114)	• BF 7–12 months: lower risk of overweight at W1 (114); • BF >12 months in FTO rs9939609 TA children was associated with reduced risk of overweight at W1 (OR = 0.41, 95% CI: 0.19–0.88) (114); • No significant effect of BF or FTO variants on BMI/overweight at W2 or W3 (114).
		Analysis from a cross-sectional study (117)	Verier et al. (117)	• Non–breastfed children (n = 173), Ala12 carriers vs. Pro12Pro registered higher BMI: +1.88 kg/m² (adjusted P = 0.007), WC: +3.8 cm (adjusted P = 0.03) (117); • Breastfed children: no significant difference in BMI, WC, or skinfolds between Ala12 carriers and Pro12Pro (117); • Protective effect of BF mitigates genetic predisposition to higher adiposity in Ala12 carriers, even for short-duration BF (117).

(Continued)

TABLE 1 (Continued)

Infant exposome domains	Exposure factors	Study design	References	Main results of the studies
	Breast milk (as a vehicle of substances) *EDCs*	Analysis from a longitudinal cohort study (78)	Vacca M. et al. (78)	• In breastfed infants (stratified by maternal urinary BPA; >0.96 mg/g creatinine = high exposure), differential gut colonization was observed at 12 months: *Ruminococcus torques* group was significantly higher in low-exposed infants ($p < 0.05$), whereas *Erysipelatoclostridium* and *Bifidobacterium breve* were enriched in high-exposed infants (78); • Retrospective β-diversity analysis (from birth to 12 months) confirmed compositional disparities between exposure groups (78); • Stratification by phthalate exposure showed no significant differences between groups (78).
	Nicotine	Cross-sectional analysis from a cohort study (82)	Napierala et al. (82)	• Smoking during lactation significantly increased the TBARS and TEAC (measure of total antioxidant potential) in colostrum and mature milk ($p < 0.05$) (82); • The activity of antioxidant enzymes, including superoxide dismutase, glutathione S-transferase, glutathione peroxidase, and catalase, was significantly elevated ($p < 0.05$) (82).
		Systematic review (81)	Macchi et al. (81)	• Maternal smoking during lactation was consistently associated with altered HBM composition. Specifically, smoking is correlated with a reduction in total lipid and PUFA levels and a higher concentration of MUFA (81).
		Analysis from a longitudinal cohort study (79)	Srivastava et al. (79)	• Maternal smoking increased obesity probability by +2.8%, paternal smoking by +2.1%, and exposure to both parents by +2.0%. Age-stratified analyses confirmed a higher risk in (4–11 years: +2.3%) children (79)
	Complementary feeding	Analysis from 2 cohort studies (119)	Homann et al. (119)	• Higher daily dietary diversity modulated the gut microbiota homeostasis and promoted taxa as *Bifidobacterium* (β = 0.28, $p < 0.01$), while reducing genera like *Veillonella* (β = −0.22, $p < 0.05$) (119).
Internal exposome	Biological responses to external exposure factors			
	ROS production (82) Adipogenesis genes expression (107) Epigenetic pathways *via* DNA methylation and CpG island regulation (104, 107) Chronic low-grade inflammation in adipose tissue (104) Gut microbiota homeostasis (116, 125) SCFAs production (105) Obesity-related genes (FTO, NRF1, LEPR) and PPAR signaling (104, 107, 111)			

Presentation of the findings referred to the exposome domains and biological responses.
PM, particulate matter; FV, fruits and vegetables; BMI, body mass index; WC, waist circumference; PFAS, per- and polyfluoroalkyl substances; GW, gestational age; EDCs, endocrine disrupting chemicals; HMW, high molecular weight; DEHP, di(2-ethylhexyl) phthalate; %BF, percentage body fat; VAT, visceral adipose tissue LMW, low molecular weight; A/G ratio, Android-to-Gynoid fat ratio; BPA, bisphenol A; PPAR, peroxisome proliferator-activated receptor; SCFAs, short-chain fatty acids; Nbs, nature-based solution; EVI, enhanced vegetation index; SGA, small for gestational age; BF, breastfeeding; EAR, early adiposity rebound; AR, adiposity rebound; EBF, exclusive breastfeeding; AP, adiposity peak; FTO, fat mass and obesity-associated gene; TBARS, thiobarbituric acid reactive substances; TEAC, trolox equivalent antioxidant capacity; HBM, human breast milk; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; ROS, reactive oxygen species; NRF1, nuclear respiratory factor 1 gene; LEPR, Leptin Receptor gene.

1.11–2.86) (134). Additionally, gut microbiota mediated the effect of smoking habits during pregnancy on the higher risk of obesity in offspring. Particularly, the Firmicutes group accounted for the largest portions (23.3–24.6%) of the total effects on BMI *z*-scores at 1 year and 12.4–15.2% at 3 years of age of the children (137). The main results are summarized in Table 1.

5 Discussion

Childhood obesity has become one of the most critical global health challenges (5). Particular attention should be directed to preventable factors during the pivotal period of the "first 1,000 days" (6). This narrative review provides a novel perspective by exploring various exposure factors within the general external exposome during the "first 1,000 days" of life (chemical compounds, air pollution, urbanization), focusing on their potential role in the development of childhood obesity (17, 23, 36).

In addition, the review also explores specific external exposome factors (infant feeding practices, children's lifestyle, SES) to which the mother, and more broadly the family, are exposed, recognizing the complex interplay of intergenerational and familial dynamics that may affect the child's development and future health (71, 93). Furthermore, exposure to environmental factors during both the prenatal period and early childhood may alter crucial metabolic processes, threatening the internal exposome homeostasis and increasing the risk of developing obesity and other metabolic disorders (102).

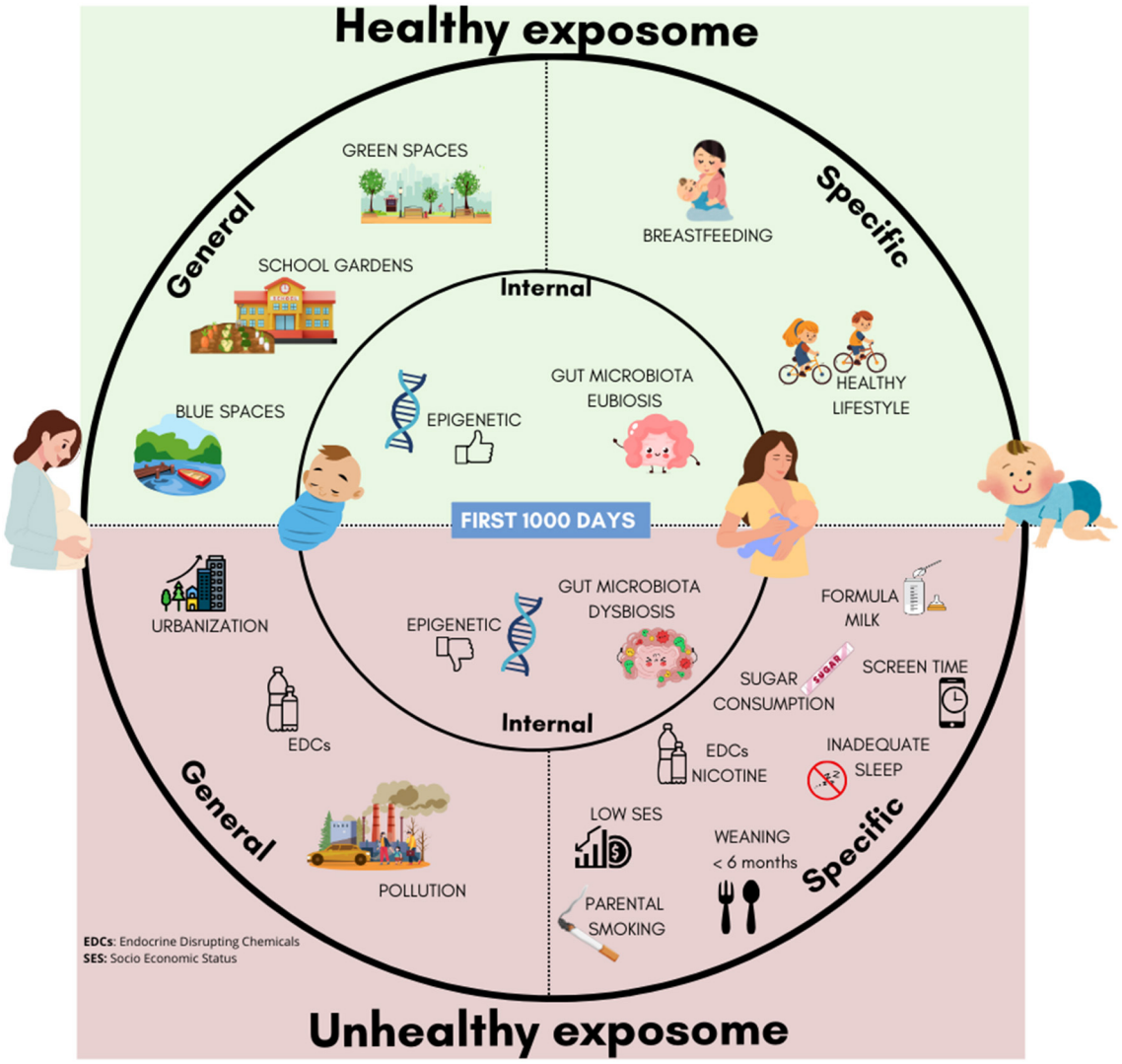


FIGURE 1
Healthy and unhealthy esposome factors in early life: general, specific, and internal determinants influencing childhood obesity risk.

According to the literature findings, the authors identified a "healthy exposome," encompassing protective factors against obesity, and an "unhealthy exposome" associated with higher obesity risk (Figure 1).

Clear evidence of protective factors emerged across different domains of the exposome (33–35). Within the general external exposome, results indicate that access to GABS protects against obesity by supporting healthier growth trajectories, increased PA, and healthier dietary patterns (33–35).

Similarly, regarding the specific external exposome, exclusive breastfeeding for at least 6 months, adequate complementary feeding, regular PA, limited screen time, and proper sleep represent robust protective factors (64, 65, 85–88). These exposures act through internal pathways, including modulation of gut microbiota composition, regulation of AR, and epigenetic effects on obesity-related genes (110–112, 117, 130).

In contrast, evidence indicates that key risk factors include maternal smoking during pregnancy and early-life exposure to EDCs, such as BPA and phthalates, which have been shown to promote obesogenic processes by disrupting adipogenesis, altering glucose and lipid metabolism, and enhancing proinflammatory signaling (103, 107–110, 134).

However, the role of several factors remains controversial, including evidence regarding SES and the exposure to PFAS, for which findings are heterogeneous and context-dependent (17, 99). Moreover, although breastfeeding is consistently protective, conflicting evidence exists regarding its biochemical composition. For instance, Vieira Queiroz De Paula et al. reported heterogeneous associations of hormones (e.g., leptin, adiponectin, insulin) and macronutrients with later obesity risk (72). These discrepancies highlight the complexity of exposome research and the need for more longitudinal cohort studies.

Addressing these exposures early in life is essential, as they may have compounding effects that influence health outcomes and potentially persist across generations (9, 138, 139).

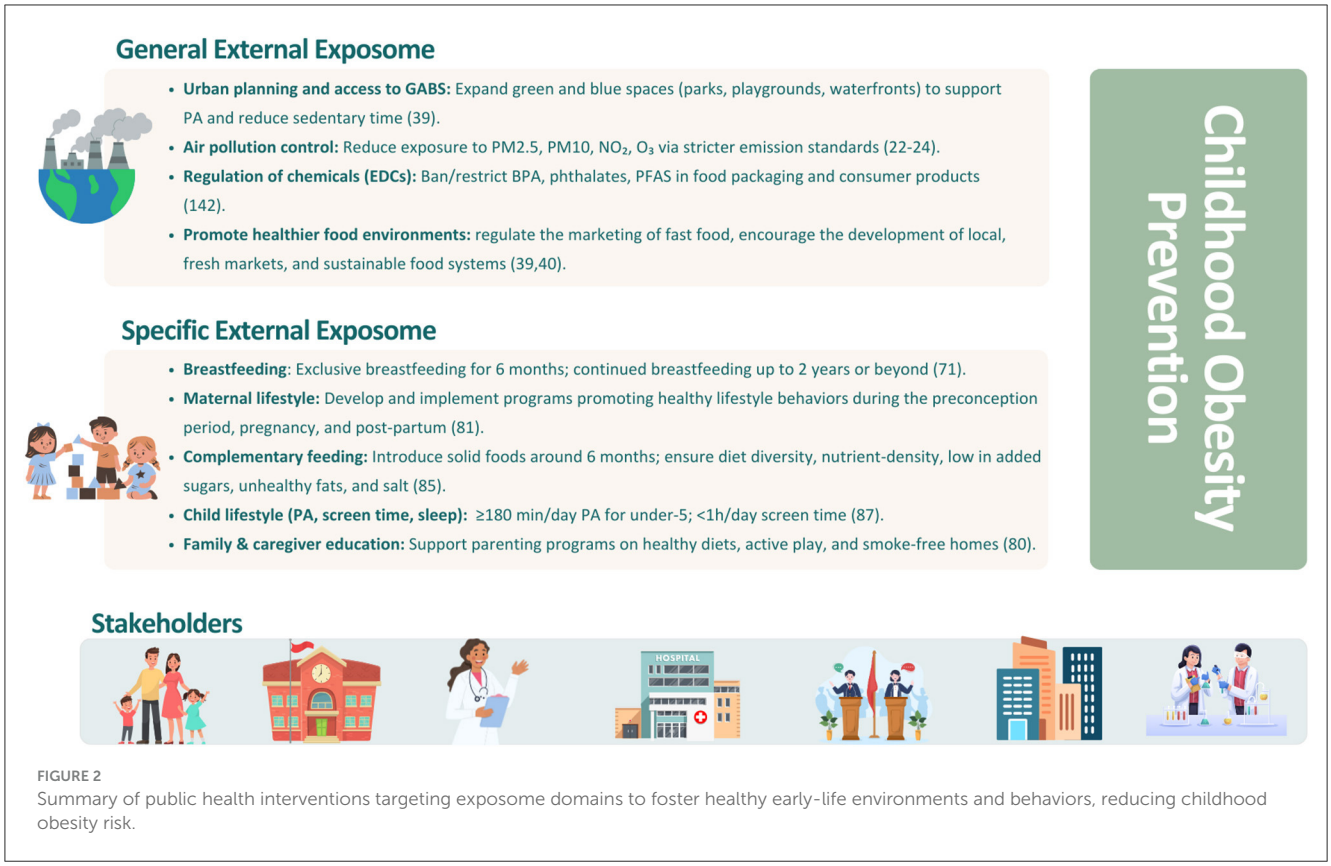


FIGURE 2
Summary of public health interventions targeting exposome domains to foster healthy early-life environments and behaviors, reducing childhood obesity risk.

Furthermore, One Health challenges, such as biodiversity loss, climate change, and ecosystem degradation, lead to exacerbation of exposure to air pollution, EDCs, and unhealthy food environments, thereby contributing to obesity and NCDs (140). To address these challenges, integrated actions are needed. Accordingly, the authors present a roadmap of actions aimed at promoting a healthy exposome during the first 1,000 days of life (Figure 2).

Accordingly, recent regulatory actions, including the 2023 EFSA safety assessment that reduced the tolerable daily intake of BPA (from 4 μg/kg bw/day to 0.2 ng/kg bw/day) and the 2024 European Commission ban on its use in food contact materials, represent key steps forward in limiting early-life exposure to these chemicals (141).

In addition, the WHO highlights six priority areas for action, such as promotion of healthy diets, increased PA, preconception and pregnancy care, early childhood nutrition, school-based interventions, and weight management, highlighting that only an integrated strategy can effectively address the modifiable risk factors identified and reduce the global burden of childhood obesity (142).

Despite the innovative approach adopted, this review has certain limitations that must be acknowledged. Primarily, not all factors of the general and specific external exposome were analyzed. This limitation arises from the inherent difficulties in assessing the complex and multifactorial nature of environmental exposures during the critical "first 1,000 days". Second, while a range of exposures were considered, not all were assessed within the prenatal period, with some factors being investigated exclusively during early childhood. Furthermore, the results concerning certain exposome components were inconsistent, which can largely be attributed to the heterogeneity of the available research. Variations in study designs, population characteristics, and exposure assessment methodologies contribute to these discrepancies, thereby limiting the ability to generalize some of the conclusions derived from the findings.

6 Conclusion

During the first 1,000 days of life, general and specific external exposures critically shape childhood obesity risk through their effects on internal biological processes. A functional exposome approach, integrating environmental, behavioral, and biological data, allows the identification of critical windows of vulnerability and supports early interventions that limit harmful exposures. Interdisciplinary collaboration is essential to unravel the complex interplay between genetic susceptibility, environmental determinants, and lifestyle-related factors, thereby enabling the development of tailored prevention strategies. Future research should prioritize the integration of big data analytics, machine learning, and epidemiological studies to clarify inconsistent findings and uncover exposure patterns not detectable with traditional methods. This approach enhances the ability to design

evidence-based policies, regulatory frameworks, and community-based initiatives that reduce disease prevalence and improve overall public health outcomes.

Author contributions

BM: Writing – original draft. FL: Writing – original draft. IB: Writing – original draft. FS: Writing – original draft. CF Writing – original draft. DEM: Writing – original draft. FV: Writing – original draft. EC: Writing – original draft. NM: writing – review & editing. CDF: Writing – review & editing. RDG: Writing – review & editing, Supervision. HC: Writing – review & editing, Supervision, Project administration.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Project funded under the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.4-Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP F13C22000720007, Project title "National Biodiversity Future Center-NBFC" and the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.3-Call for proposal No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU. Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP F13C22001210007, Project title "ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security-Working ON Foods.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

References

1. Istituto Superiore di Sanità (2025). Available online at: https://www.epicentro.iss.it/obesita/aggiornamenti (Accessed February 10, 2025).

2. Bendor CD, Bardugo A, Pinhas-Hamiel O, Afek A, Twig G. Cardiovascular morbidity, diabetes and cancer risk among children and adolescents with severe obesity. *Cardiovasc Diabetol.* (2020) 19:1–14. doi: 10.1186/s12933-020-01052-1

3. Horesh A, Tsur AM, Bardugo A, Twig G. Adolescent and childhood obesity and excess morbidity and mortality in young adulthood-a systematic review. *Curr Obes Rep.* (2021) 10:301–10. doi: 10.1007/s13679-021-00439-9

4. Vrijheid M, Fossati S, Maitre L, Márquez S, Roumeliotaki T, Agier L, et al. Early-life environmental exposures and childhood obesity: an exposome-wide approach. *Environ Health Perspect.* (2020) 128:1–14. doi: 10.1289/EHP5975

5. Ling J, Chen S, Zahry NR, Kao TSA. Economic burden of childhood overweight and obesity: a systematic review and meta-analysis. *Obes Rev.* (2022) 24:e13535. doi: 10.1111/obr.13535

6. Matvienko-Sikar K, Cooney J, Flannery C, Murphy J, Khashan A, Huizink A. Maternal stress in the first 1000 days and risk of childhood obesity: a systematic review. *J Reprod Infant Psychol.* (2021) 39:180–204. doi: 10.1080/02646838.2020.1724917

7. Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: an individual participant data meta-analysis. *PLoS Med.* (2019) 16:e1002744. doi: 10.1371/journal.pmed.1002744

8. Almeida-Toledano L, Navarro-Tapia E, Sebastiani G, Ferrero-Martínez S, Ferrer-Aguilar P, García-Algar Ó, et al. Effect of prenatal phthalate exposure on fetal development and maternal/neonatal health consequences: a systematic review. *Sci Total Environ.* (2024) 950:175080. doi: 10.1016/j.scitotenv.2024.175080

9. Pérez-Muñoz C, Carretero-Bravo J, Ortega-Martín E, Ramos-Fiol B, Ferriz-Mas B, Díaz-Rodríguez M. Interventions in the first 1000 days to prevent childhood obesity: a systematic review and quantitative content analysis. *BMC Public Health.* (2022) 22:2367. doi: 10.1186/s12889-022-14701-9

10. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev.* (2005) 14:1847–50. doi: 10.1158/1055-9965.EPI-05-0456

11. D'Errico A, Maritano S, Moccia C, Isaevska E, Pizzi C, Moirano G, et al. Esposoma: dalla definizione alle sfide future [Exposome: from defini;on to future challenges.]. *Recen Prog Med.* (2023) 114:34954. doi: 10.1701/4042.40227

12. Vrijheid M. The exposome: a new paradigm to study the impact of environment on health. *Thorax.* (2014) 69:876–8. doi: 10.1136/thoraxjnl-2013-204949

13. La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul HB, et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nat Rev Endocrinol.* (2020) 16:45–57. doi: 10.1038/s41574-019-0273-8

14. Calcaterra V, Cena H, Loperfido F, Rossi V, Grazi R, Quatrale A, et al. Evaluating phthalates and bisphenol in foods: risks for precocious puberty and early-onset obesity. *Nutr.* (2024) 16:2732. doi: 10.3390/nu16162732

15. Dong Y, Gao D, Li Y, Yang Z, Wang X, Chen M, et al. Effect of childhood phthalates exposure on the risk of overweight and obesity: a nested case-control study in China. *Environ Int.* (2022) 158:106886. doi: 10.1016/j.envint.2021.106886

16. Gutiérrez-Torres DS, Barraza-Villarreal A, Hernandez-Cadena L, Escamilla-Nuñez C, Romieu I. Prenatal exposure to endocrine disruptors and cardiometabolic risk in preschoolers: a systematic review based on cohort studies. *Ann Glob Heal.* (2018) 84:239. doi: 10.29024/aogh.911

17. Frangione B, Birk S, Benzouak T, Rodriguez-Villamizar LA, Karim F, Dugandzic R, et al. Exposure to perfluoroalkyl and polyfluoroalkyl substances and pediatric obesity: a systematic review and meta-analysis. *Int J Obes.* (2024) 48:131–46. doi: 10.1038/s41366-023-01401-6

18. Lin MH, Lee CY, Chuang YS, Shih CL. Exposure to bisphenol A associated with multiple health-related outcomes in humans: an umbrella review of systematic reviews with meta-analyses. *Environ Res.* (2023) 237:116900. doi: 10.1016/j.envres.2023.116900

19. Symeonides C, Aromataris E, Mulders Y, Dizon J, Stern C, Barker TH, et al. An umbrella review of meta-analyses evaluating associations between human health and exposure to major classes of plastic-associated chemicals. *Ann Glob Heal.* (2024) 90:52. doi: 10.5334/aogh.4459

20. Ribeiro CM, Beserra BTS, Silva NG, Lima CL, Rocha PRS, Coelho MS, et al. Exposure to endocrine-disrupting chemicals and anthropometric measures of obesity: a systematic review and meta-analysis. *BMJ Open.* (2020) 10:33509. doi: 10.1136/bmjopen-2019-033509

21. Wu Q, Li G, Zhao CY, Na XL, Zhang YB. Association between phthalate exposure and obesity risk: a meta-analysis of observational studies. *Environ Toxicol Pharmacol.* (2023) 102:104240. doi: 10.1016/j.etap.2023.104240

22. Simkova S, Veleminsky M, Sram RJ. The impact of air pollution to obesity. *Neuro Endocrinol Leh.* (2020) 41:146–53.

23. Luo C, Wei T, Jiang W, Yang YP, Zhang MX, Xiong CL, et al. The association between air pollution and obesity: an umbrella review of meta-analyses and systematic reviews. *BMC Public Health.* (2024) 24:1–8. doi: 10.1186/s12889-024-19370-4

24. Zheng J, Zhang H, Shi J, Li X, Zhang J, Zhang K, et al. Association of air pollution exposure with overweight or obesity in children and adolescents: a systematic review and meta-analysis. *Sci Total Environ.* (2024) 910:168589. doi: 10.1016/j.scitotenv.2023.168589

25. Huang C, Li C, Zhao F, Zhu J, Wang S, Sun G. The association between childhood exposure to ambient air pollution and obesity: a systematic review and meta-analysis. *Int J Environ Res Public Health.* (2022) 19:4491. doi: 10.3390/ijerph19084491

26. Shao X, Cheng H, Zhou J, Zhang J, Zhu Y, Yang C, et al. Prenatal exposure to ambient air multi-pollutants significantly impairs intrauterine fetal development trajectory. *Ecotoxicol Environ Saf.* (2020) 1:110726. doi: 10.1016/j.ecoenv.2020.110726

27. Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P, et al. Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. *Epidemiology.* (2010) 21:884–91. doi: 10.1097/EDE.0b013e3181f2f405

28. D'Agostin M, Morgia CDS, Vento G, Nobile S. Long-term implications of fetal growth restriction. *World J Clin Cases.* (2023) 11:2855. doi: 10.12998/wjcc.v11.i13.2855

29. Margetaki K, Bempi V, Michalaki E, Roumeliotaki T, Iakovides M, Stephanou E, et al. Prenatal air pollution exposure and childhood obesity: effect modification by maternal fruits and vegetables intake. *Int J Hyg Environ Health.* (2024) 256:114314. doi: 10.1016/j.ijheh.2023.114314

30. Ji N, Johnson M, Eckel SP, Gauderman WJ, Chavez TA, Berhane K, et al. Prenatal ambient air pollution exposure and child weight trajectories from the 3rd trimester of pregnancy to 2 years of age: a cohort study. *BMC Med.* (2023) 21:341. doi: 10.1186/s12916-023-03050-y

31. Kabisch N, van den Bosch M, Lafortezza R. The health benefits of nature-based solutions to urbanization challenges for children and the elderly – a systematic review. *Environ Res.* (2017) 159:362–73. doi: 10.1016/j.envres.2017.08.004

32. Bureau UC. *Urban and Rural* (2023). Available online at: https://www.census.gov/programs-surveys/geography/guidance/geo-areas/urban-rural.htmlhttps://www.census.gov/programs-surveys/geography/guidance/geo-areas/urban-rural.html (Accessed February 10, 2025).

33. Heo S, Afanasyeva Y, Liu M, Mehta-Lee S, Yang W, Trasande L, et al. Prenatal exposure to residential greenness, fetal growth, and birth outcomes: a cohort study in New York City. *Am J Epidemiol.* (2024) 194:2621–30. doi: 10.1093/aje/kwae436

34. Torres Toda M, Avraam D, James Cadman T, Fossati S, de Castro M, Dedele A, et al. Exposure to natural environments during pregnancy and birth outcomes in 11 European birth cohorts. *Environ Int.* (2022) 1:170. doi: 10.1016/j.envint.2022.107648

35. Martenies SE, Oloo A, Magzamen S, Ji N, Khalili R, Kaur S, et al. Independent and joint effects of neighborhood-level environmental and socioeconomic exposures on body mass index in early childhood: the environmental influences on child health outcomes (ECHO) cohort. *Environ Res.* (2024) 253:119109. doi: 10.1016/j.envres.2024.119109

36. Johnson JA, Johnson AM. Urban-rural differences in childhood and adolescent obesity in the United States: a systematic review and meta-analysis. *Child Obes.* (2015) 11:233–41. doi: 10.1089/chi.2014.0085

37. Dunton GF, Kaplan J, Wolch J, Jerrett M, Reynolds KD. Physical environmental correlates of childhood obesity: a systematic review. *Obes Rev.* (2009) 10:393–402. doi: 10.1111/j.1467-789X.2009.00572.x

38. Castro-Sifuentes D, Cárdenas-Villarreal VM, Zepeda-Ríos PA, Rueda-Sánchez CB, Hernández-Martínez N, Guevara-Valtier MC. Ecological determinants of obesity risk in Mexican infants: a scoping review. *Bol Med Hosp Infant Mex.* (2023) 80:223–34. doi: 10.24875/BMHIM.23000058

39. Alejandre JC, Lynch M. "Kids get in shape with nature": a systematic review exploring the impact of green spaces on childhood obesity. *J Nutr Sci Vitaminol.* (2020) 66:S129–33. doi: 10.3177/jnsv.66.S129

40. Jia P, Shi Y, Jiang Q, Dai S, Yu B, Yang S, et al. Environmental determinants of childhood obesity: a meta-analysis. *Lancet Glob Heal.* (2023) 11:S7. doi: 10.1016/S2214-109X(23)00092-X

41. Mei K, Huang H, Xia F, Hong A, Chen X, Zhang C, et al. State-of-the-art of measures of the obesogenic environment for children. *Obes Rev.* (2021) 1:e13093. doi: 10.1111/obr.13093

42. Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ, et al. Increasing CO_2 threatens human nutrition. *Nature.* (2014) 510:139–42. doi: 10.1038/nature13179

43. Soares JC, Santos CS, Carvalho SMP, Pintado MM, Vasconcelos MW. Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. *Plant Soil.* (2019) 443:1–26. doi: 10.1007/s11104-019-04229-0

44. Shahzad Z, Rouached H. Protecting plant nutrition from the effects of climate change. *Curr Biol.* (2022) 32:R725–7. doi: 10.1016/j.cub.2022.05.056

45. Owino V, Kumwenda C, Ekesa B, Parker ME, Ewoldt L, Roos N, et al. The impact of climate change on food systems, diet quality, nutrition, and health outcomes: a narrative review. *Front Clim.* (2022) 4:941842. doi: 10.3389/fclim.2022.941842

46. Smith MR, Myers SS. Impact of anthropogenic CO_2 emissions on global human nutrition. *Nat Clim Chang.* (2018) 8:834–9. doi: 10.1038/s41558-018-0253-3

47. Sulser TB, Beach RH, Wiebe KD, Dunston S, Fukagawa NK. Disability-adjusted life years due to chronic and hidden hunger under food system evolution with climate change and adaptation to 2050. *Am J Clin Nutr.* (2021) 114:550–63. doi: 10.1093/ajcn/nqab101

48. Liu J, Li L, Chen X, Lu Y, Wang D. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: a novel idea for monitoring and evaluation of heat stress — a review. *Asian-Australas J Anim Sci.* (2019) 32:1332–9. doi: 10.5713/ajas.18.0743

49. Liu Z, Ezernieks V, Wang J, Arachchillage NW, Garner JB, Wales WJ, et al. Heat stress in dairy cattle alters lipid composition of milk. *Sci Rep.* (2017) 7:961. doi: 10.1038/s41598-017-01120-9

50. El Haddad N, Choukri H, Ghanem ME, Smouni A, Mentag R, Rajendran K, et al. High-temperature and drought stress effects on growth, yield and nutritional quality with transpiration response to vapor pressure deficit in lentil. *Plants.* (2021) 11:95. doi: 10.3390/plants11010095

51. Muehe EM, Wang T, Kerl CF, Planer-Friedrich B, Fendorf S. Rice production threatened by coupled stresses of climate and soil arsenic. *Nat Commun.* (2019) 10:4985. doi: 10.1038/s41467-019-12946-4

52. Chi L, Bian X, Gao B, Tu P, Ru H, Lu K. The effects of an environmentally relevant level of arsenic on the gut microbiome and its functional metagenome. *Toxicol Sci.* (2017) 160:193–204. doi: 10.1093/toxsci/kfx174

53. O'Donnell DR, Du ZY, Litchman E. Experimental evolution of phytoplankton fatty acid thermal reaction norms. *Evol Appl.* (2019) 12:1201–11. doi: 10.1111/eva.12798

54. Vecellio DJ, Kong Q, Kenney WL, Huber M. Greatly enhanced risk to humans as a consequence of empirically determined lower moist heat stress tolerance. *Proc Natl Acad Sci U S A.* (2023) 120:e2305427120. doi: 10.1073/pnas.2305427120

55. Sepulveda J, Moeller AH. The effects of temperature on animal gut microbiomes. *Front Microbiol.* (2020) 11:384. doi: 10.3389/fmicb.2020.00384

56. Manser CN, Paul M, Rogler G, Held L, Frei T. Heat waves, incidence of infectious gastroenteritis, and relapse rates of inflammatory bowel disease: a retrospective controlled observational study. *Am J Gastroenterol.* (2013) 108:1480–5. doi: 10.1038/ajg.2013.186

57. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature.* (2014) 510:417–21. doi: 10.1038/nature13421

58. Part C, Filippi V, Cresswell JA, Ganaba R, Hajat S, Nakstad B, et al. How do high ambient temperatures affect infant feeding practices? A prospective cohort study of postpartum women in Bobo-Dioulasso, Burkina Faso. *BMJ Open.* (2022) 12:e061297. doi: 10.1136/bmjopen-2022-061297

59. Ma J, Li Z, Zhang W, Zhang C, Zhang Y, Mei H, et al. Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: a study of 91 term infants. *Sci Rep.* (2020) 10:15792. doi: 10.1038/s41598-020-72635-x

60. Neves PAR, Vaz JS, Maia FS, Baker P, Gatica-Domínguez G, Piwoz E, et al. Rates and time trends in the consumption of breastmilk, formula, and animal milk by children younger than 2 years from 2000 to 2019: analysis of 113 countries. *Lancet Child Adolesc Health.* (2021) 5:619–30. doi: 10.1016/S2352-4642(21)00163-2

61. Qiao J, Dai LJ, Zhang Q, Ouyang YQ. A meta-analysis of the association between breastfeeding and early childhood obesity. *J Pediatr Nur.* (2020) 53:57–66. doi: 10.1016/j.pedn.2020.04.024

62. Qureshi R, Jadotte Y, Zha P, Porter SA, Holly C, Salmond S, et al. The association between prenatal exposure to environmental tobacco smoke and childhood obesity:

a systematic review. *JBI Database Syst Rev Implement Reports.* (2018) 16:1643–62. doi: 10.11124/JBISRIR-2017-003558

63. Fang K, Mu M, Liu K, He Y. Screen time and childhood overweight/obesity: a systematic review and meta-analysis. *Child Care Health Dev.* (2019) 45:744–53. doi: 10.1111/cch.12701

64. Li W, Yuan J, Wang L, Qiao Y, Liu E, Wang S, et al. The association between breastfeeding and childhood obesity/underweight: a population-based birth cohort study with repeated measured data. *Int Breastfeed J.* (2022) 17:82. doi: 10.1186/s13006-022-00522-4

65. Ma J, Qiao Y, Zhao P, Li W, Katzmarzyk PT, Chaput JP, et al. Breastfeeding and childhood obesity: a 12-country study. *Matern Child Nutr.* (2020) 16:e12984. doi: 10.1111/mcn.12984

66. Bianco I, Ferrara C, Romano F, Loperfido F, Sottotetti F, El Masri D, et al. The influence of maternal lifestyle factors on human breast milk microbial composition: a narrative review. *Biomed.* (2024) 12:2423. doi: 10.3390/biomedicines12112423

67. Szyller H, Antosz K, Batko J, Mytych A, Dziedziak M, Wrześniewska M, et al. Bioactive components of human milk and their impact on child's health and development, literature review. *Nutrients*. (2024) 16:1487. doi: 10.3390/nu16101487

68. Zheng M, D'Souza NJ, Atkins L, Ghobadi S, Laws R, Szymlek-Gay EA, et al. Breastfeeding and the longitudinal changes of body mass index in childhood and adulthood: a systematic review. *Adv Nutr.* (2024) 15:100152. doi: 10.1016/j.advnut.2023.100152

69. Cheema AS, Gridneva Z, Furst AJ, Roman AS, Trevenen ML, Turlach BA, et al. Human milk oligosaccharides and bacterial profile modulate infant body composition during exclusive breastfeeding. *Int J Mol Sci.* (2022) 23:2865. doi: 10.3390/ijms23052865

70. Rito AI, Buoncristiano M, Spinelli A, Salanave B, Kunešová M, Hejgaard T, et al. Association between characteristics at birth, breastfeeding and obesity in 22 countries: the WHO European childhood obesity surveillance initiative - COSI 2015/2017. *Obes Facts.* (2019) 12:226–43. doi: 10.1159/000500425

71. World Health Organization. *Breastfeeding reduces child obesity risk by up to 25%* (2019). Available online at: https://www.unicef.org.uk/babyfriendly/breastfeeding-reduces-child-obesity-risk-by-up-to-25-per-cent/ (Accessed February, 2025).

72. Vieira Queiroz De Paula M, Grant M, Lanigan J, Singhal A. Does human milk composition predict later risk of obesity? A systematic review. *BMC Nutr*. (2023) 9:89. doi: 10.1186/s40795-023-00742-9

73. Iribarne-Durán LM, Peinado FM, Freire C, Castillero-Rosales I, Artacho-Cordón F, Olea N. Concentrations of bisphenols, parabens, and benzophenones in human breast milk: a systematic review and meta-analysis. *Sci Total Environ*. (2022) 806:150437. doi: 10.1016/j.scitotenv.2021.150437

74. Onyena AP, Bede-Ojimadu O, Abaate TJ, Amachree D, Frazzoli C, Folorunso OM, et al. Lactational exposure of human infants to metal(loid)s in Sub-Saharan Africa and Mediterranean Europe: a systematic review and meta-analysis. *Curr Res Toxicol.* (2024) 7:100201. doi: 10.1016/j.crtox.2024.100201

75. Braun JM. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. *Nat Rev Endocrinol.* (2017) 13:161–73. doi: 10.1038/nrendo.2016.186

76. Dualde P, Pardo O, Corpas-Burgos F, Kuligowski J, Gormaz M, Vento M, et al. Biomonitoring of parabens in human milk and estimated daily intake for breastfed infants. *Chemosphere*. (2020) 240:124829. doi: 10.1016/j.chemosphere.2019.124829

77. Biagi E, Quercia S, Aceti A, Beghetti I, Rampelli S, Turroni S, et al. The bacterial ecosystem of mother's milk and infant's mouth and gut. *Front Microbiol.* (2017) 8:1214. doi: 10.3389/fmicb.2017.01214

78. Vacca M, Calabrese FM, Loperfido F, Maccarini B, Cerbo RM, Sommella E, et al. Maternal exposure to endocrine-disrupting chemicals: analysis of their impact on infant gut microbiota composition. *Biomedicines.* (2024) 12:234. doi: 10.3390/biomedicines12010234

79. Srivastava P, Trinh TA, Hallam KT, Karimi L, Hollingsworth B. The links between parental smoking and childhood obesity: data of the longitudinal study of Australian children. *BMC Public Health*. (2024) 24:68. doi: 10.1186/s12889-023-17399-5

80. Cummings JR, Lumeng JC, Miller AL, Hyde LW, Siada R, Gearhardt AN. Parental substance use and child reward-driven eating behaviors. *Appetite.* (2020) 144:104486. doi: 10.1016/j.appet.2019.104486

81. Macchi M, Bambini L, Franceschini S, Alexa ID, Agostoni C. The effect of tobacco smoking during pregnancy and breastfeeding on human milk composition-a systematic review. *Eur J Clin Nutr.* (2021) 75:736–47. doi: 10.1038/s41430-020-00784-3

82. Napierala M, Merritt TA, Miechowicz I, Mielnik K, Mazela J, Florek E. The effect of maternal tobacco smoking and second-hand tobacco smoke exposure on human milk oxidant-antioxidant status. *Environ Res.* (2019) 170:110–21. doi: 10.1016/j.envres.2018.12.017

83. Philips EM, Santos S, Trasande L, Aurrekoetxea JJ, Barros H, von Berg A, et al. Changes in parental smoking during pregnancy and risks of adverse birth outcomes and childhood overweight in Europe and North America: an individual participant data meta-analysis of 229,000 singleton births. *PLoS Med.* (2020) 17:e1003182. doi: 10.1371/journal.pmed.1003182

84. Papoutsou S, Savva SC, Hunsberger M, Jilani H, Michels N, Ahrens W, et al. Timing of solid food introduction and association with later childhood overweight and obesity: the IDEFICS study. *Matern Child Nutr*. (2018) 14:e12471. doi: 10.1111/mcn.12471

85. United Nations international children's emergency fund. *Improving Young Children's Diets During the Complementary Feeding Period – UNICEF Programming Guidance* (2020). Available online at: https://www.unicef.org/documents/improving-young-childrens-diets-during-complementary-feeding-period-unicef-programming (Accessed February 10, 2025).

86. Rousham EK, Goudet S, Markey O, Griffiths P, Boxer B, Carroll C, et al. Unhealthy food and beverage consumption in children and risk of overweight and obesity: a systematic review and meta-analysis. *Adv Nutr.* (2022) 13:1669–96. doi: 10.1093/advances/nmac032

87. World Health Organization. *To grow up healthy, children need to sit less and play more* (2019). Available online at: https://www.who.int/news/item/24-04-2019-to-grow-up-healthy-children-need-to-sit-less-and-play-morehttps://www.who.int/news/item/24-04-2019-to-grow-up-healthy-children-need-to-sit-less-and-play-more (Accessed February 10, 2025).

88. World Health Organization. *Report of the commission on ending childhood obesity* (2016). Available online at: https://iris.who.int/bitstream/handle/10665/204176/9789241510066_eng.pdf?sequence=1 (Accessed February 10, 2025).

89. Jia P, Dai S, Rohli KE, Rohli RV, Ma Y, Yu C, et al. Natural environment and childhood obesity: a systematic review. *Obes Rev*. (2021) 22:e13097. doi: 10.1111/obr.13097

90. Edwards NM, Myer GD, Kalkwarf HJ, Woo JG, Khoury PR, Hewett TE, et al. Outdoor temperature, precipitation, and wind speed affect physical activity levels in children: a longitudinal cohort study. *J Phys Act Health.* (2015) 12:1074–81. doi: 10.1123/jpah.2014-0125

91. Morrissey B, Taveras E, Allender S, Strugnell C. Sleep and obesity among children: a systematic review of multiple sleep dimensions. *Pediatr Obes*. (2020) 15:e12619. doi: 10.1111/ijpo.12619

92. Devaux M, Sassi F. Social inequalities in obesity and overweight in 11 OECD countries. *Eur J Public Health.* (2013) 23:464–9. doi: 10.1093/eurpub/ckr058

93. Jang M, Owen B, Lauver DR. Different types of parental stress and childhood obesity: a systematic review of observational studies. *Obes Rev.* (2019) 20:1740–58. doi: 10.1111/obr.12930

94. Bertrand-Protat S, Chen J, Jonquoy A, Frayon S, Thu Win Tin S, Ravuvu A, et al. Prevalence, causes and contexts of childhood overweight and obesity in the Pacific region: a scoping review. *Open Res Eur*. (2023) 3:52. doi: 10.12688/openreseurope.15361.2

95. Alruwaili BF, Bayyumi DF, Alruwaili OS, Alsadun RS, Alanazi AS, Hadi A, et al. Prevalence and determinants of obesity and overweight among children and adolescents in the middle east and north African countries: an updated systematic review. *Diabetes Metab Syndr Obes.* (2024) 17:2095–103. doi: 10.2147/DMSO.S458003

96. Mcgillivray J, Mcvilly K, Skouteris H, Boganin C. Parental factors associated with obesity in children with disability: a systematic review. *Obes Rev.* (2013) 14:541–54. doi: 10.1111/obr.12031

97. Avery A, Anderson C, McCullough F. Associations between children's diet quality and watching television during meal or snack consumption: a systematic review. *Matern Child Nutr.* (2017) 13:e12428. doi: 10.1111/mcn.12428

98. Spiga F, Tomlinson E, Davies AL, Moore TH, Dawson S, Breheny K, et al. Interventions to prevent obesity in children aged 12 to 18 years old. *Cochrane database Syst Rev*. (2024) 5:CD015330. doi: 10.1002/14651858.CD015330.pub2

99. Johnson KA, Showell NN, Flessa S, Janssen M, Reid N, Cheskin LJ, et al. Do neighborhoods matter? A systematic review of modifiable risk factors for obesity among low socio-economic status black and hispanic children. *Child Obes.* (2019) 15:71–86. doi: 10.1089/chi.2018.0044

100. Sacre H, Haddad C, Hajj A, Zeenny RM, Akel M, Salameh P. Development and validation of the socioeconomic status composite scale (SES-C). *BMC Public Health.* (2023) 23:1–13. doi: 10.1186/s12889-023-16531-9

101. Wild CP. The exposome: from concept to utility. *Int J Epidemiol.* (2012) 41:24–32. doi: 10.1093/ije/dyr236

102. Martin RM, Patel R, Kramer MS, Guthrie L, Vilchuck K, Bogdanovich N, et al. Effects of promoting longer-term and exclusive breastfeeding on adiposity and insulin-like growth factor-I at age 115 years: a randomized trial. *JAMA*. (2013) 309:1005–13. doi: 10.1001/jama.2013.167

103. Dalamaga M, Kounatidis D, Tsilingiris D, Vallianou NG, Karampela I, Psallida S, et al. The role of endocrine disruptors bisphenols and phthalates in obesity: current evidence, perspectives and controversies. *Int J Mol Sci.* (2024) 25:675. doi: 10.3390/ijms25010675

104. Longo M, Zatterale F, Naderi J, Nigro C, Oriente F, Formisano P, et al. Low-dose bisphenol-A promotes epigenetic changes at pparγ promoter in adipose precursor cells. *Nutrients.* (2020) 12:3498. doi: 10.3390/nu12113498

105. Naomi R, Yazid MD, Bahari H, Keong YY, Rajandram R, Embong H, et al. Bisphenol A (BPA) leading to obesity and cardiovascular complications: a compilation of current *in vivo* study. *Int Jour of Mol Sciens.* (2022) 23:2969. doi: 10.3390/ijms23062969

106. Otaka H, Yasuhara A, Morita M. Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and cleanup by solid-phase extraction. *Anal Sci.* (2003) 19:1663–6. doi: 10.2116/analsci.19.1663

107. Ye X, Bishop AM, Needham LL, Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk. *Anal Chim Acta.* (2008) 622:150–6. doi: 10.1016/j.aca.2008.05.068

108. Webb MD, Park JW, Day DB, Trabulsi JC, Sathyanarayana S, Melough MM. Associations of phthalate exposure with adiposity and metabolic syndrome in US adolescents and adults, NHANES 2013 to 2018. *J Endocr Soc*. (2024) 8:bvae189. doi: 10.1210/jendso/bvae189

109. World Health Organization. *Breastfeeding* (2025). Available online at: https://www.who.int/health-topics/breastfeeding#tab=tab_1 (Accessed February 10, 2025).

110. Kumari P, Bisht A. The protective effects of breastfeeding on maternal lifestyle disorders: a systematic review. *Int J Heal Sci Res.* (2023) 13:2249–9571. doi: 10.52403/ijhsr.20230315

111. Lin D, Chen D, Huang J, Li Y, Wen X, Wang L, et al. Pre-birth and early-life factors associated with the timing of adiposity peak and rebound: a large population-based longitudinal study. *Front Pediatr.* (2021) 9:742551. doi: 10.3389/fped.2021.742551

112. Wu Y, Lye S, Dennis CL, Briollais L. Exclusive breastfeeding can attenuate body-mass-index increase among genetically susceptible children: a longitudinal study from the ALSPAC cohort. *PLoS Genet*. (2020) 16:e1008790. doi: 10.1371/journal.pgen.1008790

113. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer*. (2023) 22:138. doi: 10.1186/s12943-023-01827-6

114. Kanders SH, Nilsson KW, Åslund C. Breastfeeding moderates the relationship between fat mass and obesity-associated gene rs9939609 and body mass index among adolescents. *Obes Sci Pract.* (2021) 8:66. doi: 10.1002/osp4.546

115. Górczyńska-Kosiorz S, Kosiorz M, Dziegielewska-Gesiak S. Exploring the interplay of genetics and nutrition in the rising epidemic of obesity and metabolic diseases. *Nutr.* (2024) 16:3562. doi: 10.3390/nu16203562

116. Wu YY, Lye S, Briollais L. The role of early life growth development, the FTO gene and exclusive breastfeeding on child BMI trajectories. *Int J Epidemiol.* (2017) 46:1512–22. doi: 10.1093/ije/dyx081

117. Verier C, Meirhaeghe A, Bokor S, Breidenassel C, Manios Y, Molnár D, et al. Breast-feeding modulates the influence of the peroxisome proliferator-activated receptor-γ (PPARG2) Pro12Ala polymorphism on adiposity in adolescents: the healthy lifestyle in europe by nutrition in adolescence (HELENA) cross-sectional study. *Diabetes Care.* (2010) 33:190–6. doi: 10.2337/dc09-1459

118. Sun C, Mao S, Chen S, Zhang W, Liu C. PPARs-orchestrated metabolic homeostasis in the adipose tissue. *Int J Mol Sci*. (2021) 22:8974. doi: 10.3390/ijms22168974

119. Homann CM, Rossel CAJ, Dizzell S, Bervoets L, Simioni J, Li J, et al. Infants' first solid foods: impact on gut microbiota development in two intercontinental cohorts. *Nutrients*. (2021) 13:2639. doi: 10.3390/nu13082639

120. Huh SY, Rifas-Shiman SL, Taveras EM, Oken E, Gillman MW. Timing of solid food introduction and risk of obesity in preschool-aged children. *Pediatrics*. (2011) 127:e544–51. doi: 10.1542/peds.2010-0740

121. Sun C, Foskey RJ, Allen KJ, Dharmage SC, Koplin JJ, Ponsonby AL, et al. The impact of timing of introduction of solids on infant body mass index. *J Pediatr*. (2016) 179:104–110.e1. doi: 10.1016/j.jpeds.2016.08.064

122. Thompson AL. Evaluating the pathways linking complementary feeding practices to obesity in early life. *Nutr Rev.* (2020) 78:13–24. doi: 10.1093/nutrit/nuz057

123. Tang M, Hendricks AE, Krebs NF. A meat- or dairy-based complementary diet leads to distinct growth patterns in formula-fed infants: a randomized controlled trial. *Am J Clin Nutr.* (2018) 107:734–42. doi: 10.1093/ajcn/nqy038

124. World Health Organization. *WHO guideline for complementary feeding of infants and young children 6-23 months of age* (2023). Available online at: https://www.who.int/publications/i/item/9789240081864 (Accessed February 10, 2025).

125. Redsell SA, Edmonds B, Swift JA, Siriwardena AN, Weng S, Nathan D, et al. Systematic review of randomised controlled trials of interventions that aim to reduce the risk, either directly or indirectly, of overweight and obesity in infancy and early childhood. *Matern Child Nutr.* (2016) 12:24–38. doi: 10.1111/mcn.12184

126. Koletzko B, von Kries R, Monasterolo RC, Subías JE, Scaglioni S, Giovannini M, et al. Infant feeding and later obesity risk. *Adv Exp Med Biol.* (2009) 646:15–29. doi: 10.1007/978-1-4020-9173-5_2

127. Haschke F, Binder C, Huber-Dangl M, Haiden N. Early-life nutrition, growth trajectories, and long-term outcome. *Nestle Nutr Inst Workshop Ser.* (2019) 90:107–20. doi: 10.1159/000490299

128. Davis EC, Wang M, Donovan SM. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. *Gut Microbes.* (2017) 8:143–71. doi: 10.1080/19490976.2016.1278104

129. Davis EC, Castagna VP, Sela DA, Hillard MA, Lindberg S, Mantis NJ, et al. Gut microbiome and breast-feeding: implications for early immune development. *J Allergy Clin Immunol.* (2022) 150:523–34. doi: 10.1016/j.jaci.2022.07.014

130. Verduci E, Bronsky J, Embleton N, Gerasimidis K, Indrio F, Köglmeier J, et al. Role of dietary factors, food habits, and lifestyle in childhood obesity development: a position paper from the European society for paediatric gastroenterology, hepatology and nutrition committee on nutrition. *J Pediatr Gastroenterol Nutr.* (2021) 72:769–83. doi: 10.1097/MPG.0000000000003075

131. Shi Y, Yin R, Pang J, Chen Y, Li Z, Su S, et al. Impact of complementary feeding on infant gut microbiome, metabolites and early development. *Food Funct*. (2024) 15:10663–78. doi: 10.1039/D4FO03948C

132. Haddad EN, Comstock SS. Archive for research in child health (ARCH) and baby gut: study protocol for a remote, prospective, longitudinal pregnancy and birth cohort to address microbiota development and child health. *Methods Protoc*. (2021) 4:52. doi: 10.3390/mps4030052

133. Rampelli S, Guenther K, Turroni S, Wolters M, Veidebaum T, Kourides Y, et al. Pre-obese children's dysbiotic gut microbiome and unhealthy diets may predict the development of obesity. *Commun Biol*. (2018) 1:222. doi: 10.1038/s42003-018-0221-5

134. Usuda H, Okamoto T, Wada K. Leaky gut: effect of dietary fiber and fats on microbiome and intestinal barrier. *Int J Mol Sci.* (2021) 22:7613. doi: 10.3390/ijms22147613

135. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie*. (2016) 124:11–20. doi: 10.1016/j.biochi.2015.06.020

136. Wrzosek L, Miquel S, Noordine ML, Bouet S, Joncquel Chevalier-Curt M, Robert V, et al. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol.* (2013) 11:61. doi: 10.1186/1741-7007-11-61

137. Peng Y, Tun HM, Ng SC, Wai HK, Zhang X, Parks J, et al. Maternal smoking during pregnancy increases the risk of gut microbiome-associated childhood overweight and obesity. *Gut Microbes.* (2024) 16:2323234. doi: 10.1080/19490976.2024.2323234

138. Isaevska E, Moccia C, Asta F, Cibella F, Gagliardi L, Ronfani L, et al. Exposure to ambient air pollution in the first 1000 days of life and alterations in the DNA methylome and telomere length in children: a systematic review. *Environ Res*. (2021) 193:110504. doi: 10.1016/j.envres.2020.110504

139. Loperfido F, Vincenti A, Basilico S, Conti MV, Orsini F, Franchi C, et al. Rethinking food policy framework to tackle childhood obesity in the Italian context. *Nutr Rev*. (2025) 83:e1–3. doi: 10.1093/nutrit/nuae059

140. Cena H, Labra M, and NBFC Collaborator Group. Biodiversity and planetary health: a call for integrated action. *Lancet.* (2024) 403:1985–6. doi: 10.1016/S0140-6736(24)00292-7

141. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Lambré C, Barat Baviera JM, Bolognesi C, Chesson A, Cocconcelli PS, Crebelli R, et al. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA J.* (2023) 21:e06857. doi: 10.2903/j.efsa.2023.6857

142. Wickramasinghe K, Chatterjee S, Williams J, Weber MW, Rito AI, Rippin H, et al. Childhood overweight and obesity abatement policies in Europe. *Obes Rev.* (2021) 22:e13300. doi: 10.1111/obr.13300

Appendix D: questionnaires of paper 1 empirical

MEDI-LITE

Qual è il consumo dei seguenti gruppi di alimenti?	**Opzione:**	**1**	**2**	**3**
FRUTTA *(es: una mela, pera, arancia; 3 prugne; mandarini)*	1 porzione = **150 g**	**0**	**1**	**2**
VERDURA *(es: 1 piatto di insalata, 2 pomodori, ½ vaschetta di verdura cotta)*	1 porzione = **100 g**	**0**	**1**	**2**
LEGUMI *(es: mezza scatoletta di fagioli, ceci, lenticchie o piselli)*	1 porzione = **70 g**	**0**	**1**	**2**
CEREALI *(pane, pasta, biscotti, ecc.) (es: pasta 80 g; 4 biscotti ≈ 50 g)*	1 porzione = **130 g**	**0**	**1**	**2**
PESCE *(eccetto molluschi e crostacei)*	1 porzione = **100 g**	**0**	**1**	**2**
CARNE E SALUMI *(1 porzione carne = 100 g; salumi = 50 g)*	1 porzione = **80 g**	2	1	0
LATTE E LATTICINI *(es: latte 150 g; yogurt 125 g)*	1 porzione = **180 g**	2	1	0
ALCOL *(1 U.A. = 1 bicchiere di vino o 1 lattina di birra)*		1	2	0
OLIO DI OLIVA	Condimento abituale	0	1	2

- Punteggio: 0-18
- cut-off: 9
- Score: ≥ 9 c'è una buona aderenza alla dieta mediterranea.
- Maggiore è lo score, maggiore è l'aderenza.

ATTIVITA' FISICA - TURCONI

SEZIONE C. ATTIVITA' FISICA E IMPIEGO DEL TEMPO LIBERO

C. 1 Svolge regolarmente qualche attività fisica?

□ sempre, tutto l'anno **3**

□ solo in alcune stagioni **2**

□ talvolta **1**

□ mai **0**

C. 2 Quanto tempo dedica all'attività fisica ?

□ 1h-2h alla settimana **1**

□ 3h-4h alla settimana **2**

□ più di 4h alla settimana **3**

□ non svolgo nessuna attività fisica **0**

C. 3 Quando ha del tempo libero, cosa preferisce fare ?

□ ascoltare musica/ leggere/usare il computer/ guardare la TV/andare a cinema, teatro **0**

□ praticare sport **3**

□ visitare mostre, musei **1**

□ passeggiare, fare shopping **2**

C. 4 Quanto tempo trascorre davanti al computer e/o televisore e/o in automobile ?

□ 1h-2h al giorno **3**

□ 3h-4h al giorno **2**

□ 5h-6h al giorno **1**

□ più di 6h al giorno **0**

C. 5 Il suo stile di vita, secondo lei, è:

□ molto sedentario **0**

□ sedentario **1**

□ moderatamente attivo **2**

□ molto attivo **3**

Appendix E: Supplementary Material

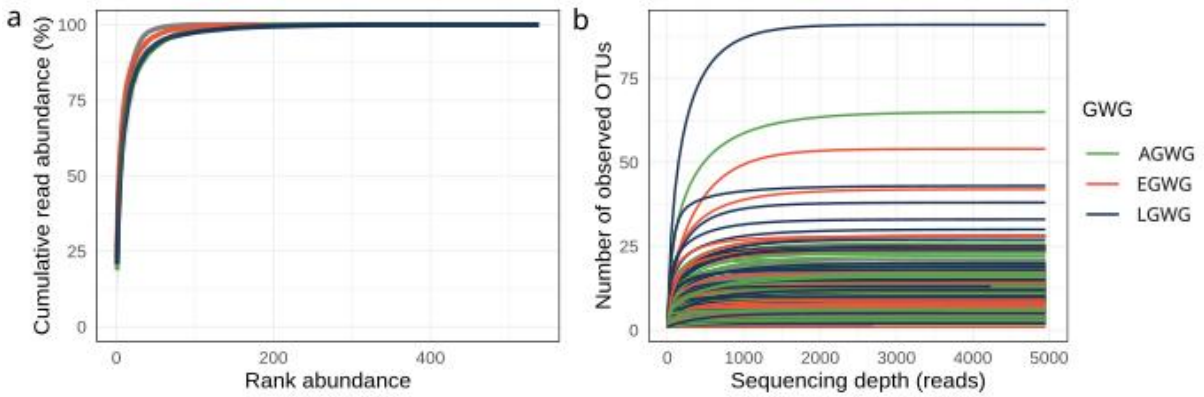
Paper 2 empirical:

Dataset quality assessment

DNA extraction and sequencing library preparation was successful for 168 / 183 sample analyses (91.8 %) and yielded between 2'688 and 126'205 DNA reads after QC and bioinformatic processing. Failed samples were those yielding significantly less mapped DNA reads (mappedReads) than 10'000; here mappedReads < 2'000. Low-read samples were disregarded in all subsequent analyses.

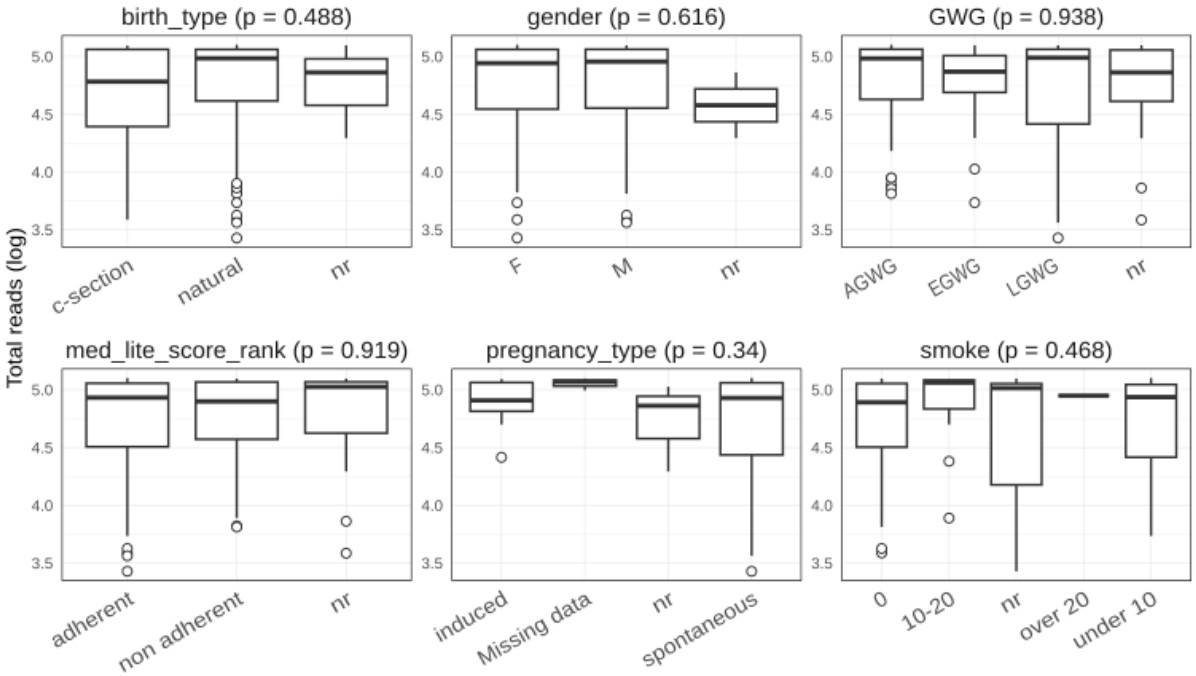
The bioinformatic workflow produced an OTU reference sequences file in FASTA format, loaded using the "Biostrings" package v2.70.3.[1] Several sequences were flagged for containing non-standard nucleotide characters outside the IUPAC DNA alphabet. These sequences were inspected, then in order to preserve biological interpretability while ensuring compatibility with downstream functions, non-IUPAC characters were removed, resulting in a cleaned FASTA format sequence object, combined into ampvis2 object using "ampvis2" package v2.8.9,[2] together with the OTU count data table and metadata from the study cohort.

After quality filtering the dataset was composed of 168 samples of 12'706'791 reads (minimum: 2'688, maximum: 126'205, median: 87'214) collapsed in a total of 538 OTUs. To explore community structure and sequencing depth adequacy, both rank-abundance and rarefaction curves were generated using the R package "ampvis2", highlighting that sequencing was effective in adequately sampling the microbial diversity (Figure S1). Moreover, the total number of reads did not differ across the main study variables, excluding sequencing-related biases and validating the subsequent statistical analyses (Figure S2).



Supplementary figure 1. (a) Rank-abundance curves showing the abundance percentage of OTUs ranked by decreasing abundance across samples grouped by GWG categories. The x-axis represents the rank of each OTU (following abundance rank), while the y-axis shows the corresponding abundance percentage on a linear scale. Shaded areas indicate standard deviations across replicates within each group. (b) Rarefaction curves depicting the relationship

between sequencing depth (total number of reads) on x-axis and observed OTU richness (total number of OTUs) on y-axis, for each sample, coloured according to GWG categories. The step size for calculating rarefaction points was set to 50 reads and the x-axis was constrained to a maximum of 5'000 reads.



Supplementary figure 2. Boxplot reports the sequencing depth expressed as logarithm of the total number of reads per sample among the main categorical variables considered in the study. Differences are assessed using anova test after fitting the model formula using linear model. P-values from the analysis are reported on top of each panel. All the categories within each variable in which the metadata are not annotated during the enrollment are indicated as not reported (nr).

To properly perform the downstream statistical analysis according to the study project purposes, the dataset underwent a cleaning process illustrated as follows. We kept only the subjects that presented a category within the GWG variable, as the main variable considered in the study, thus eliminating 13 samples with no GWG reference, keeping a total of 155 samples with 531 associated OTUs. The birth type was initially included in the permutation model showing a significant effect (Adonis PERMANOVA: $R^2 = 0.02$, $p < 0.001$), this was likely attributable to both the imbalance in the dataset as the c-section samples were 23 against 132 samples with normal birth and for a real biological effect of delivery mode on meconium microbiota as already documented.[3] To reduce the bias resulting from the dataset imbalance, a

permutation analysis was performed by increasing the number of permutations to 9'999 and stratified within the birth type, that is, the birth type was set as a group within which to constrain permutations. This analysis highlights a significant effect albeit reduced (Adonis PERMANOVA: $R^2 = 0.02$, $p = 0.04$). In line with this evidence and the aim of the study, all subsequent analyses were conducted exclusively on samples from individuals born via natural delivery and with an assigned classification within the GWG category. Therefore, samples related to c-section delivery mode and lacking GWG information were excluded in order to minimize potential confounding effects that could lead to misleading interpretations. After this further quality filtering the dataset was composed of 132 samples, of 10'246'006 reads (minimum: 2'688, maximum: 126'205, median: 96'442.5) collapsed in a total of 436 OTUs. A further sample with missing values in key metadata variables (i.e. GWG, gender, pre pregnancy BMI, pre pregnancy weight, weight gain, medi lite score, medi lite score rank, PA, DH, FFQ and IPAQ) was also excluded to ensure complete case analysis and improve the robustness and interpretability of downstream multivariate statistical analyses, obtaining a final clean dataset of 131 samples suitable for the all subsequent analyses. The adonis PERMANOVA analyses described were carried out by using the *adonis2* function of "vegan" package v2.6.10,[4] based on Bray-Curtis distance matrix made from normalised counts (relative abundance).

Bacterial diversity analysis according to the mother's diet and lifestyle variables

Performing the *Adonis2* function by using a model formula which include several factors and potentially collinear variables could affect the outcome of the analysis, so an analysis to test the potential collinearity was first performed by using Variance Inflation Factor (VIF) analysis, in order to remove predictors with collinearity and select an appropriate number of factors to include in the model formula of permutational multivariate analysis. This analysis was performed by using the *vif* function of the "car" package.[5] We considered as potentially collinear variables those with a Generalized VIF (GVIF) value > 5, among these we identified the pre pregnancy BMI and pre pregnancy weight variables which reported a GVIF value of 6.01 and 5.43 respectively, and their correlation was also tested (Pearson; corr: 0.894, $p < 0.001$). Therefore, we exclude the pre pregnancy weight from the model formula. Therefore, the adonis PERMANOVA analysis was carried out producing the output reported in table S1, highlighting the effect of the main variables selected on the bacterial diversity. The adonis PERMANOVA analysis was performed on Bray-Curtis distance matrix produced from normalised count (relative abundance) and setting 1'000 number of permutations.

Supplementary table 1. Adonis PERMANOVA table. The table reports the results of the analysis of adonis PERMANOVA. Factors considered in the model formula are reported with the related adonis output (Sum of Squares, R^2 and P-value). Continuous variables (Pre pregnancy BMI, PA, DH, FFQ, IPAQ) are added in the model as covariates.

Factor	SumOfSqs	R^2	P-value
GWG	0.541	0.011	0.8991
Gender	0.235	0.005	0.8591
Pre pregnancy BMI	0.385	0.008	0.4426
Weight gain	0.284	0.006	0.7473
Medi lite score	0.346	0.007	0.5544
Medi lite score rank	0.234	0.005	0.8641
Mother celiac condition	0.958	0.019	0.1788
PA	0.391	0.008	0.4296
DH	0.297	0.006	0.7143
FFQ	0.228	0.005	0.8891
IPAQ	0.355	0.007	0.5155
Residual	45.372	0.914	
Total	49.626	1	

To deeply inspect the relationship between bacterial diversity and continuous variables of nutritional and clinical data, the environmental fitting analysis was performed by using the *envfit* function of the "vegan" package v2.6.10. The analysis was performed on relative abundances -transformed counts and converted into a distance matrix using the Bray-Curtis index, then the principal coordinate axes were calculated using the *cmdscale* function. The continuous variables related to diet and lifestyle were normalized by using z-score transformation to remove scale bias which can affect the distance-based analysis. The analysis was carried out setting 1’000 numbers of permutations. Environmental fitting analysis does not reveal significant correlations between diversity and the tested variables, confirming the absence of significant effects of these variables on the bacterial diversity (Table S2).

Supplementary table 2. The table reports the results from environmental fitting analysis considering the main variables related to diet and lifestyle conditions. Each vector is reported together with the related R^2 and p-values from permutation testing.

Factor	R^2	Pr(>r)
Pre pregnancy weight	0.017	0.3556
End pregnancy weight	0.010	0.5285
Weight gain	0.008	0.6114
Pre pregnancy BMI	0.025	0.2208
End pregnancy BMI	0.018	0.3457
FFQ	0.001	0.9530
DH	0.002	0.9091
PA	0.024	0.2228
Medi lite score	0.011	0.4735

To assess differences in bacterial diversity (β-diversity) between GWG groups, a pairwise adonis PERMANOVA was carried out. For each pairwise comparison, the subset of the distance matrix corresponding to the two groups (see Group 1 and 2 in table S3) was extracted, and a PERMANOVA test was performed using the *adonis2* function from the "vegan" package v2.6.10, setting 1'000 number of permutations. The analysis was performed on Bray-curtis distance matrix produced from normalised (relative abundance) counts table. To facilitate the interpretation, the resulting R^2 values, indicating the proportion of variance explained by group membership, were displayed as heatmap in the figure 1b.

Supplementary table 3. Table reports the results from pairwise adonis PERMANOVA testing differences between different gestational weight gain (GWG) categories. For each comparison, the proportion of variance explained (R^2) and the associated p-value are reported.

Group 1	Group 2	R^2	P-value
LGWG	AGWG	0.006	0.849
LGWG	EGWG	0.008	0.903

AGWG	EGWG	0.013	0.466

The α-diversity metrics, i.e. Shannon, Simpson, and inverse Simpson indices were generated for each sample using the *amp_alphadiv* function of the "ampvis2" package. Differences in α-diversity among GWG categories were assessed using Kruskal-Wallis test for each index. Differences in α-diversity among GWG categories were assessed using Kruskal-Wallis test for each index. No statistically significant differences in α-diversity were observed between GWG groups (Shannon: $p = 0.349$, Simpson: $p = 0.594$, and inverse Simpson: $p = 0.594$).

Unsupervised learning approach highlight hidden structure of meconium microbiota

To identify latent structure in the multivariate space, unsupervised learning was carried out by using Partitioning Around Medoids (PAM) algorithm implemented in the *pam* function of the "cluster" package v2.1.4,[6] using the Bray-Curtis dissimilarity matrix on relative abundance transformed counts as input. The optimal number of clusters (k) was empirically determined by evaluating the average silhouette width (ASW) for k ranging from 2 to 10. ASW values were computed iteratively and the value of k maximising mean silhouette width was selected as the optimal clustering solution. Clustering performance using the PAM algorithm was evaluated based on the minimisation of the objective function, which reflects the total dissimilarity between observations and their assigned medoids. The initial configuration, generated by the "build" phase, yielded a dissimilarity value of 0.532, and subsequent optimisation through the "swap" phase reduced this value to 0.516. The internal cohesion and external separation for each cluster were reported in table S4. Cluster sizes ranged from 13 (Cluster 4) to 48 (Cluster 1). The average within-cluster dissimilarities varied between 0.381 (Cluster 5) and 0.624 (Cluster 2), with lower values suggesting higher internal similarity. Notably, Cluster 5 exhibited the lowest average dissimilarity, indicating tight grouping of its members, whereas Cluster 2 reported the highest average dissimilarity, suggesting greater internal variability.

Supplementary table 4. The table reports, for each cluster, the number of observations (Cluster size), maximum within-cluster dissimilarity (Max diss), average within-cluster dissimilarity (Av diss), and minimum separation from other clusters (Separation).

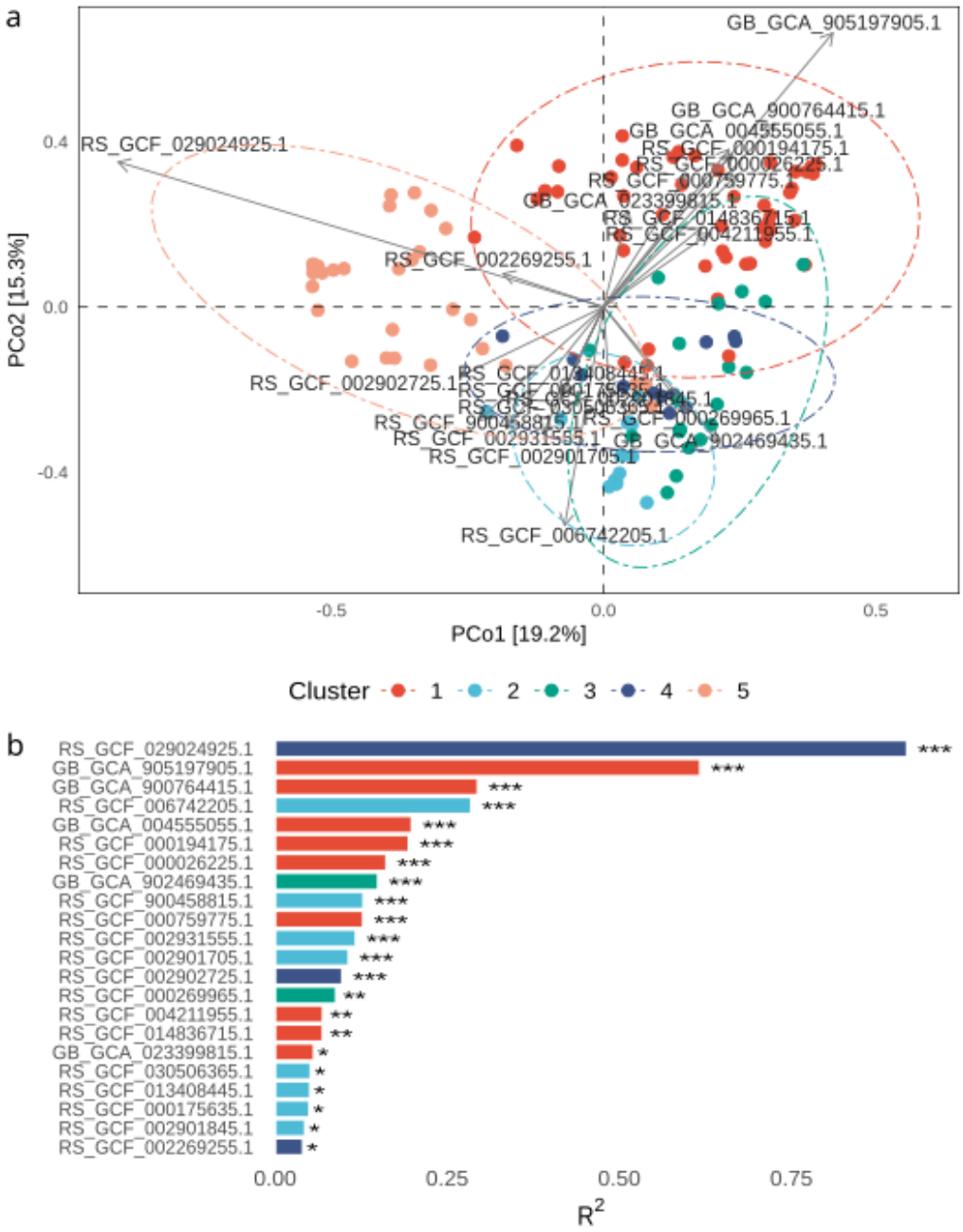
Cluster size	**Max diss**	**Av diss**	**Separation**

48	0.9975	0.541	0.2193
17	0.9858	0.6244	0.2404
19	0.8298	0.5756	0.2193
13	1	0.5382	0.3825
34	0.9895	0.381	0.2404

Cluster membership was then overlaid on the first two axes of PCoA space for visualisation and interpretation. Additionally, PCoA results were extended to four dimensions in order to capture a higher proportion of total β-diversity variance and to evaluate inter-cluster separation along supplement ordination axes (i.e. PCo1 vs PCo4 in figure 2b). The effect of Cluster in explaining bacterial diversity was statistically assessed by using permutational multivariate analysis adonis PERMANOVA using the *adonis2* function of "vegan" package v2.6.10, setting 1'000 as the number of permutations. The adonis PERMANOVA highlights that the identified clusters explained a significant proportion of the variance in microbial community composition that reflects biologically relevant meaning in the dataset ($R^2 = 0.349$, $p = 0.001$).

To define the influence of the main OTUs on the separation of the clusters, we applied a constrained environmental fitting approach on the ordination space. The OTU relative abundance matrix was filtered to remove OTUs with zero variance or those absent across all samples and OTUs present in less than 5% of the samples were removed to reduce noise from rare taxa. A PCoA was then performed based on a Bray-curtis distance matrix using the filtered community composition data described above. Significant OTUs were identified by fitting OTU vectors onto the PCoA ordination using the *envfit* function from the "vegan" package v2.6.10, setting 1'000 permutations to assess statistical significance. Only OTUs with a permutation-based p-value < 0.05 were retained and projected as vectors in the multidimensional space (Figure S4a).

The orientation of each OTU reflects its maximal correlation with the ordination axes. To associate OTUs with specific bacterial clusters, we calculated the cosine similarity between each significant OTU vector and the centroids of predefined sample clusters, defined as the mean coordinates on the first two ordination axes. Each OTU was assigned to the cluster whose centroid exhibited the highest directional similarity, providing an interpretable link between environmental fitting analysis and cluster-associated OTUs (Figure S3b and table S5). The cosine similarity formula was: $\cos(\theta) = A \cdot B / \|A\| \|B\|$; where $A \cdot B$ is the dot product of the two vectors, and $\|A\|$ and $\|B\|$ are their euclidean norms. This metric quantifies the cosine of the angle (θ) between the vectors, with values closer to 1 indicating higher similarity in direction.



Supplementary figure 3. OTUs-Clusters association. (a) PCoA (Bray-Curtis distance) reports the sample distribution according to the clusters produced by the PAM algorithm. The OTUs significantly selected by environmental fitting analysis ($p < 0.05$) are fitted as directional vectors into the multidimensional space. Clusters are represented using color code in the legend and ellipses represent 95% confidence intervals of the clusters. (b) Barplot reports the coefficients of determination (R^2) from the OTUs selected by the environmental fitting analysis ($p < 0.05$). Each bar is colored based on the best alignment between significant OTU and cluster using a cosine analysis. The asterisks highlight significance levels of each OTU (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

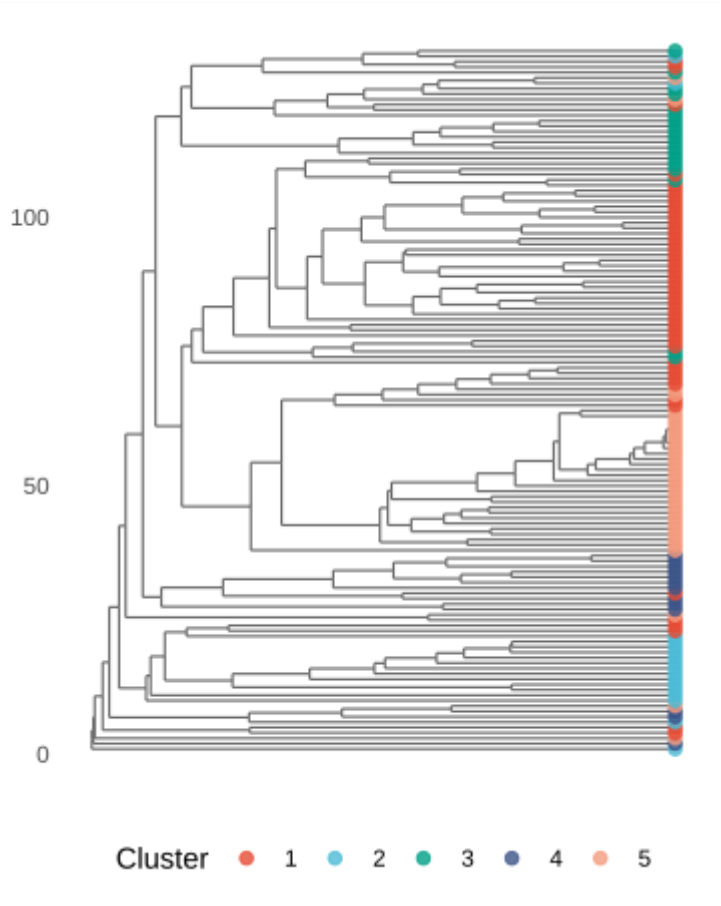
Supplementary table 5. Table reports the results of the environmental fitting analysis depicted in figure S3. Table reports the significant OTUs together with the species-level taxonomic assignment, the related cluster calculated using cosine similarity and the output values from *envfit* analysis, i.e. coefficients of determination (R^2) values and p-values. Significant effect are also highlighted using asterisks (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

OTU	Species-level assignment	Cluster	R^2	p-value	Sign
RS_GCF_029024925.1	*Enterococcus faecalis*	5	0.9182	0.000999	***
GB_GCA_905197905.1	*Sutterella sp905197905*	1	0.6175	0.000999	***
RS_GCF_006742205.1	*Staphylococcus epidermidis*	2	0.2849	0.000999	***
GB_GCA_902469435.1	*Collinsella sp902469435*	4	0.1497	0.000999	***
GB_GCA_004555055.1	*Alloprevotella sp004555055*	1	0.1987	0.000999	***
GB_GCA_900764415.1	*Collinsella sp900764415*	1	0.2945	0.000999	***
RS_GCF_000194175.1	*Escherichia coli F*	1	0.1944	0.000999	***
RS_GCF_000026225.1	*Escherichia fergusonii*	1	0.1618	0.000999	***
RS_GCF_002901705.1	*Staphylococcus lugdunensis*	2	0.1069	0.000999	***
RS_GCF_002902725.1	*Staphylococcus caprae*	5	0.0979	0.000999	***

RS_GCF_900458815.1	*Staphylococcus saccharolyticus*	2	0.1285	0.000999	***
RS_GCF_002931555.1	*Pseudoclavibacter sp002931555*	2	0.1173	0.000999	***
RS_GCF_000759775.1	*Escherichia albertii*	1	0.1279	0.000999	***
RS_GCF_000269965.1	*Bifidobacterium infantis*	4	0.0887	0.003996	**
RS_GCF_014836715.1	*Escherichia whittamii*	1	0.0693	0.003996	**
RS_GCF_004211955.1	*Escherichia sp004211955*	1	0.0695	0.004995	**
RS_GCF_030506365.1	*Corynebacterium tuberculostearicum E*	2	0.0519	0.015984	*
RS_GCF_013408445.1	*Corynebacterium tuberculostearicum*	2	0.0511	0.016983	*
RS_GCF_000175635.1	*Corynebacterium tuberculostearicum C*	2	0.0498	0.018981	*
GB_GCA_023399815.1	*Stenotrophomonas sp023399815*	1	0.0564	0.021978	*
RS_GCF_002901845.1	*Staphylococcus hominis*	2	0.0437	0.022977	*

To assess similarity between microbial community clusters, hierarchical clustering was performed on a Bray-Curtis dissimilarity matrix. The Bray-Curtis distances were computed from normalised abundance (relative abundance) data using the *vegdist* function from the "vegan" package. Hierarchical clustering was then conducted using the *hclust* function with the average linkage method (UPGMA). To evaluate the fidelity of the dendrogram in representing the original dissimilarities, cophenetic distances were calculated. The correlation between the original Bray-Curtis distance matrix and the cophenetic distance matrix was computed using Pearson's correlation. A correlation coefficient (r) greater than 0.75 (r = 0.83) was interpreted

as indicating a good fit between the dendrogram structure and the original dissimilarities. The dendrogram structure was produced by using the "ggdendro" package v0.2.[7]

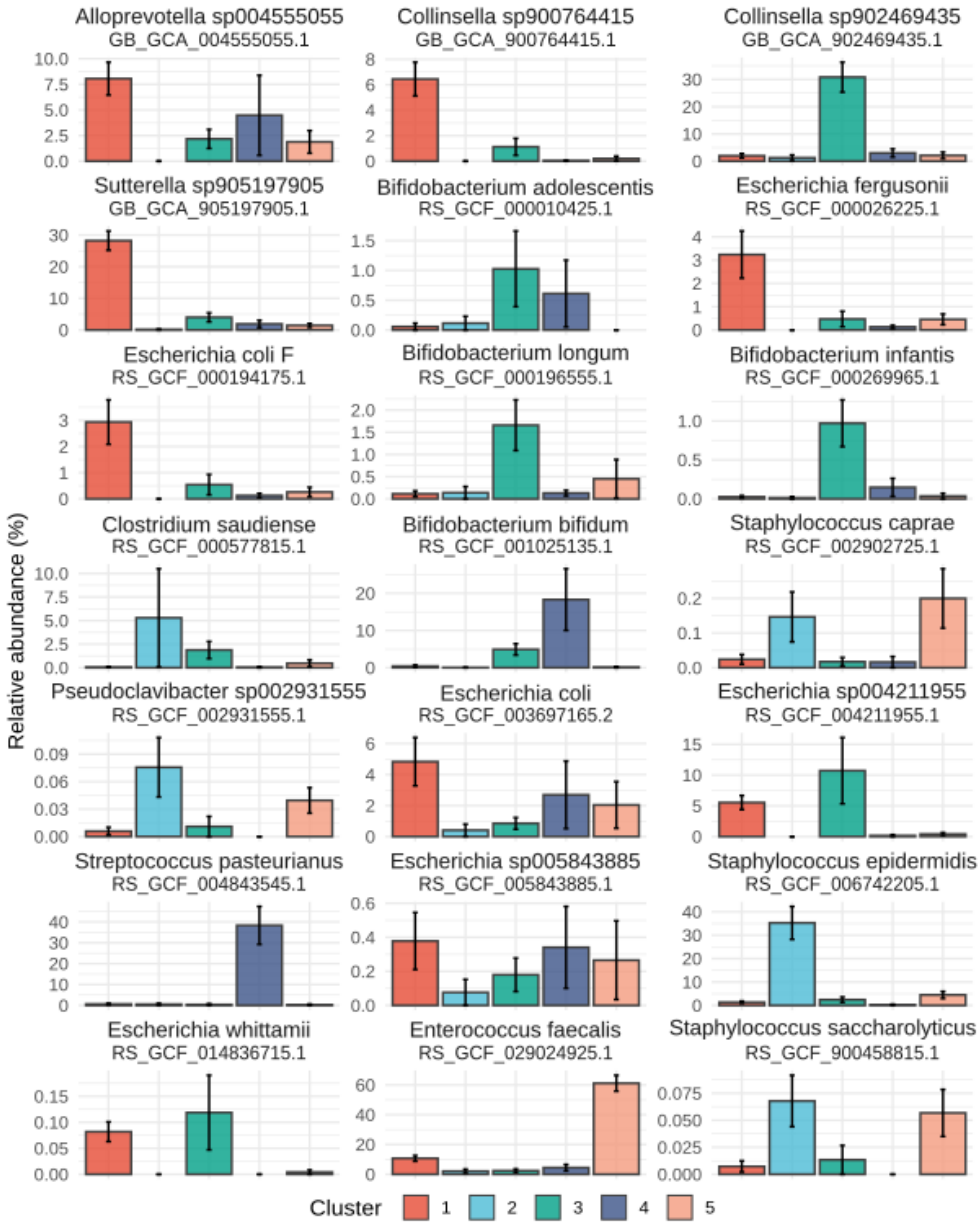


Supplementary figure 4. A dendrogram represents hierarchical clustering analysis performed using the average linkage method and based on a Bray-Curtis dissimilarity matrix from normalised abundance. Tree branches were drawn as line segments, and sample nodes were displayed as coloured points, according to the assigned cluster.

Taxonomic signature associated to the clusters

To identify differentially abundant operational taxonomic units (OTUs) among clusters, the OTUs present in at least 10% of the samples were filtered to avoid bias related to rare OTUs, then transformed in relative abundance percentages. Differences among clusters were inspected by using Kruskal-Wallis rank sum test adjusting the p-value using the false discovery rate (FDR) method. OTUs with FDR-adjusted p-values < 0.05 were considered significantly different across clusters and retained for post hoc pairwise testing. For each significant OTU, pairwise Wilcoxon rank-sum test (FDR correction) were performed between clusters (Table

S7). Significantly different OTUs were further explored through heatmap analysis of percentage abundance. Heatmaps generated with the Significant OTUs were displayed using hierarchical clustering analysis produced using package "ComplexHeatmap" v2.18,[8] applying Euclidean distance and hierarchical clustering with the Ward D2 method (Figure 3d).



Supplementary figure 5. Different OTUs abundances among clusters. Bar plots represent the mean OTU relative abundance percentages and standard error across clusters (color scheme). The OTUs are selected by using Kruskal-Wallis test ($p < 0.05$) then with the Wilcoxon rank test (FDR correction method). For each OTU the corresponding species-level taxonomic assignment is reported.

Supplementary table 6. Table reports the differences in OTUs relative abundances among clusters assessed by using Wilcoxon rank sum test. The p-values are adjusted by using the FDR correction method. The table reports the OTUs annotations and related species-level taxonomic assignment. Significant comparisons are highlighted using asterisks (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

All figures were produced using the graphics package "ggplot2" v3.5.2[9] and where necessary the graphic rendering was improved using the open source software Inkscape (https://inkscape.org/).

References of supplementary materials for Paper 2 Empirical

1. Pagès H, Aboyoun P, Gentleman R, DebRoy S (2024). *Biostrings: Efficient manipulation of biological strings*. R package version 2.70.3, https://bioconductor.org/packages/Biostrings.

2. ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. Andersen K.S., Kirkegaard R.H., Karst S.M., Albertsen M. (2018) bioRxiv. doi: https://doi.org/10.1101/299537.

3. Turunen, J., Tejesvi, M.V., Paalanne, N. *et al.* Investigating prenatal and perinatal factors on meconium microbiota: a systematic review and cohort study. *Pediatr Res* **95**, 135–145 (2024). https://doi.org/10.1038/s41390-023-02783-z.

4. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J, Borman T (2025). vegan: Community Ecology Package. R package version 2.6-10, https://CRAN.R-project.org/package=vegan.

5. Fox J, Weisberg S (2019). An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. https://www.john-fox.ca/Companion/.

6. Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., Hornik, K.(2022). cluster: Cluster Analysis Basics and Extensions. R package version 2.1.4.

7. De Vries A, Ripley BD (2024). ggdendro: Create Dendrograms and Tree Diagrams Using 'ggplot2'. R package version 0.2.0, https://CRAN.R-project.org/package=ggdendro.

8. Gu, Z., Eils, R., & Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics, 32(18), 2847-2849.

9. H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.