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THE IMPACT OF DIETARY INTAKES ON GUT MICROBIOTA OF WOMEN AFFECTED BY GESTATIONAL DIABETES MELLITUS AND OF THEIR OFFSPRING

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INTRODUCTION

1. GESTATIONA DIABETES MELLITUS (GDM)

1.1 Definition, diagnosis and epidemiology

Gestational Diabetes Mellitus (GDM) is defined as any glucose intolerance with the onset or first recognition during pregnancy, usually resolving after delivery [1]. This condition is associated with adverse outcomes both for the mother and the offspring [2].

For over 50 years, there has been a lack of consensus over the appropriate diagnostic criteria for GDM. The first diagnostic criteria were provided by O´Sullivan and Mahan in 1964 and were based on a 3-hour 100 g oral glucose tolerance test (OGTT) [3]. Between 1990 and 2005, there was a proliferation of guidelines with different diagnostic criteria, many based on a one-step method using a 75-g OGTT [4-5].

The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study, a large multinational and multicenter study on 23000 pregnant women, was designed with the goal to achieve consensus in the diagnosis of GDM by investigating the impact of maternal glycemia on the risk of adverse pregnancy and neonatal outcomes [6]. The HAPO study demonstrated a linear increase in the risk of primary outcomes, such as Large for Gestational Age (LGA), cord C-peptide, neonatal hypoglycemia (NH), and Caesarian Section (CS), with increasing degrees of hyperglycemia, as assessed by a 2-hours 75-g OGTT.

In 2010, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) released new diagnostic criteria for GDM, based on the HAPO study outcomes [7]. The IADPSG criteria were later adopted by the American Diabetes Association (ADA), the International Federation of Gynecology and Obstetrics, and the Endocrine Society [8].

At the first antenatal visit, IADPSG recommends screening pregnant women by using standard criteria to diagnose diabetes mellitus to identify women with "pre-existing diabetes". A diagnosis of overt diabetes can be established in women who meet any of the following criteria: fasting plasma glucose level (FPG) ≥7.0 mmol/L (126 mg/dL), a casual plasma glucose of 11.1 mmol/L (≥200 mg/dL), or glycated hemoglobin (HbA1c) levels ≥6.5. The confirmation of the diagnosis precludes the need for an OGTT.

If the early screening is negative, the IADPSG recommends universal screening to be performed at 24-28 gestational weeks with a 2-hours 75-g OGTT (the "one-step approach"). Gestational diabetes is diagnosed, if one or more values equal, or exceeds the following thresholds: FPG≥5.1 mmol/L (92 mg/dL), 1-h plasma glucose≥10 mmol/l (180 mg/dL) and 2-h plasma glucose ≥8.5 mmol/L (153 mg/dL).

The OGTT should be performed after an overnight fasting of 8-14 hours, with the usual carbohydrate intake [7].

In Italy, in 2011 a panel of experts drew up national guidelines including most of IADPSG recommendations; the screening is recommended to women at risk of GDM only [21]. High-risk women (individuals with obesity, prior GDM or a first-trimester FPG between 5.5 and 6.9 mmol/L (100–125 mg/dL)) should perform a selective screening at 16-18 weeks of gestational age. Medium-risk women, i.e. those with previous fetal macrosomia, a first-degree relative with diabetes, age ≥ 35 years, high-risk race/ethnicity or overweight, should perform the OGTT at 24-28 weeks. High-risk women with a negative screening should be tested again at 24–28 weeks.

GDM is the most frequent pregnancy complications, affecting around 14% of pregnant women worldwide according to the 2017 International Diabetes Federation (IDF) estimates [9]. The prevalence of GDM is in a direct proportion to the prevalence of type 2 diabetes mellitus (T2DM) and is higher in African, Hispanic, Indian, and Asian

women than in the Caucasian population [10]. Recently, its prevalence has increased by 2-3 times, owing to the adoption of the new IADPSG diagnostic criteria [11-12]. This dramatic rise in the GDM prevalence will have a major impact on the health care systems, and the consequences of labeling a large number of women with a GDM diagnosis are not known at present [13].

In Italy, GDM prevalence ranges between 3% and 10.8% [14-15]. In a multicenter study on 2750 pregnant women, the prevalence of GDM and impaired glucose tolerance (IGT) were 6.3% and 6.1%, respectively [16].

1.2 Risk factors for GDM

Several conditions increase the risk to develop GDM. Epidemiological studies are limited by methodological bias and the use of different diagnostic criteria and measurements, making difficult the comparisons among studies.

The most common risk factors for GDM include: overweight/obesity [17], excessive gestational weight gain [9], westernized diet [18], ethnicity [19], genetic polymorphisms [20], advanced maternal age [21], intrauterine environment (low or high birthweight) [22], familial and/or personal history of GDM [23], and diseases related to insulin resistance, such as polycystic ovarian syndrome (PCOS) [24]. Each of these risk factors are either directly or indirectly associated with impaired β-cell function and/or insulin sensitivity.

Dietary habits are associated with GDM, even independently of body mass index (BMI) and overall caloric intake. Diets that are high in saturated fats, refined sugars and red and processed meats have been associated with an increased risk of GDM [25-26], while an increased consumption of fiber, micronutrients and polyunsaturated fats has been associated with a reduced risk [27,28,29]. The inverse association between dietary

fiber and GDM may be the result of the reduction of appetite and glucose absorption [20]. Saturated fats directly interfere with insulin signaling [30] and induce inflammation and endothelial dysfunction that are both pathogenic factors for GDM [31]. On the other hand, omega-3 polyunsaturated fatty acids (PUFA) have anti-inflammatory properties. A relationship between the intake of processed meat and GDM was found and seems to be independent from the content of fatty acids, cholesterol, heme iron and protein [32]. It has been suggested that nitrates (a common preservative in processed meats) and/or advanced glycation end products (AGEs), deriving from meat processing, could both play a role being implicated in β-cell toxicity [33]. Moreover, high-protein diets were found to be associated with GDM, independently of meat consumption [34-35]. This could be due to the role of amino acids as substrates for hepatic glucose production and hepatic lipotoxicity [36]. However, recent data failed to find a relationship between dietary intake of total and major protein sources and the risk of GDM [37].

1.3 Consequences of GDM

GDM has wide-ranging consequences for both the mother and the fetus.

Maternal consequences

GDM increases the risk for short-term and long-term maternal health issues. There is an increased risk of pregnancy complications, including preterm birth, preeclampsia and C-section delivery [2].

It is estimated that 30–70% of women with a history of GDM will develop a Type 2 Diabetes Mellitus (T2DM) within 15 years [38,39,40]. Even among women with normal weight, a history of GDM was related to more than a 6-fold increased risk of developing

T2DM later in life [41]. Each additional pregnancy confers a three-fold increase risk of T2DM in women with a history of GDM [42].

Moreover, an increased risk for cardiovascular disease (CVD) among women with a history of GDM has been reported, which is only partly explained by the increased BMI of those women [43].

Child consequences

GDM causes short and long-term consequences for the infant. The increased placental transport of glucose, amino acids and fatty acids stimulate the fetus's endogenous production of insulin and insulin-like growth factor 1 (IGF-1). These conditions can cause fetal overgrowth and macrosomia at birth [44]. Excess fetal insulin production can stress the developing pancreatic β-cells, contributing to β-cell dysfunction and insulin resistance [45]. Finally, fetal macrosomia is a risk factor for shoulder dystocia [46].

The newborn form GDM mothers are at increased risk of hypoglycemia, which is likely due to the fetal hyperinsulinemia and can contribute to brain injury if not properly managed [46].

In the long term, the offspring of GDM women are at increased risk of obesity, T2DM, CVD. They show an almost double risk of developing childhood obesity when compared with offspring from nondiabetic mothers, even after adjusting for confounders such as maternal BMI [47]. The risk of developing an impaired glucose tolerance greatly increases, being this impairment detectable at very young ages [48]. Furthermore, the daughters of GDM women are themselves more likely to experience GDM in their own pregnancies, thus contributing to a vicious inter-generational cycle of hyperglycemic complications [49].

1.4 Management of GDM

The cornerstone of GDM management is glycemic control. The first approach is the intervention on lifestyle habits, that include medical nutrition therapy and daily exercise. If the glycemic goals are not achieved by means of this approach, a medical therapy should be initiated. This latter will not be treated as my research focuses on diet.

Blood glucose monitoring

Women are instructed to carry out self-monitoring of blood glucose 4 times a day: fasting glucose and 1- or 2-hours post-meals. Monitoring after meals blood glucose should be preferred as the risk of macrosomia increases with increased post-meals maternal glucose levels, as demonstrated by randomized controlled trials [50-51].

HbA1C values are lower in pregnant women due to the insulin-independent glucose uptake by the fetus and placenta and the rise in red cell mass and turnover during pregnancy. For this reason, monitoring HbA1C values during pregnancy is not useful [52].

Glycemic targets

The glycemic goals for GDM women are: fasting glucose ≤5-5.3 mmol/L (90-95 mg/dL), 1-hour post-meal glucose values ≤ 7.8 mmol/L (140 mg/dL), and 2-hours post-meal values ≤ 6.7 mmol/L (120 mg/dL) [52].

Lifestyle interventions

Medical nutritional therapy (MNT) is the keystone of GDM treatment and allows to reach glycemic goals in 80-90% of GDM women [53]. The optimal dietary prescription

would be a diet providing adequate nutrition to support fetal and maternal well-being and appropriate weight gain, while maintaining normoglycemia without ketosis [54].

Exercise has been shown to improve glycemic control in GDM. Daily moderate exercise (at least 30 minutes) is recommended in the absence of medical or obstetric contraindications [52].

1.5 Medical Nutritional Therapy for GDM

Lifestyle interventions, that includes diet and exercise recommendations, are the cornerstone of prevention and correction of the hyperglycemia in pregnancy [52]. MNT includes an individualized nutrition plan developed by a registered dietitian with expertise in the management of GDM. The food plan should provide adequate calorie intake to promote fetal/neonatal and maternal health, achieve glycemic goals, and promote appropriate gestational weight gain [52]. Excessive weight gain should be discouraged as it further increases the risk of adverse pregnancy outcomes, fetal macrosomia and childhood obesity [55]. The recommended weight gain during singleton pregnancy depends on pre-pregnancy BMI: 12.5-18 kg of weight gain for women with underweight (BMI <18.5 kg/m^2); 11.5-16 kg for normal weight (BMI 18.5-24.9 kg/m^2); 7-11.5 kg for overweight (BMI 25-29.9 kg/m^2), and 5-9 kg for obesity (BMI ≥30.0 kg/m^2) [56].

However, the efficacy of MNT is often scarce and there is no consensus about the optimal composition of the diet [57]. Most of the available studies were limited by small sample sizes, differences in maternal ethnicities or short duration of the interventions [58].

Guidelines dietary recommendation

Dietary recommendations greatly varied among the different guidelines and are often based on a low-grade of evidence [59].

The principal dietary recommendations are summarized in **Table 1**.

Table 1. Nutritional recommendation in the available guidelines for GDM treatment

Clinical practice guideline	Energy restriction	Macronutrient contribution to the energy intake	Carbohydrate (CHO) Intake	Protein intake
American College of Obstetricians and Gynecologists [60]		CHO: 33%-40% Proteins: 20% Fats: 40%	Complex CHO, ↓ glycemic index, ↑ fiber, CHO allocated in 3 meals and 2-3 snacks	
American Diabetes Association [52]			CHO: >175 g/d Fiber: 28 g/d	>71 g/d
Academy of Nutrition and Dietetics [61]	Obesity: –30% of Estimated Energy Requirements	CHO: 36.7%-60% (↓/medium GI) CHO: >65% in the Dietary Approaches to Stop Hypertension diet	175 g/d, fiber 28 g/d, ↓GI (<55), CHO distributed in 3 meals and ≥2 snacks	>71 g/d (or 1.1 g/kg/d)
Endocrine Society [62]	Overweight/Obesity Energy: 1600-1800 kcal/d Underweight/normal weight: No restriction	CHO: 35%-45%	Distributed in 3 small-to-medium sized meals and 2-4 snacks, with 1 evening snack	
International Federation of Gynecology and Obstetrics [63]	Energy: 2050 kcal/d, irrespective of body weight	CHO: 35%-45%	>175 g CHO/d, ↓ GI, CHO distributed in 3 small-to-medium sized meals and 2-4 snacks. Evening snack needed to prevent overnight ketosis. Fiber: >28 g/d	Diabetic nephropathy: ↓ proteins to 0.6-0.8 g/kg ideal body weight
Italian Association of Diabetes/ Italian Society for Diabetes [64]	Energy: >1500 kcal/d Underweight: 40 kcal/kg/d Normal weight: 30 kcal/kg/d Overweight: 24 kcal/kg/d	CHO: 50% Proteins: 20% Fats: 30% Fiber: 28 g/d Night snack: 25 g CHO, 10 g proteins	CHO >40%	
International Diabetes Federation [65]	Overweight: <30% Estimated Energy Requirements		↓ GI	

EI= Energy Intake; PRO= protein; CHO=Carbohydrates; GI= Glycemic Index; OW= overweight; OB= obese.

There is no definitive agreement about the optimal calorie intake for GDM women. Based on American Diabetes Association guideline, the food plan should be based on a nutrition assessment according to the Dietary Reference Intakes (DRI). The DRI for all pregnant women recommends a minimum of 175 g of carbohydrate, a minimum of 71 g of protein and 28 g of fiber. On the other hand, Italian guidelines do not provide a restriction in carbohydrates intakes, which should be around 50% of the whole energy intake and not <40% to avoid the risk of ketosis. A night/evening snack is recommended to prevent overnight ketosis and it should include 25 g carbohydrates and 10 g proteins [64]. The evening snack is recommended by Endocrine Society guidelines too, that, on the contrary, suggest a carbohydrates restriction (35-45%) [62]. Similarly, the International Federation of Gynecology and Obstetrics and by American College of Obstetricians and Gynecologists recommends a carbohydrates reduction around 35-45% and 33-40% of whole energy respectively [60,63].

The indication to use low or medium Glycemic Index (GI) foods is provided by the Academy of Nutrition and Dietetics, the International Federation of Gynecology and Obstetrics and the International Diabetes Federation [63,65].

Low-carbohydrates diets

Historically, the main focus of MNT in GDM has been carbohydrates restriction, in order to reduce postprandial maternal blood glucose levels and the fetal glucose exposure and the risk of macrosomia [66]. Nevertheless, evidences of carbohydrates restriction in GDM are scarce and recently this strategy has been questioned due to the unintended consequences of the inevitable increase in fat intake [58,67].

In 1990, based on decades of clinical experience, Jovanovic-Peterson reported that carbohydrates restriction to 30–40% of total calories can be sufficient to control

postprandial hyperglycemia avoiding starvation ketosis [66]. The next year, the same authors reported in obese women with GDM a correlation between the percentage of carbohydrates in a meal and 1-hour postprandial glucose, and suggest a carbohydrates intake <45% to keep the postprandial glucose level < 120 mg/dL [50]. Although not supported by strong evidence, the ADA included carbohydrates restriction (≤40% of total daily caloric intake) in the 1995 practice guidelines for GDM [68].

In 1998, a non-randomized study demonstrated that diets with <42% of carbohydrates decreased the risk of postprandial hyperglycemia and fetal macrosomia and reduced insulin requirement [69]. Few years later, the low-carbohydrates strategy was questioned [70]. In a correlational study on 80 women with GDM or mild hyperglycemia, a higher carbohydrate intake was unexpectedly associated with a decreased incidence of newborn macrosomia [70]. Infant birth weight was negatively correlated with carbohydrates intake and there were no large-for-gestational-age infants among women whose carbohydrate intake exceeded 210 g/day. This study was affected by poor diet adherence and high rate of insulin requirement (38%). The authors concluded that a carbohydrate intake of at least 250 g/day should be recommend in GDM women, with a low-fat consumption in order to limit energy intake [70].

Cypryk et al. compared low and high-carbohydrates diets (45% vs 65%, respectively) but failed to find differences in blood glucose values, concluding that both diets are effective and safe [71].

Thus, a decade ago, doubts about the effectiveness of low-carbohydrates diet in GDM began to emerge.

In 2011, the ADA removed the recommendation of carbohydrates restriction from the guidelines [72], while other scientific societies, such as the Endocrine Society and the

American College of Obstetricians and Gynecologists (ACOG), were still recommending a carbohydrates reduction (33-40% of total calories) [60,62].

In 2013, Moreno-Castilla et al. randomly assigned 152 GDM patients to either a low-carb diet (40% of total kcal) or a control diet (55% of total kcal). The low-carb diet did not reduce either the need for insulin or the obstetric and perinatal outcomes [73].

Few years later, Tout el al. investigated the effects of a maternal carbohydrate-restricted diet (35–40% of energy) *versus* the usual-care diet (carbohydrate intake 50–55%) and did not observe differences in terms of blood glucose or maternal-infant outcomes [74].

By lowering the carbohydrates content, dietary fats increase, if the protein consumption does not change (15-20% of total energy).

It is well known that the amount and type of dietary fatty acids have a significant role in the modulation of insulin resistance [75]. High-fat diets, in particular high saturated fat diets, may promote insulin resistance through several mechanisms: interference with insulin signaling [75]; promoting inflammation through TNF-alpha production [76]; increasing intestinal permeability and endotoxemia by lipopolysaccharide (LPS) serum levels increase [77] and enhancement of oxidative stress [78].

Metabolic adaptations during pregnancy are finalized to spare glucose and amino acids for the fetal growth. In the last trimester, fetal growth accelerates, and the concentrations of several counter-regulation hormones increase [79]. Lipolysis and glucose production are increased in comparison with non-pregnant women, and the maternal use of fatty acids as energy substrates is promoted to save glucose for the fetus [80]. As a consequence, a high-fat diet could worsen the insulin resistance already characterizing the late pregnancy. Moreover, the increased gestational lipolysis determines a rise in free fatty acids (FFA). Two decades ago, Sivan et al [81] showed that a physiologic increase in plasma FFA was able to inhibit insulin-stimulated glucose uptake in healthy pregnant women further worsening insulin resistance.

The role of maternal lipids serum concentrations in excess fetal growing is emerging [82]. Shaefer-Graf first reported that maternal lipids are strong predictors of fetal lipid serum values and fetal growth in GDM patients with optimal metabolic control [83]. Although the target of GDM therapy has always been glycemic control, maternal triglycerides and FFA have been recently recognized as stronger predictors of excess fetal growth than maternal glucose since they can cross the placenta and constitute substrates for fetal fat accretion [84].

Low Glycemic Index and High-Complex Carbohydrate Diet

In the last decade, the focus of the research in nutritional therapy for GDM has moved from carbohydrate restriction to new strategies that includes diet with a low Glycemic Index (GI) and rich in complex unrefined carbohydrates.

A careful distribution of carbohydrates throughout the day and the use of sugars that are digested more slowly may help in attenuate postprandial hyperglycemia [85].

Different types of carbohydrates have varying effects on postprandial glucose. Sugars lead to an acute increase in blood glucose levels while the polysaccharides and starches from whole grains, vegetables and legumes are able to limit the postprandial glucose rise [86].

GI is a system of classifying carbohydrate-containing foods according to their postprandial blood glucose increment [87]. Several studies have evaluated the effects of low-GI diets on maternal and fetal outcomes in women with GDM, reporting a good feasibility and acceptability [88-89], the reduction in insulin treatment [89, 95], the prevention of excessive maternal weight gain [90, 95], the improvements in fasting [91] and postprandial glucose values [92]. Other studies failed to find significant differences in gestational/neonatal outcomes [91-93] and low-GI diets were associated with an

increased prematurity risk [94]. Indeed, the risk for adverse newborn outcomes has been refuted by a recent large metanalysis [95].

The effects of complex carbohydrates/fiber on postprandial glycemia, satiety and energy intake are well known [96]. The possibility to avoid carbohydrates restriction by increasing complex carbohydrates and reducing fat intake, is currently a discussed topic. Hernandez hypothesized that pregnancy-induced insulin resistance and excessive fetal growth can be attenuated by a low-fat diet with high-quality carbohydrates [97-98]. The CHOICE (Choosing Healthy Options in Carbohydrates Energy) diet was developed; it contains 60% of energy from "complex carbohydrate" (polysaccharides and starches from grains, vegetables, and fruit). This diet determined lower circulating FFA levels, an increased adipose tissue sensitivity and a decreased expression of pro-inflammatory gene expression when compared to low-carb diets [98].

At present, the optimal percentage of dietary carbohydrates to reduce adverse maternal/neonatal outcomes and obtaining an adequate compliance of the women to the nutritional scheme has not yet been defined [99].

Mediterranean diet

The Mediterranean diet is characterized by a high consumption of vegetables, fruit, nuts, seeds, legumes, unprocessed grains and olive oil, a moderate consumption of fish and a limited consumption of dairy products, red meat, processed meats and refined sugars. This diet is rich in antioxidants and anti-inflammatory foods with a low GI [100].

The Mediterranean diet has proven to display beneficial effects on cardiovascular and metabolic health [100-101]. At present, a few studies are available about pregnancy and no trials have evaluated the efficacy of Mediterranean diet in the GDM treatment.

The St Carlos GDM Prevention Study, a randomized controlled trial conducted in Spain, compared the early adoption of a Mediterranean diet (8–12 weeks' gestation) to a conventional diet on the development of GDM in 874 pregnant women. The Mediterranean diet reduced the incidence of GDM and decreased several perinatal outcomes including insulin treatment, preterm delivery, emergency cesarean-sections, perineal trauma, and the incidence of small- and large- for gestational age births [102]. A post-hoc analysis comparing women with GDM to women with normal glucose tolerance found that the Mediterranean diet was not significantly associated with difference in weight gain, pregnancy-induced hypertension, mode of delivery, perineal trauma, preterm delivery, and neonatal macrosomia between groups [103].

Randomized controlled trial in large samples are therefore needed to define the potential role of the Mediterranean diet in GDM. Similarly, the best diet for this frequent pregnancy complication has yet to be defined.

2. MICROBIOTA

2.1 Definition and composition of gut microbiota

The term gut microbiota refers to the microorganisms colonizing the human gastrointestinal tract [104]. The human gastrointestinal (GI) tract contains more than 100 trillion microorganisms [105] that encodes over 3 million genes whereas the human genome consists of approximately 23000 genes [106].

The gut microbiota is composed of several species of microorganisms, including bacteria, yeast and viruses. Taxonomically, bacteria are classified according to phyla, classes, orders, families, genera and species. The dominant gut microbial phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia, with the two phyla Firmicutes and Bacteroidetes representing 90% of gut microbiota (**Figure 1**). The Firmicutes phylum is composed of more than 200 different genera including *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus* and *Ruminicoccus*. *Clostridium* genera represent 95% of the Firmicutes phyla. Bacteroidetes consists of predominant genera such as *Bacteroides* and *Prevotella*. The Actinobacteria phylum is proportionally less abundant and mainly represented by the *Bifidobacterium* genus [107].

The gut microbiota varies according to the intestine anatomical regions, which differ in terms of physiology, pH and O_2 availability, digesta flow rates, substrate availability and host secretions. The microorganism amount increases steadily along the gastrointestinal tract, with small numbers in the stomach and very high concentrations in the colon. Aerobic bacteria progressively decrease in the colon with predominance of strictly anaerobic bacteria [108].

Figure 1. Taxonomic gut microbiota composition

Source: Rinninella et al, Microorganisms 2019, 7, 14

The stomach and proximal duodenum are exceptionally inhospitable and very few bacteria are resistant to this acidic condition. The stomach harbors only 101 bacteria per gram; increasing densities and bacterial diversities are found in the duodenum (103/g), jejunum (104/g), ileum (107/g) and colon (1012 bacteria/gram) [109]. Mucosa-associated bacteria from distal small intestine and colon are dominated by the phyla Bacteroidetes and Firmicutes. An enrichment of *Lactobacillus*, *Veillonella* (both Firmicutes), and *Helicobacter* (Proteobacteria) is present in the proximal gut, whereas *Bacilli*, *Streptococcaceae* (both Firmicutes), *Actinomycinaeae* and *Corynebacteriaceae* (both Actinobacteria) are abundant in duodenum, jejunum and ileum. Increased proportions of *Lachnospiraceae* (Firmicutes) and Bacteroidetes are found in the colon [110].

Most of the studies analyzed bacteria in feces, while only limited information is available for mucosa-associated microorganisms because the isolation of mucosa-associated bacteria is much more complex and needs intestinal biopsies. Significant differences in bacterial composition have been shown between fecal and biopsy samples from same individual [110]. This is a great limitation of most of the studies on gut microbiota in humans.

Gut microbiota composition varies also dramatically between individuals. The composition of the gut microbiota is strongly influenced by a range of factors, such as maternal prenatal factors [111], delivery mode [112], breast- or formula-feeding [113], host genetics [114-115], dietary habits [116], the administration of antibiotics and other drugs [117], and environmental exposure [118].

For more than a century, neonatal gut has been considered sterile at birth. However, the bacterial composition of amniotic fluid seems similar to the meconium microbiome, suggesting than meconium reflects the intrauterine environment [119].

During the first days of life, the gut microbiota of healthy full-term newborns is composed of facultative anaerobic species, such as *Escherichia coli*, *Enterococcus spp*, a-hemolytic streptococci, and *Staphylococcus spp*. Subsequently, oxygen consumption and nutrients in milk shift the microbial composition towards a predominance of anaerobic bacteria, such as *Bacteroides*, *Bifidobacterium* and *Clostridium spp* [120].

Both the composition and function of the early infant microbiota are primarily defined by birth mode, antibiotic exposure and early-life feeding practices.

The microbiota of the healthy newborns closely matches the maternal stool, vaginal, or skin microbiota, depending on the delivery mode. Vaginal birth exposes infants to the microbes that are colonizing the mother's birth canal. After C-section birth, the neonatal microbiota is more similar to the maternal cutaneous flora, and it is influenced by the hospital environment [121]. Several studies indicate that infants born by C-section tend to have a lower number of anaerobes, such as Bacteroidetes, and a less diverse microbiota than infants born by unassisted vaginal birth [122,123,124]. However, these studies are affected by ethnic and geographic diversity and differences in the analytical methodologies. Moreover, not all studies have found an association between mode of birth (vaginal versus C-section), and the development of the gut microbiota [125,126]. The clinical decision to perform a C-section is often determined by the underlying maternal or fetal medical complications and is accompanied by varying use of medications, including antibiotics, all conditions potentially influencing the microbial composition [127].

Diet may affect the composition of the gut microbiota. Formula-fed infants tend to have relatively stable microbiota with higher alpha-diversity and relative abundance of facultative anaerobes and strict anaerobes when compared to breast-fed infants [128]. Breast-fed fecal samples are less complex with a higher number of aerobic organisms and exhibit more dramatic changes in the microbial composition over their first year

after birth [128]. *Bifidobacteria* and *Clostridium difficile* predominate in breast-fed neonates, whereas *Bacteroides* and *Clostridium perfrigens* prevail in formula-fed infants [129].

Moreover, early breastfeeding has long-lasting effects on the gut microbiota and its effects persist even after weaning [130].

2.2 Diet and the microbiota

Diet is one of the most powerful factors that modify within-days the intestinal microbiota and probably the easier to be manipulated [131]. Specific foods and dietary patterns can influence the relative abundance of different types of bacteria in the gut, which in turn can affect health. The human colonic microbiota is considered to be relatively stable and generally reverts to the original status quo after short-term dietary changes or antibiotic therapy [131]. Changing the macronutrient composition of the diet can significantly affect the gut microbiota. Host enzymes are not able to degrade the structural polysaccharides found in dietary plant material. Approximately 40 g of dietary carbohydrates reach the colon each day having escaped digestion by host enzymes [132]. The main categories are resistant starches (RS), non-starch polysaccharides (NSP) and oligosaccharides. Fermentation of these complex undigested polysaccharides results in the production of short chain fatty acids (SCFA), primarily acetate, propionate and butyrate, generally in a ratio of 3:1:1 [133]. Butyrate is the major energy source for the colonocytes while propionate is transported to the liver where it has a role in gluconeogenesis, whilst acetate enters systemic circulation and is used in lipogenesis [133].

The intakes of different undigested carbohydrates were associated with different microbe growth: prebiotics fiber, such as fructans, polydextrose, fructo-oligosaccharides

(FOS), galacto-oligosaccharides (GOS) were linked to the growth of intestinal *Bifidobacteri* and *Lactobacilli*; resistant starch were reported to increase the abundance of *Ruminococcus*, *E. rectale*, and *Roseburia* [134]. Thus, changing the amount and/or type of carbohydrate could have a profound and rapid influence on the composition of the gut microbiota and its metabolic products [135].

Protein fermentation can lead to the production of deleterious metabolites. Approximately only 12–18 g proteins reach the human colon daily, corresponding to around 10% of the ingested proteins [136]. In the colon, proteins provide nitrogen for the growth of saccharolytic bacteria, and amino acids for fermentation by a-saccharolytic species [137]. The predominant proteolytic bacteria identified in human feces are *Bacteroides* species, *Clostridium*, *Propionibacteria*, Streptococci, Bacilli and Staphylococci [131].

The main pathway of amino acid fermentation in the human colon is deamination, leading to the production of SCFA and ammonia. Most of the ammonia produced is rapidly absorbed, metabolized into urea by the liver and excreted in urine. Ammonia alters the morphology of intestinal tissues and may act as a tumor promoter in the gut. Another pathway of amino acid fermentation is decarboxylation of amino acids and peptides, leading to the formation of amines. Their toxicological significance is not well understood; amines might act as precursors in nitrosamine formation, which are known carcinogens [131].

The consumption of a high-fat diet increases total anaerobic microbes and *Bacteroides*, while a low-fat diet increases *Bifidobacterium* [138]. However, a high-fat diet is generally low in carbohydrates, while a low-fat diet is significantly higher in digestible carbohydrates and fiber. Thus, the observed changes in the microbiota composition may be due to the changes in carbohydrates intake.

Several studies have investigated the impact on microbiota composition of some dietary patterns, such as Western diet or Mediterranean diet. Western diets seem to decrease the number of total bacteria and beneficial bacteria, such as *Bifidobacterium* and *Eubacteria*. In contrast, the Mediterranean diet is associated with a healthy gut microbiota, with an increase in *Bifidobacterium* and *Lactobacillus spp* [138].

The principal effects of single nutrients and diets on the microbiota composition are summarized in the table below.

Table 2. Effects of different dietary patterns on the microbiota composition [138]

	Bifidobacterium	***Lactobacillus***	***Bacteroides***	***Akkermansia***	***Prevotella***	***Eubacteria***
High-fat		↓	↑			
High saturated fatty acids			↑			
High unsaturated fatty acids	↑	↑		↑		
Animal proteins	↓		↑			
Vegetable proteins	↑	↑				
High fiber	↑	↑			↑	↑
Rich in polyphenols	↑	↑	↓			
Western diet	↓	↓	↑			↓
Mediterranean diet	↑	↑	↑		↑	↑

2.3 Roles of the microbiota in metabolic diseases

The gut microbiota plays an important role in the regulation of the host's metabolism; it maintains a symbiotic relationship with the gut mucosa with substantial metabolic, immunological and gut protective functions. However, an altered microbiota (dysbiosis) could contribute to the development of metabolic diseases through different mechanisms, such as increased harvest of energy from the diet, abnormal SCFA production, abnormal gut permeability and increasing absorption of lipopolysaccharide (LPS) and production of bacterial toxic substances, such as trimethylamine [139-140].

The composition and metabolic actions of the microbiota play a significant role in energy processing of dietary intake, with increased energy harvest [141]. The role of SCFA on energy homeostasis is ambiguous. Fecal SCFA has been found to be increased in the gut microbiota of individual with obesity [142] and this might indicate a greater ability to extract energy from undigested foods.

Butyrate and propionate are generally considered beneficial [143] while the role of acetate is still controversial. A beneficial regulatory role in energy metabolism and insulin sensitivity is suggested by most studies. Acetate could play a role in weight control by downregulating the sub-clinic chronic inflammation associated with obesity and the production of anorexigenic hormones [144]. On the contrary, some animal data suggest that acetate could promote the development of obesity and insulin resistance by increasing appetite and the lipid storage capacity of adipose tissue by inhibiting intracellular lipolysis and increasing adipogenic differentiation [144-145].

SCFA in the intestine activate G-protein-coupled receptors (GPR), such as GPR41 (free fatty acid receptor 3; FFAR3) and GPR43 (free fatty acid receptor 2; FFAR2). Both those receptors trigger the secretion of gut hormones (GLP-1 and PYY). Leptin is also released from adipocytes when SCFA bind to GPR41 [146]. PYY, GLP1 and leptin can

decrease appetite [147]. GLP-1 increases insulin secretion from pancreatic β-cells and decreases glucagon secretion, with enhanced peripheral glucose uptake and a lower hepatic neoglucogenesis.

SCFA can inhibit lipolysis and suppress the inflammatory mediators, such as NO (nitric oxide), TNF-α (Alpha Tumor Necrosis Factor), IL-1β (Interleukin 1 beta), IL-6 (Interleukin 6) [148-149].

The impairment in the balance between gut microbiota and the host's immune response could lead to the intestinal translocation of bacterial fragments (such as LPS through the gut barrier into the blood) and the development of "metabolic endotoxemia" with systemic inflammation. These molecules can stimulate macrophage infiltration and activate the synthesis of inflammatory cytokines. Current views suggest that low-grade chronic systemic inflammation contributes to the development of insulin resistance and metabolic diseases [150].

Another mechanism by which microbiota could play a role in metabolic disorders is the production of Trimethylamine-*N*-oxide (TMAO). Gut microbes are able to use the dietary methylamines (choline, l-carnitine, and phosphatidylcholine) producing trimethylamine (TMA), which is further oxidized to TMAO by the hepatic enzyme flavin-containing monooxygenase 3 (FMO3) [151]. Red meat, eggs, dairy products and salt-water fish are the main dietary sources of TMA.

Elevated plasma levels of TMAO have been associated with increased risk of cardiovascular diseases and T2DM [152]. In a large cross-sectional study, TMAO plasma concentrations measured in early and mid-pregnancy were positively related to increased odds of developing GDM [153]. At present, the mechanisms of action of TMAO are unknown. In mice fed with a high-fat/high-sugar diet, TMAO promoted impaired glucose tolerance and adipose tissue inflammation [154]. Moreover, scarce

information is available about taxonomic composition of TMA-producing bacteria in humans which include either Firmicutes, Actinobacteria or Proteobacteria species [155].

3. MICROBIOTA AND GESTATIONAL DIABETES

Herein, I summarize the content of my recent narrative review on this topic [156].

3.1 The modifications of the gut microbiota during pregnancy

Studies on gut microbiota composition in healthy pregnancy have documented profound alterations from the first to the third trimester [157-158]. Increased between-individual diversity (β-diversity) and decreased richness (intra-individual or α-diversity) have been reported, and a microbial pattern similar to that of non-pregnant adults with metabolic syndrome was found in late pregnancy [157,159].

During gestation, profound hormonal, immunological, and metabolic changes take place and the variations in the gut microbial composition may contribute to the metabolic changes typical of late pregnancy that promote maternal weight gain, increasing circulating pro-inflammatory cytokines, hyperglycemia and insulin resistance [160]. Current literature on microbiota composition in pregnancy is highly discordant reporting no variations [161], increased Proteobacteria/Actinobacteria abundance, *Roseburia* intestinalis and *Faecalibacterium prausnitzii* reduction, and α-diversity decrease [157]. Several studies have evaluated the relationship of BMI and maternal weight gain with microbiota. Mothers' pre-pregnancy weight and BMI have been correlated with increased relative abundance of *Bacteroides* [162], *Clostridium* [163], *Staphylococcus* [162-163], reduced *Bifidobacteria* [162]. A lower α-diversity was found among women with overweight/obesity [164].

Gestational weight gain has been associated with higher abundance of *Bacteroides* species [163], *Staphylococcus* [162-163], *Enterobacteriaceae*, *Escherichia coli*, reduced

abundance of *Bifidobacterium*, *Akkermansia muciniphila* [162] and lower α-diversity [164].

Many correlations among specific taxa and gestational metabolic variables have been found, such as direct relationships between *Collinsella* and circulating levels of insulin, triglycerides, and very-low-density lipoproteins; *Sutterella* and C-reactive protein; *Ruminococcaceae/Lachnospiraceae* and leptin; *Bacteroidaceae* and ghrelin; *Coprococcus* and gastrointestinal polypeptide (GIP). Moreover, inverse relationships between *Blautia* and insulin values; *Faecalibacterium/Fusobacterium* ratios and blood glucose; *Odoribacter* and arterial blood pressure; *Ruminococcaceae* and GIP, and *Prevotellaceae* and ghrelin have been reported [165,166].

In conclusion, the gut microbiota seems to contribute to maternal metabolic changes through mechanisms that remain at present largely undefined.

3.2 The gut microbiota in pregnancy complicated by GDM

Some studies have observed that the gut microbiota may be involved in the development of pre-diabetic conditions outside of pregnancy. Overall, insulin resistance has been associated with a higher Firmicutes/Bacteroidetes ratio [167] and with a reduced abundance of butyrate-producing bacteria such as *Roseburia* and *Faecalibacterium prausnitzii* [168].

Only few studies have evaluated the microbiota of GDM patients with highly contrasting results.

Koren et al [157] reported that both healthy and GDM women display changes in the composition of their gut microbiota with advancing gestational age. The women who later developed GDM have a reduced microbial richness in the first trimester although their microbiota composition did not differ from that of the healthy controls.

Accordingly, two recent Chinese studies reported a lower α-diversity in GDM women with respect to normoglycemic women [169-170].

Specific differences between GDM and normoglycemic women were reported by a few studies. Increased gut abundance of *Parabacteroides distasonis*, *Klebsiella variicola* [171], *Ruminococcus*, *Eubacterium*, *Prevotella* [172], *Collinsella*, *Rothia*, *Desulfovibrio*, *Actinobacteria* [159], Firmicutes [172] and reduced gut richness of *Methanobrevibacter smithii*, *Alistipes* species, *Bifidobacterium* species, *Eubacterium* species [171] *Akkermansia*, *Bacteroides*, *Parabacteroides*, *Roseburia*, and *Dialister* [172] were reported in GDM patients compared to normoglycemic controls. Some of the changes in the bacterial species related to GDM have been reported also in T2DM patients, such as the reduction in *Roseburia* and *Akkermansia muciniphila*, and the increment in Proteobacteria [173].

Functional analyses showed a greater abundance of membrane transport and energy metabolism pathways, lipopolysaccharide and phosphotransferase systems, and lower amino acid metabolic pathways in the microbiome of GDM patients [171].

If the changes in the gut microbiota contribute to or are a consequence of the development of GDM is a debated question. A different microbiota composition was found to precede the onset of GDM in early pregnancy, since both reduced microbial richness and increased abundance of *Ruminococcaceae* family have been reported with a supposed subsequent increased energy harvest, pro-inflammatory status, and impaired insulin signaling [174]. Therefore, the gut microbiota composition in women who will develop the GDM in late pregnancy seems to be impaired early during pregnancy.

These findings suggest that an early modulation of the microbiota in pregnancy might be a new approach for the prevention and management of gestational hyperglycemia.

If the aberrant microbiota might contribute to the development of T2DM after pregnancy has not yet been clarified. A few studies described a different microbiota within 3–16 months after delivery in women with a previous GDM compared to those with a previous normoglycemic pregnancy. In particular, GDM+ women showed a higher abundance of *Prevotellaceae*, *Collinsella*, *Olsenella*, and *Clostridium* and a reduction in Firmicutes, *Fusobacterium* and the parent family *Fusobacteriaceae*, and genus *Ruminococcus* (*Ruminococcus* from *Lachnospiraceae* family) [159, 175]. Indeed, no gut microbiota differences between GDM+ and GDM- women were found 5-years postpartum [176]. Therefore, the possibility that aberrant microbiota could contribute to the future development of T2DM in the GDM+ women becomes less probable.

3.3 The gut microbiota of the offspring from mothers with GDM

Initial colonization of the infant gut, starting from intrauterine life, sets the stage for the lifelong relatively stable adult microbiome. For this reason, pregnancy offers a window of opportunity to determine long-term health consequences for the child and maternal.

An interesting hypothesis is that the gut microbiota of the fetus may reflect pathophysiological processes occurring during pregnancy or represent a direct transmission of maternal intestinal bacteria. In this context, it would be interesting to understand if the gut microbiota of the offspring from mothers with GDM may be influenced by maternal glycemic status and if it may have an impact on their future health.

Few studies have analyzed the gut microbiota composition of the offspring of mothers with GDM with contrasting results.

The gut microbiota composition of the newborns was reported to be either not different [157] or abnormal [166,176, 177, 178] in relation to the maternal glycemic status.

In particular, the microbiota α-diversity of the newborns from GDM mothers was described as similar [157] or lower [178] than that of newborns from non-GDM mothers. An increased abundance of the phyla of Proteobacteria and Actinobacteria, a reduction of Bacteroidetes and a decreased abundance of *Prevotellae* and *Lactobacilli* were reported in newborns of mothers with GDM [178].

In 5-years children, the gut microbiome of GDM offspring remained different from that on non-GDM offspring. In particular, the *Anaerotruncus* genus was observed to be more abundant in the children of GDM+ women; this genus has been positively associated with both glucose intolerance and gut permeability, suggesting a role in the pathogenesis of diabetes [176]. These results suggested that the maternal influences on the gut microbiome persisted over time.

Prospective studies would be necessary to further clarify the mode of the mother-to-baby microbes transmission during pregnancy, as well as the impact of the influence of the maternal microbiota on the offspring adult-onset diseases.

3.4 The influence of diet on the microbiota in pregnancy

Despite the importance of diet-microbiota associations, only few studies have evaluated the role of nutrition on gut microbiota during the crucial period of pregnancy. A lower fiber intake has been associated with reduced gut microbiota diversity and richness [179], greater abundance of *Collinsella*, a genus associated with T2DM [180,181], and greater abundance of *Sutterella*, a Proteobacteria with known pro-inflammatory capacity [182].

In pregnancy, vegetarian diets resulted in increased relative abundances of *Roseburia* and *Lachnospiraceae*, but no difference in α-diversity when compared to omnivorous diets [181]. A lower bacterial richness was found in pregnant women with high-fat, low-fiber intakes [179]. In women with overweight/obesity, a high intake of saturated fatty acids (SFAs) during early pregnancy was associated with the reduction of all indexes of microbiota richness [179], while, early after delivery, an increased SFA consumption was associated with reductions in Proteobacteria and Firmicutes relative abundance [182]. Monounsaturated fatty acids (MUFAs) have been associated with increased abundance of Firmicutes, Proteobacteria, and Bacteroidetes [182].

In pregnant women, fat-soluble vitamins seem to act as modulators of gut microbiota. Higher intake of vitamin D was associated with reduced microbial α-diversity. The consumption of retinol and vitamin D was associated with a relative increase in abundance of the pro-inflammatory Proteobacteria phylum. On the contrary, vitamin E intake was associated with a relative decrease in the abundance of Proteobacteria [182].

In pregnant women with overweight, dietary fiber and n–3 polyunsaturated fatty acids (PUFAs) were associated with higher microbiota richness and lower serum zonulin levels [183], a protein that adversely modulates the permeability of gut tight junctions. Indeed, caution should be used in the interpretation of these results, since intakes were assessed by food frequency questionnaires [182] or food diaries [183], and, owing to the subjective nature of these data, associations between microbiota and micronutrients might be overestimated.

Owing to the emerging evidence of the potential role of human gut microbiota on metabolism and inflammation, future research is warranted in order to test the intriguing possibility that microbiota manipulation may improve maternal (and consequently neonatal) health.

3.5 Diet-microbiota interaction in GDM

Because of the ability of the gut microbiota to modulate the metabolism and inflammation of the host, the possibility of manipulation of the intestinal microbiota is emerging as a promising therapeutic strategy for many chronic medical conditions [184], including pathological pregnancy.

As previously mentioned, GDM is characterized by changes in the composition of the microbiota, however it is still unknown if these changes are a consequence or a cause in determining the increased maternal insulin resistance.

Based on current knowledge on diet-microbiota relationship outside of pregnancy, a diet rich in fiber with high content of complex and unrefined carbohydrates could result in a shift of the microbiota towards a potentially favorable enterotype [185].

The classical nutritional approach with carbohydrates restriction inevitably leads to the increase of dietary fat, if protein consumption remains in the recommended levels (15-20% total energy) and could potentially increase pro-inflammatory bacteria resulting in increased insulin resistance. In several human studies, high-fat diet has been suggested to increase total anaerobic microbes and in particular *Bacteroides*. Low-fat diets had been reported to increase the *Bifidobacterium* abundance with concomitant fasting glucose and total cholesterol reductions [186]. Animal studies have shown that high SFA intake resulted in considerably lower amounts of *Lactobacillus intestinalis* and increased in propionate and acetate producing bacteria [187]. Moreover, high-fat diet has been associated with endotoxemia and an increased concentration of the plasmatic pro-inflammatory LPS [77].

To date, there is a lack of clinical trials specifically focused on the effect of different nutritional strategies on the microbiota composition in pregnancy complicated by GDM. The potential impact of specific dietary interventions on the gut bacteria composition

and function is of considerable interest in the search for the optimal strategy to prevent and treat GDM.

4. THE RESEARCH STUDY

4.1 Background

GDM is one of the most common pregnancy complications and it is associated with a moderately increased risk of maternal and perinatal outcomes [2]. Lifestyle interventions were reported to provide benefits to the health of GDM women and their babies and it has been hypothesized that at least some of these beneficial effects might be due to the modulation of the maternal microbiota during pregnancy. Indeed, variations in nutrient and energy intake were associated to specific bacterial abundance [131]. However, to date only few studies have evaluated the microbiota of GDM patients, showing contrasting results [156]. Moreover, it is well known that the maternal environment affects the offspring health. The newborn gut microbiota is strongly influenced by maternal health and pregnancy conditions. Early disruption of the infant microbiota has been associated with many inflammatory, immune-mediated, allergic, and dysmetabolic diseases in later life [188-189]. Children's obesity, non-alcoholic hepatic liver diseases, aberrant cardiac growth are, among others, the conditions that have been associated with maternal/newborn dysbiosis [190-191]. Nevertheless, uncertainty still exists about the microbiota offspring colonization, and either the modality or the timing of microbial exposure for the fetus/newborn are controversial [192].

Only a few data are available about the associations between maternal characteristics and newborn microbiota pattern. This knowledge is important, since it underscores the possibility of early modulation of the offspring gut microbiota by acting on specific maternal factors and/or characteristics.

The data herein presented have been recently published [193,194].

4.2 Methods

Study design

This is a prospective observational explorative study.

Aims

The aims of the study are:

- To evaluate the associations between diet and microbiota in pregnancies complicated by GDM and whether patients with greater adherence to dietary recommendations present a different microbial pattern than less adherent patients
- To compare the gut microbiota of GDM patients with normoglycemic pregnant women
- To evaluate whether the gut microbiota composition of the offspring of GDM patients is associated with the maternal nutritional habits or with the type of feeding (breastfeeding *vs* artificial milk).
-

Patients recruitment

The participants were 50 patients with GDM and their offspring consecutively recruited from the "Città della Salute e della Scienza" Hospital of Turin from October 2016.

Inclusion criteria were:

-gestational age between 24-28 weeks

- Caucasian race

- GDM diagnosed by a 75g oral glucose tolerance test (OGTT).

Women who had the following criteria were excluded from the study:

-twin pregnancy

-use of prebiotics/probiotics, antibiotics or any drug during pregnancy

-any pathological conditions before or during pregnancy (known diabetes mellitus, hypertension, cardiovascular, pulmonary, autoimmune, joint, liver or kidney diseases, thyroid dysfunction, cancer, any other disease/condition)

-no compliance to the study protocol.

All women were taking folic acid supplementation.

GDM was diagnosed by OGTT performed at 24-28 gestational weeks in the morning, after at least 8h-overnight fast, when the fasting plasma glucose was ≥92 mg/dL and/or 1h post-OGTT glycemia ≥180 mg/dL and/or 2h post-OGTT glycemia ≥153 mg/dL, according to international criteria [52].

Patients were instructed to self-monitor finger-prick capillary blood glucose at least 4 times per day. Insulin therapy was added to diet when fasting blood glucose levels were consistently ≥90 mg/dL, 1-hour levels consistently ≥130 mg/dL, or 2-hour levels ≥120 mg/dL, according to guidelines [52].

Dietary intervention

In our cohort, all the patients with GDM routinely received dietary counselling and nutritional recommendations in line with guidelines (carbohydrates 45% total energy, rapidly absorbed sugars <10% total energy, proteins 18-20% total energy, fats 35% total energy, at least 20-25 g/day fibre intake, no alcohol) [62]. Furthermore, 30-min daily moderate exercise was recommended (i.e. brisk walking). Patients were considered to be compliant to the given dietary recommendations in the presence of all the following

criteria: at least 20 g/day fiber consumption (or increasing fiber intake more than 50% than enrolment), sugar reduction to less than 10% of total energy and abolition of alcohol intake.

Finally, all mothers were suggested to breastfeed their children.

Sample collection, anthropometric measurements and dietary information

Questionnaires, anthropometric values, fasting blood samples and stool samples were collected for all participants both at 24-28 weeks of gestational age at the time of GDM diagnosis (enrolment), and at 38 weeks, or before delivery, in the case of preterm delivery (study end). The researchers were in continuous contact with the patients, through weekly telephone contact. In this way, they were aware of the progress of pregnancy.

Fecal samples of the offspring were collected between the 3rd and the 5th day of life, after meconium expulsion. Data relative to the type of delivery, birth weight, gestational age and the type of feeding were extracted from medical records.

Stool samples were self-collected by the patients. Briefly, the subjects were instructed on how to self-collect the samples, and all materials were provided in a convenient, refrigerated, specimen collection kit. Patients were provided with sterile containers to collect the feces (VWR, Milan, Italy). The fecal samples were collected at home and transferred to the sterile sampling containers using a polypropylene spoon (3 spoons of about 10g) and immediately stored at 4°C. The specimens were transported to the laboratory within 12 hours of collection at a refrigerated temperature. Containers were immediately stored at -80°C for DNA extraction. No storage medium was used.

Participants completed a 3-day food record (2 weekdays and 1 weekend day) and the Minnesota-Leisure-Time-Physical Activity Questionnaire [195] at enrolment and at the

study end. Detailed information on how to record food and drink consumed by using common household measures was provided to all participants. Two dieticians checked all questionnaires for completeness, internal coherence and plausibility.

The 3-days food record questionnaires were analyzed using Winfood 3 Pro Software (release 3.4.4; 2011 version; Medimatica, Teramo, Italy) according to the table of food consumption of the Italian National Institute of Nutrition [196] and the Food Composition Database for Epidemiologic Study in Italy [197].

Data relative to pre-pregnancy weight was self-reported; weight, height, and arterial blood pressure (BP) were measured at time of enrolment, and weight and BP at the study end. Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm with a stadiometer (SECA model 711, Hamburg, Germany), with the participants wearing light clothes and no shoes. Arterial BP was measured from the left arm, in a sitting position, after at least 10 min of rest, with a mercury sphygmomanometer with appropriate cuff sizes (ERKA Perfect-Aneroid, Germany). Two measurements were taken by trained personnel with arm supported at heart level and the values reported were the means of the two. Glucose levels were self-measured by the patients by the BGSTAR® glucometer (Sanofi-Deutscland GmbH, Frankfurt, Germany). The average of the values measured 1-hour after each meal during the third trimester have been reported.

Determination of the gut microbiota

The analyses were carried out at the Microbiology Laboratory of the Department of Agricultural, Forestry and Food Sciences at the University of Turin.

Nucleic acid was extracted from the feces collected. Total DNA from the samples was extracted using the RNeasy Power Microbiome KIT (Qiagen, Milan, Italy) following

the manufacturer's instructions. One microliter of RNase (Illumina Inc. San Diego. CA) was added to digest RNA in the DNA samples, with an incubation of 1 h at 37°C. DNA was quantified using the QUBIT dsDNA Assay kit (Life Technologies, Milan, Italy) and standardized at 5 ng/μL.

DNA directly extracted from fecal samples was used to assess the microbiota by the amplification of the V3-V4 region of the 16S rRNA gene using the primers and protocols described by Klindworth *et al* [198]. PCR amplicons were cleaned using Agencourt AMPure kit (Beckman Coulter, Milan, Italy) and the resulting products were tagged by using the Nextera XT Index Kit (Illumina Inc. San Diego. CA) according to the manufacturer's instructions. After the 2nd purification step, amplicons products were quantified using a QUBIT dsDNA Assay kit (Life Technologies). Subsequently, equal amounts of amplicons from different samples were pooled. The pooled sample was run on an Experion workstation (Biorad, Milan, Italy) for quality analysis prior to sequencing. The sample pool (4 nM) was denatured with 0.2 N NaOH, diluted to 12 pM, and combined with 20% (vol/vol) denatured 12 pM PhiX, prepared according to Illumina guidelines. The sequencing was performed with a MiSeq Illumina instrument (Illumina) with V3 chemistry and generated 250 bp paired-end reads according to the manufacturer's instructions.

Bioinformatics analysis

Paired-end reads were first assembled using FLASH software [199] with default parameters. Joint reads were further quality filtered (at Phred < Q20) using QIIME 1.9.0 software [200] and short reads (< 250 bp) were discarded through Prinseq [201]. Chimera filtering was performed through USEARCH software version 8.1 [202]. Operational Taxonomic Units (OTUs) were picked at 97% of similarity threshold by

UCLUST algorithms [203] and centroids sequences of each cluster were matched to the Greengenes 16S rRNA gene database version 2013. After sequencing, a total of 2,100,009 raw reads (2 X 250 bp) were obtained. After joining, a total of 1,919,311 reads passed the filters applied with QIIME, with an average value of 23,406 ± 31,535 reads/sample and a sequence length of 457 bp. The rarefaction analysis and Good's coverage, expressed as percentages, indicated that there was satisfactory coverage for all the samples (Good's coverage average, 92%). In order to avoid biases due to the different sequencing depth, OTU tables were rarefied to the lowest number of sequences per sample (4078 reads/sample). The OTU table displays the higher taxonomy resolution that was reached; when the taxonomy assignment was not able to reach the genus, family name was displayed.

Ethical aspects

The present study conforms to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the "Città della Salute e della Scienza" hospital of Turin (approval 707/2016). All patients provided written informed consent prior to participation in the study protocol.

4.3 Results

Characteristics of the participants

Nine women did not return stool samples and were lost at follow-up. Data of 41 patients were therefore analyzed. The clinical characteristics of the participants did not differ from those of the 9 women who dropped out. Seven women (17.1%) gave birth before the 38^{th} week, either by induced vaginal delivery or Caesarean section. These patients provided the fecal and blood samples and the food questionnaire about a week before all

the others (37th week); they did not differ with regard to nutritional, anthropometric, or metabolic characteristics when compared to the others.

Most participants were overweight women, with excessive fat intake and lower than recommended fiber consumption (**Table 3**). From enrolment (24-28 weeks of gestational age) to the study end (38 weeks), weight and Body Mass Index (BMI) increased, and metabolic and inflammatory patterns of participants worsened, as usually occurs during the third trimester of pregnancy (**Table 3**).

Adherence to the dietary recommendations

After the dietary counselling, 34.1% (14/41) of the participants declared to be adherent to the given dietary recommendations. Characteristics at enrolment did not significantly differ between adherents and non-adherents, even if adherents showed increased values of weight and BMI (**Table 4**). Adherent women showed reduced intakes of sugars, and increased consumption of fiber, oligosaccharides, polyunsaturated fatty acids (PUFA) than non-adherents. All participants had abolished alcohol consumption. Adherents had a better metabolic and inflammatory pattern, with a significantly greater reduction in fasting glucose and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) levels at the end of the study (**Table 4**).

Microbiota signature between dietary adherences

The microbiota α- and β-diversity values were not significantly different between adherent and non-adherent subjects (data not shown). Similarly, there was no significant separation of the microbiota composition. We performed a number of analyses investigating the shift in microbiota as a function of the adherence to diet. Taking into account the shift in the microbiota between enrolment and study end in adherents and non-adherents (**Figure 2**) a common microbiota signature was observed. *Blautia,*

Coprococcus, *Dorea* and *Lachnospiraceae* significantly increased in both groups during the progression of pregnancy while *Rikenellaceae* decreased. We observed that the delta (study-end minus baseline) values of those OTUs was significantly higher in adherent patients. Between the two groups, we detected a specific microbiota shift at the study end: an impressive decrease in *Bacteroides* in adherents, and higher abundance of *Faecalibacterium* and L-*Ruminococcus* together with minor OTUs in non-adherents (**Figure 2**).

Associations between microbiota and nutrient intakes and metabolic variables

Several associations between nutrients variables and microbiota could be detected both at enrolment and at the study end (**Figure 3**). At enrolment, *Alistipes* was found positively related with fat intakes (β=0.10; 95%CI 0.06 0.14; *P*<0.001) in a regression model, after adjusting for age and weight values (**Table 5**). At the end of the study, many associations among specific microbiota relative abundance and nutrient intakes and their changes across pregnancy were detected in multiple regression analyses, after adjusting for age, weight change, and adherence to the given recommendations. Among the relationships, we underline the direct associations between *Roseburia* and fiber intake (β=0.09; 95%CI 0.02 0.16; *P*=0.01), and that between *L-Ruminococcus* and oligosaccharides (β=0.02; 95%CI 0.01 0.03; *P*=0.006), that however did not reach the established statistical cut-offs (**Table 5**). Results did not change significantly, after adjusting for pre-pregnancy BMI, educational level and exercise.

Comparison of gut microbiota of GDM patient with normoglycemic pregnant women

In order to find differences between GDM+ and normoglycemic (GDM-) subjects, we compared our data to comparable subjects from the American Gut Project (AGP)

dataset. The microbiota α-diversity values were significantly lower in GDM+ when compared to GDM- subjects ($P < 0.001$) (**Figure 4**) Weighted Unifrac distance matrix showed a separation of the microbiota between GDM+ and GDM- (Anosim R = 0.14, P=0.049) (**Figure 5**). GDM+ displayed a higher abundance ($P<0.001$) of the Firmicutes phyla, and of *Collinsella*, *Dorea*, *Odoribacter* and *Ruminococcaceae* ($P<0.001$) than GDM- subjects, while Enterobacteriaceae abundance was increased in GDM- (**Figure 6**).

Infant gut microbiota and maternal dietary habits

As the mode of collection and/or storage of the offspring stool samples by 12 mothers resulted inappropriate, data of 29 infants (70.7%) could be analyzed only.

No clear separation of the infant gut microbiota was observed (anosim $P > 0.05$) according to the maternal compliance to dietary recommendations (**Figure 7**). The correlation plot between maternal nutrient intakes and infant gut microbiota showed a higher number of significant associations with maternal variables at enrolment than at the study-end (**Figure 8**).

Maternal oligosaccharides derived mainly from dairy products, cereals, fruit and vegetables, while saturated fatty acids (SFA) derived mainly from meat and cheese. In a multiple regression model, after adjusting for weight change, breastfeeding and Cesarean section, associations between infant *Ruminococcus* with maternal oligosaccharide (positive association) and with maternal intake of SFA (inverse association) were found, even if not reaching the defined cutoff of p-values. The inverse relationship between *Rikenellaceae* and maternal SFA intake remained statistically significant (**Table 6**). In the multivariate model, no significant association between infant gut microbiota and maternal dietary habits at the study-end was found.

Infant gut microbiota and breastfeeding

Ten out of the 29 (34.5%) newborns were breastfed by their mothers, while 19 (65.5%) were formula-fed, using formulas without added probiotic/prebiotic compounds.

We observed a non-significant reduction in microbial richness in breastfed infants when compared to infants fed with artificial milk.

The β–diversity calculation based on unweighted UniFrac distance matrices assessed by Principal coordinate analysis (PCoA) showed a separation of the infant microbiota only as a function of the type of feeding (breast *vs* formula) as confirmed by ANOSIM and ADONIS statistical test (P <0.001) (**Figure 9**).

At phylum level, we observed that breastfed infants showed a higher abundance of Actinobacteria and Proteobacteria, while in formula-fed infants we observed a higher proportion of Firmicutes phyla (**Figure 10**).

At genus level, breastfed infants displayed an increased abundance of *Escherichia* and *Bifidobacterium,* while formula-fed infants had a varied microbiota mainly composed of *Bacteroides*, *Clostridium*, *Enterococcaceae*, *Escherichia*, *Faecalibacterium*, *Staphylococcus* and *Streptococcus* (**Figure 10**).

In multiple regression analyses, a significant association between breastfeeding and the relative abundance of *Bifidobacterium* in the infant gut microbiota was found (β = 22.9; 95%CI = 10.1–35.7; P = 0.0017).

4.4 Discussion and conclusion

Maternal gut microbiota

Several associations between specific bacterial abundance and dietary variables were detected.

Overall, our patients consumed a low-fiber and high-fat diet, an unhealthy dietary pattern which has been associated with GDM [204]. Most of them (about 2/3) did not change substantially their dietary habits after having received nutritional recommendations and showed a worse metabolic and inflammatory pattern than the adherent women. Literature data reported a poor adherence to nutritional therapy in pregnant women [205]. Changing eating habits is difficult and it's probable that our patients had an unbalanced diet even before pregnancy that might have contributed to the onset of GDM. Despite the unhealthy dietary pattern of our women, the baseline energy intake and the weight gain in our sample are lower than expected and this might reflect the generic recommendation to control weight in pregnancy given by gynecologists.

As usual clinical practice, the participants received a single nutritional counseling with the dietician. Our results suggest that the current practice of providing general advices is not sufficient and there is a need for the development of more effective intervention strategies. Maybe, a structured approach that includes a personalized diet and a planned follow-up could increase the adherence to nutritional therapy.

We found few associations between nutrient intake and microbial abundance. Fat intake was associated with *Alistipes* among *Bacteroidetes*, while *Roseburia* and L-*Ruminococcus* among the Firmicutes appeared related, though not significantly, with nutrients related to vegetable foods (oligosaccharides, fiber). This observation is in agreement with DNA-based studies evaluating the fecal microbiota during pregnancy in

healthy overweight Finnish women at early pregnancy stage (17 week) [183] as well as in normal-weight Norwegian women during the second trimester of pregnancy [182]. On the opposite, we observed a positive association between protein intake and *Faecalibacterium* which is in disagreement with previous studies [182-183].

Research about gut microbiota composition and dietary intakes during pregnancy showed controversial results. Either no relationships between bacterial groups and dietary intakes [206] or associations between dietary fat and vitamin D with Proteobacteria increase [183] and higher gut microbiota richness and lower abundance of Bacteroidaceae with increased dietary fiber intake have been reported [206]. Those findings confirm the great heterogeneity of results on this topic and highlight difficulties in the comparison of results from the studies, probably due to the different dietary habits, the microbiota remodeling during pregnancy owing to hormonal changes, the additional insulin resistance determined by the presence of GDM.

Short-term changes in dietary pattern have been demonstrated to modulate quickly the microbiota composition. Rapid, but transient changes occur following dietary variations [131], although longer and persistent modifications are needed to shape the human gut microbiota. In addition, the effects of diet on gut microbiota, rather than being direct, are hypothesized to be the consequence of the weight change and the subsequent variation in white adipose tissue inflammation and insulin resistance [207].

We found a significantly lower α-diversity values in GDM+ patients when compared to GDM- women, as expected and according with literature data [169,177]. Low bacterial richness was associated with obesity and insulin resistance even outside of pregnancy [208]. During late gestation, the reduced insulin sensitivity is considered beneficial to support fetal growth and increased nutrient absorption, even if it is associated with metabolic impairment and inflammation. Women who developed GDM have greater

reduction in insulin sensitivity and their insulin secretion is not sufficient to maintain euglycemia, leading to glucose intolerance [209].

We found a higher abundance of the Firmicutes phyla in GDM+ subject when compared to GDM- subjects, in particular with respects to specific taxa (*Collinsella*, *Dorea* and *Ruminococcaceae*). Firmicutes harvest more energy from the diet [210] and it can be hypothesized that those enriched function could be related with the progressive weight gain and could be a feature in hyperglycemic phenomena.

Infant gut microbiota

We observed that the infant gut microbiota composition is influenced by nutritional maternal habits. The offspring relative abundance of *Ruminococcus* was directly associated with the maternal intake of oligosaccharides and inversely with the maternal intake of SFA, as previously reported in infants from healthy women [182]. The *Ruminococcus* genus produces both butyrate and a bacteriocin, ruminococcin A, able to inhibit the growth of the potentially harmful *Clostridium* species, therefore potentially playing a beneficial role for the newborns [211]. Furthermore, maternal SFA intake was inversely associated with the relative abundance of *Rikenellaceae*, a butyrate-producers family [212] which has been associated with favorable metabolic outcomes and a healthy gut [213]. These associations are remarkable and could explain the previously reported adverse impact of SFA on maternal [214] and neonatal health [215-216].

Nutritional recommendations were given to our women between 24-28 weeks of gestational age, at the time of the oral glucose challenge test and GDM diagnosis. Only one third of our participants resulted adherents to the nutritional advice; indeed, dietary adherence and nutritional habits at the end of pregnancy did not influence the offspring microbiota. Contrarily to a few previous human studies showing that the infant gut microbiota was associated with the last-trimester maternal diet [217], we found stronger

associations between infant gut bacteria composition and early maternal nutrition. Even if the mechanisms by which the maternal diet affects the offspring microbiota remain unclear, the nutritional habits of late pregnancy might have a lower impact, owing to the lower maternal-fetal time of contact and seeding possibility in a period when fetal growth is already advanced. This hypothesis needs to be confirmed by further studies. Our results indeed highlight the importance of a proper maternal nutrition, low in SFA and with a high content of fiber and prebiotic oligosaccharides, starting from early pregnancy and probably even before gestation, in order to favorably modulate the offspring microbiota.

A clear separation of infant microbiota according to the type of feeding (breast *vs* bottle-milk) was evident, as previously reported [218]. In particular, our bottle-fed infants showed increased microbial complexity and levels of strict anaerobes and facultative anaerobes, and higher abundance of Firmicutes, such as *Clostridium, Streptococcus* and *Staphylococcus*, in line with literature [219-220]. On the other hand, our breastfed infants showed a less complex microbiota with higher abundance of Proteobacteria and Actinobacteria, being the latter mainly derived from the *Bifidobacterium* genus that resulted to be strongly associated with breastfeeding, accordingly with most studies [221]. The higher abundance of *Bifidobacterium* in breast-fed infants can be explained by the presence of human milk oligosaccharides (HMOs), sugar polymers with prebiotic effects that promote the growth of specific microbial communities, including *Bifidobacterium spp.* with beneficial protective effects on the infant health [222-223].

Limitations of the study

Several limitations of the present study should be recognized. One limitation of is the small sample size; nevertheless, the power of our study to detect differences in alpha diversity was 0.84 with α=0.01. We could analyze the fecal samples of 73% of the offspring of the initially enrolled women. Therefore, we could have not been allowed to detect modest differences in bacterial composition. However, the post-hoc power estimated on the measured effect sizes was 0.80 with α=0.05.

The fecal samples were used as proxies for the microbial content of the entire gastrointestinal tract; it is reasonable to consider that mouth and skin microbiota could vary too.

Infant fecal samples were collected within the first week of life. We cannot exclude that the difference of a few days could have influenced the results due to the high instability of the infant microbiota.

The limitations of the food questionnaires must be recognized, even if these were widely used. An underestimation of energy intake of approximately 15% was observed in previous studies [224] and that could partially explain the low baseline energy intake in our sample.

Most of our patients had a very low fiber intake and consumed a high-fat diet; indeed, the dietary intakes of our patients resembled those of other pregnant women [225], and this finding is in line with the well-known associations between GDM and unhealthy diet [204].

Owing to the observational design of this study, the presence of unmeasured confounding factors could not be excluded.

Conclusions

We detected a different microbiota shift based on the adherence to dietary recommendation and some associations between nutrients intake and the abundance of specific OUTs. Moreover, we found that maternal eating habits of the first part of pregnancy have a greater influence on fetal microbiota than those of last trimester.

Collectively, these findings suggest that the development of nutritional interventions to modulate the gut microbiota, already starting from early stage of pregnancy, might be a potential new strategy in order to impact on maternal and infant metabolic health.

Table 3. Characteristics of the participants at enrolment and the study end

	At enrolment	Study end	P*
Number	41	41	
Age	37.1±4.2		
Pre-pregnancy weight (kg)	69.3±14.6		
Pre-pregnancy BMI (kg/m^2)	25.8±5.9		
Education (%)			
Primary school	17.1		
Secondary school	41.5		
University degree	41.5		
METS (h/week)	27.0 (36.4)	27.0 (26.5)	0.74**
Weight (kg)	75.8±12.9	79.0±13.3	<0.001
BMI (kg/m^2)	28.2±5.3	29.4±5.4	<0.001
Fasting glucose (mg/dL)	97.9±19.2	96.6±19.1	0.57
Fasting insulin (µU/mL)	10.1 (8.4)	11.6 (10.0)	0.02**
HOMA-IR (mmol/L*µU/mL)	2.3 (1.9)	2.8 (2.7)	0.15**
CRP (mg/L)	4.1 (4.2)	4.5 (7.5)	0.007**
Dietary intakes			
Energy (kcal)	1605.8±254.4	1766.1±306.7	0.009
Carbohydrates (% total kcal)	44.4±6.6	43.1±6.4	0.27
Sugars (% total kcal)	8.8±4.7	6.2±4.5	0.008
Sugars (g/day)	35.3±20.1	27.9±21.3	0.08
Oligosaccharides (g/day)	36.7±19.7	54.2±23.2	<0.001
Starch (g/day)	107.3±28.9	109.7±38.7	0.73
Fiber (g/day)	14.5±4.2	15.1±5.3	0.48
Proteins (% total kcal)	15.6±2.3	16.6±5.3	0.22
Total fats (% total kcal)	42.2±5.2	42.3±6.3	0.89
SFA (% total kcal)	11.3±2.2	11.1±2.7	0.65
PUFA (%kcal)	4.9±1.7	4.4±1.1	0.09

BMI=body mass index, METS=metabolic equivalent of activity, BP=blood pressure, HOMA-IR=Homeostasis Model Assessment-Insulin Resistance, CRP=C-reactive protein, SFA=saturated fatty acids, PUFA=polyunsaturated fatty acids.. Values are expressed as mean±standard deviation or median (interquartile range) *paired-sample *t*-test, **Wilcoxon matched pairs test

Table 4. Characteristics of the participants by adherence to the lifestyle recommendations and median changes (deltas) from enrolment (right)

	Baseline			Study End			Delta		
	Adherent	**Not adherent**	**P**	**Adherent**	**Not adherent**	**P**	**Adherent**	**Not adherent**	**P***
Number	14	27		14	27		14	27	
Age	35.5±3.8	38.0±4.3	0.08						
Pre-pregnancy weight (kg)	73.1±18.0	67.4±12.5	0.24						
Pre-pregnancy BMI (kg/m^2)	28.0±8.0	24.7±4.3	0.09						
Education (%)									
Secondary school	42.9	40.7							
University degree	42.9	40.7	0.94						
METS (h/week)	32.3 (37.0)	24.5 (38.0)	0.39*	27.9 (31.5)	23.3 (32.3)	0.08*	0.0	0.0	0.17
Weight (kg)	78.6±16.7	74.3±10.4	0.31	80.9±17.0	77.9±11.2	0.50	+2.0	+3.0	0.10
BMI (kg/m^2)	30.1±7.4	27.2±3.6	0.10	30.9±7.4	28.6±3.8	0.18	+0.7	+1.2	0.10
Fasting glucose (mg/dL)	99.8±29.3	96.9±11.4	0.65	88.9±25.3	100.6±13.8	0.06	-6.0	+1.0	<0.001
Fasting insulin (µU/mL)	11.3 (11.3)	9.0 (6.1)	0.83*	11.4 (10.8)	11.6 (12.4)	0.66*	-0.20	+2.0	0.003
HOMA-IR (mmol/L*µU/mL)	2.7 (2.8)	2.1 (1.3)	0.19*	2.4 (3.0)	3.1 (2.5)	0.38*	-0.45	+0.47	<0.001
CRP (mg/L)	3.2 (5.2)	4.3 (4.4)	0.76*	3.2 (3.1)	8.4 (8.3)	0.008*	-0.02	+2.5	0.003

	Baseline			Study End			Delta		
	Adherent	**Not adherent**	**P**	**Adherent**	**Not adherent**	**P**	**Adherent**	**Not adherent**	**P***
Dietary intakes									
Energy (kcal)	1659.2±309.6	1578.1±222.0	0.34	1828.6±200.7	1733.6±348.5	0.35	+161.5	+88.0	0.44
Carbohydrates (% total kcal)	44.2±5.0	44.5±7.3	0.90	43.0±5.1	43.2±7.1	0.92	-2.0	-2.0	0.74
Sugars (% total kcal)	9.7±3.7	8.3±5.1	0.36	3.9±2.2	7.5±5.0	0.015	-6.9	-1.5	0.005
Sugars (g/day)	40.7±18.4	32.5±20.8	0.22	17.5±9.7	33.4±23.7	0.02	-20.2	-7.6	0.004
Oligosaccharides (g/day)	39.7±19.8	35.2±19.9	0.50	66.7±22.6	47.8±21.1	0.01	+14.7	+13.8	0.41
Starch (g/day)	104.3±24.3	108.8±31.3	0.64	112.9±44.9	108.1±35.9	0.71	+23.1	-13.8	0.33
Fiber (g/day)	15.2±5.4	14.2±3.5	0.43	20.5±2.1	12.4±4.2	<0.001	+5.8	-0.94	<0.001
Proteins (% total kcal)	15.9±1.6	15.4±2.6	0.48	19.4±6.4	15.2±4.1	0.016	+1.9	-0.1	0.26
Total fats (% total kcal)	41.7±4.2	42.4±5.7	0.67	39.7±5.4	43.7±6.4	0.06	+1.4	+1.1	0.39
SFA (% total kcal)	11.6±2.4	11.2±2.2	0.60	9.7±1.6	11.8±2.9	0.017	-3.0	+0.6	0.03
PUFA (%kcal)	5.2±2.6	4.7±1.1	0.32	5.0±1.1	4.0±0.9	0.003	+0.1	-0.5	0.07

BMI=body mass index, METS=metabolic equivalent of activity, BP=blood pressure, HOMA-IR=Homeostasis Model Assessment-Insulin Resistance, CRP=C-reactive protein, SFA=saturated fatty acids, PUFA=polyunsaturated fatty acids. Values are expressed as mean±standard deviation or median (interquartile range) *paired-sample *t*-test, **Wilcoxon matched pairs test.

Table 5. Statistically significant associations between maternal gut microbiota composition at the study end and dietary variables in a multiple regression model.

		Rho	Beta	95% CI	P
Dietary intakes*					
Oligosaccharides (g/day)					
	L-Ruminococcus	0.37	0.11	0.02	<0.001
	Parabacteroides	-0.48	-0.02	0.008	0.005
Fiber (g/day)					
	Roseburia	0.37	0.46	0.15	0.004
Proteins (% total kcal)					
	Faecalibacterium	0.32	0.81	0.18	<0.001
PUFA %kcal					
	Roseburia	0.32	2.02	0.73	0.008

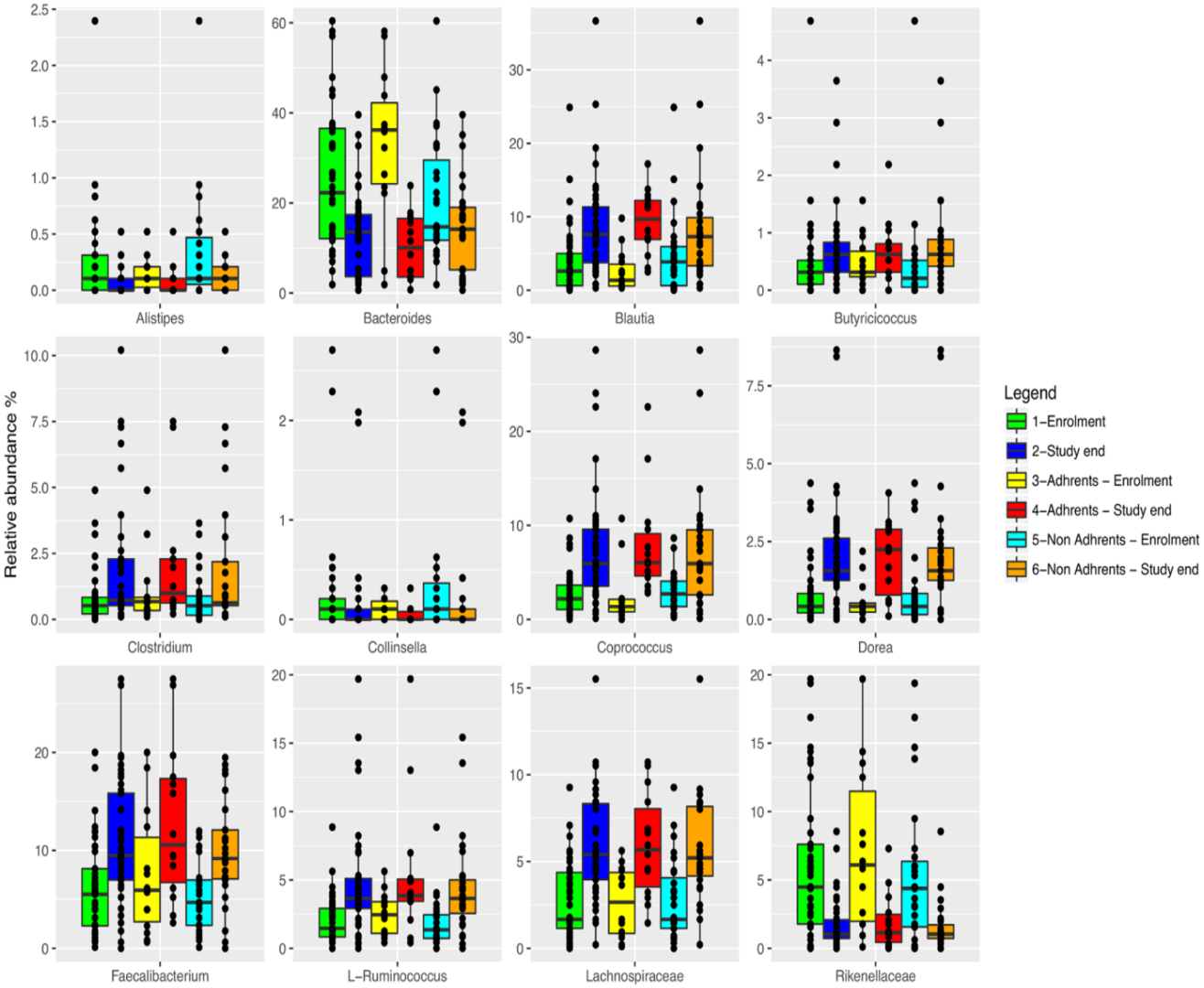
*Multiple regression model evaluating the association between bacteria (dependent variable) and the specific nutrient (independent variable), after adjusting for age, weight change

Table 6. Statistically significant associations between infant gut microbiota composition and maternal dietary habits by Spearman's correlations (left) and multiple regression analyses (right).

		Rho	Beta	95% CI	*P*
Dietary habits at enrolment					
Oligosaccharides					
	Ruminococcus	0.55	0.07	0.04 0.11	<0.001
Saturated fatty acids (%kcal)					
	Rikenellaceae	-0.61	-0.26	-0.39 -0.14	<0.001
	Ruminococcus	-0.44	-0.86	-1.31 -0.41	0.001

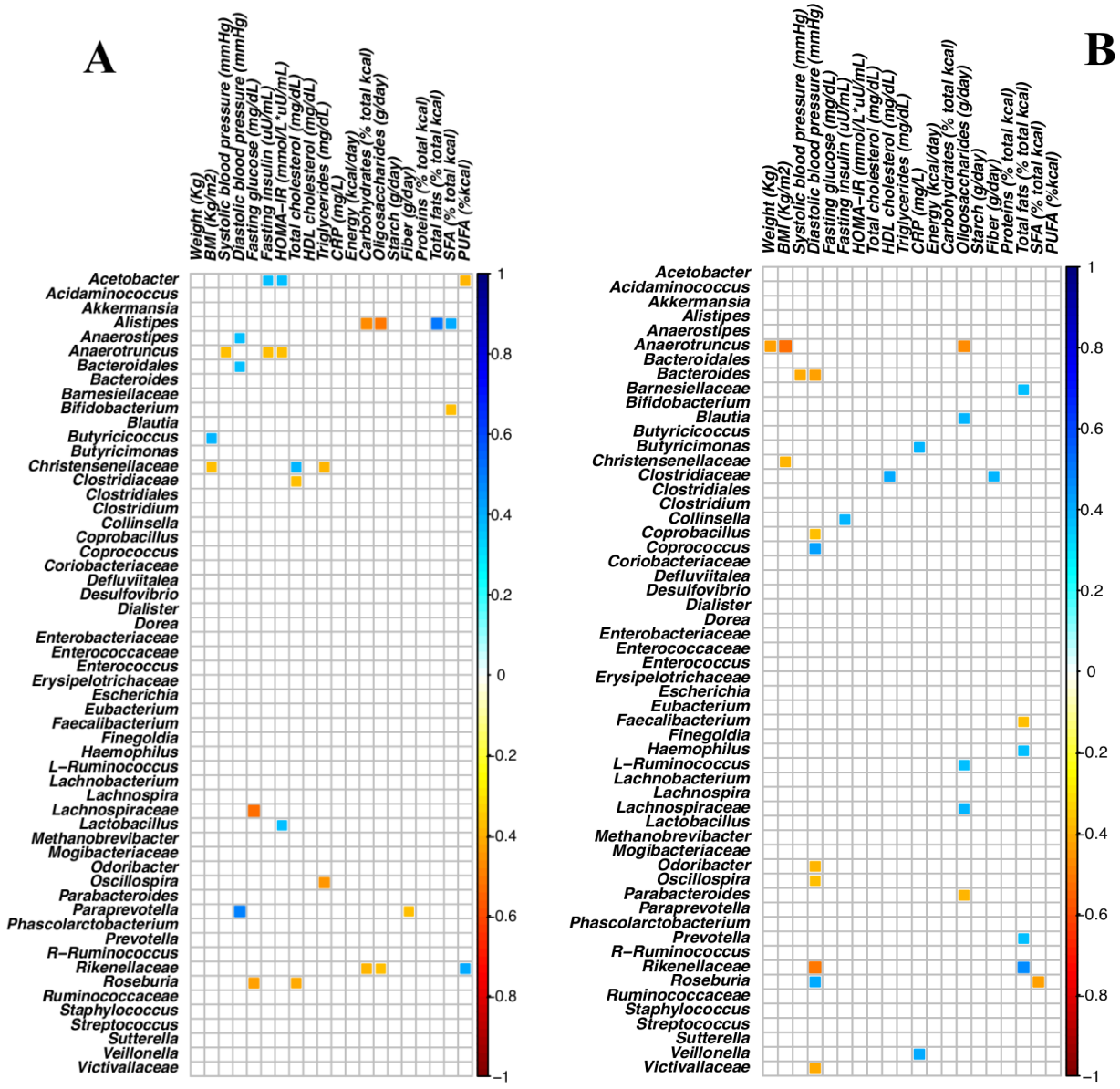
Model adjusted for maternal weight change and Cesarean section.

Figure 2. Differences in relative abundance of OTUs between adherents and not adherents to dietary recommendation at enrolment and at study end



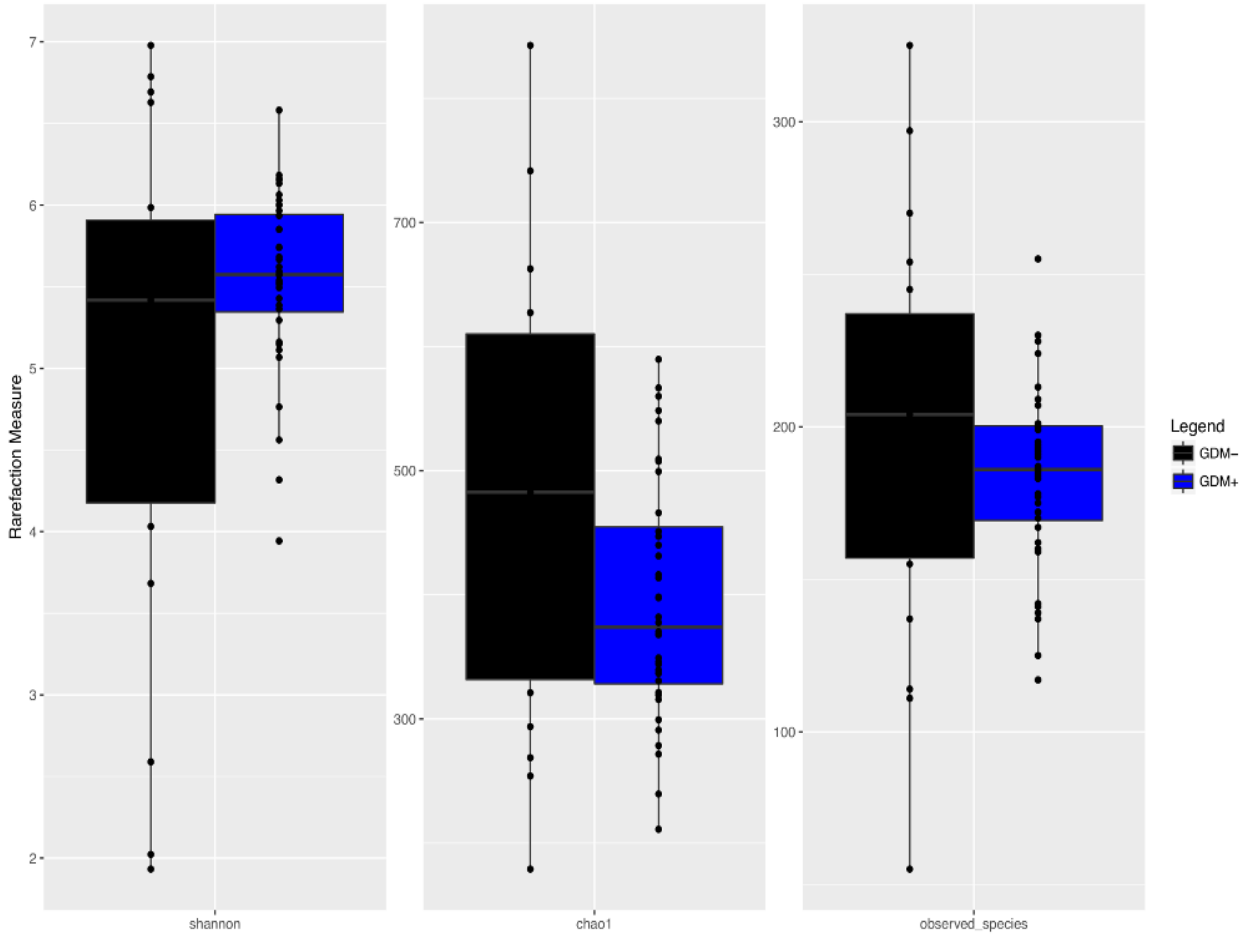
Boxplots showing the relative abundance at genus or family level of the OTUs based on Wilcoxon matched pairs test ($P \leq 0.002$) in fecal samples between: GDM patients at enrolment (green bars) and at the study end (blue bars); adherents to the dietary recommendations at enrolment (yellow bars) and at the study end (red bars); non-adherents to the dietary recommendations at enrolment (cyan bars) and at the study end (orange bars).

Figure 3. Spearman's rank correlation matrix of OTUs, dietary information and blood variables.



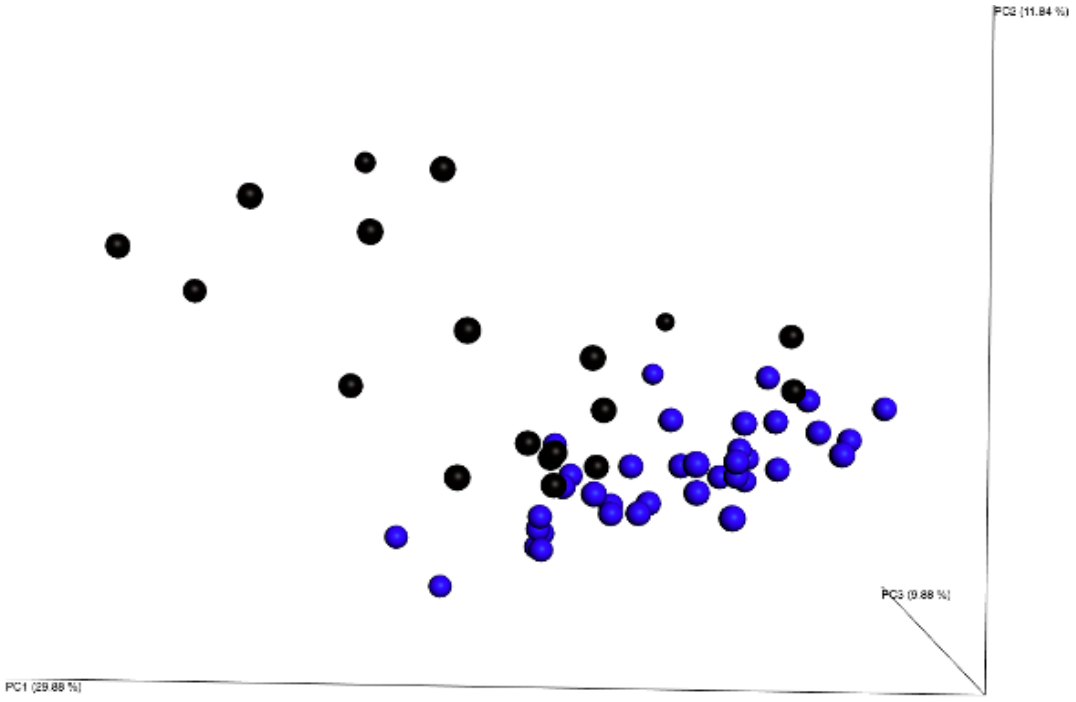
The colors of the scale bar denote the nature of the correlation, strong positive correlation in dark blue and perfectly negative correlation in dark red. Only significant correlations (P <0.01) are shown.

Figure 4. Boxplots to describe α-diversity measures of fecal microbiota of GDM+ patients and GDM-



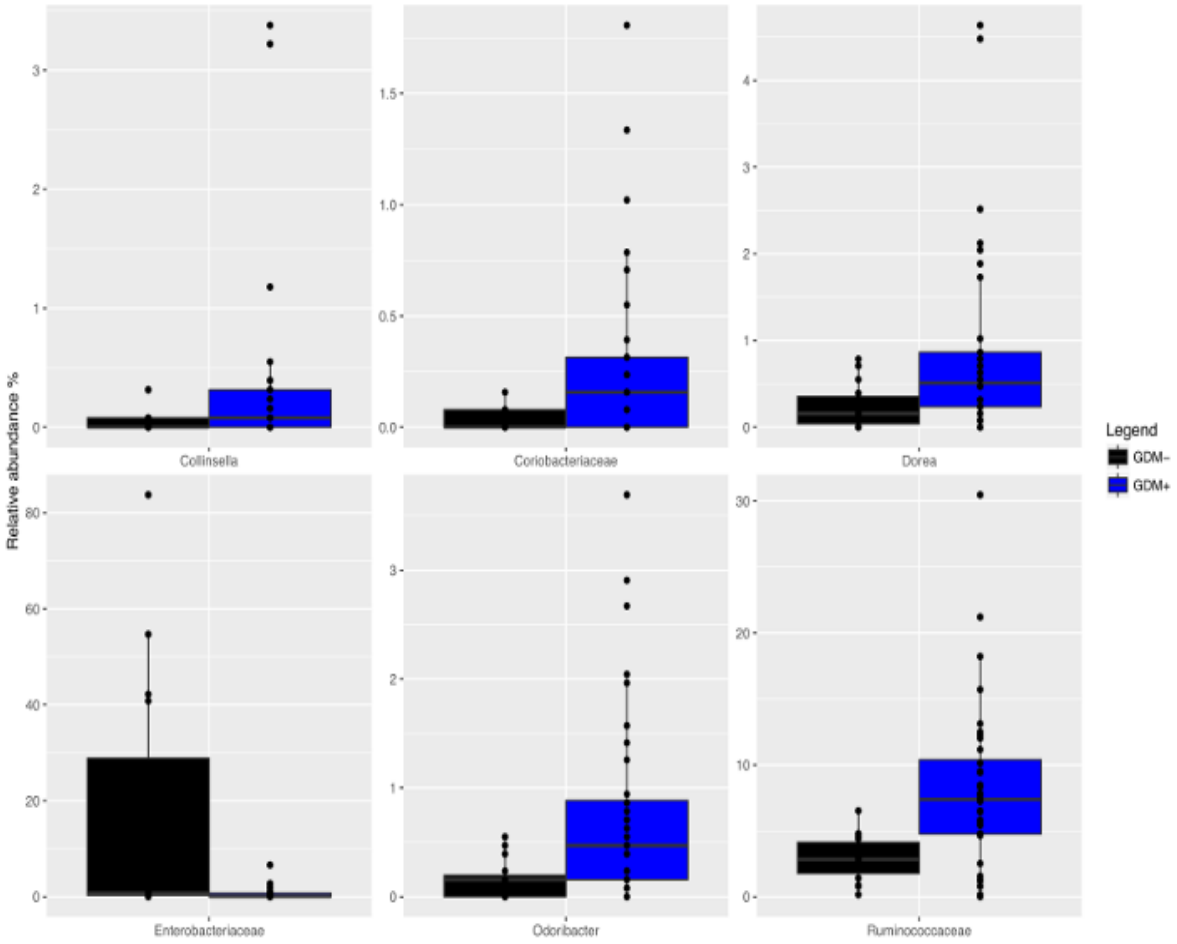
Individual points and brackets represent the richness estimate and the theoretical standard error range, respectively.

Figure 5. Principal coordinates analysis of weighted UniFrac distances for 16S rRNA gene sequence data.



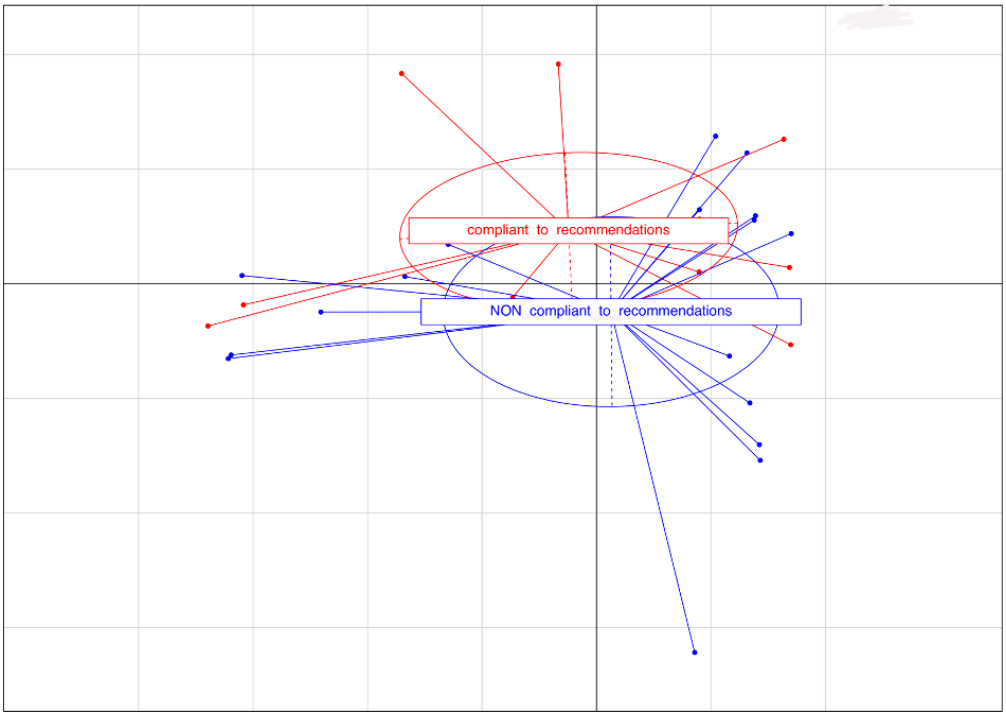
GDM+ patients blue dots; GDM- patients black dots.

Figure 6. Boxplots of differentially abundant OTUs in fecal samples between GDM+ patients and GDM- women



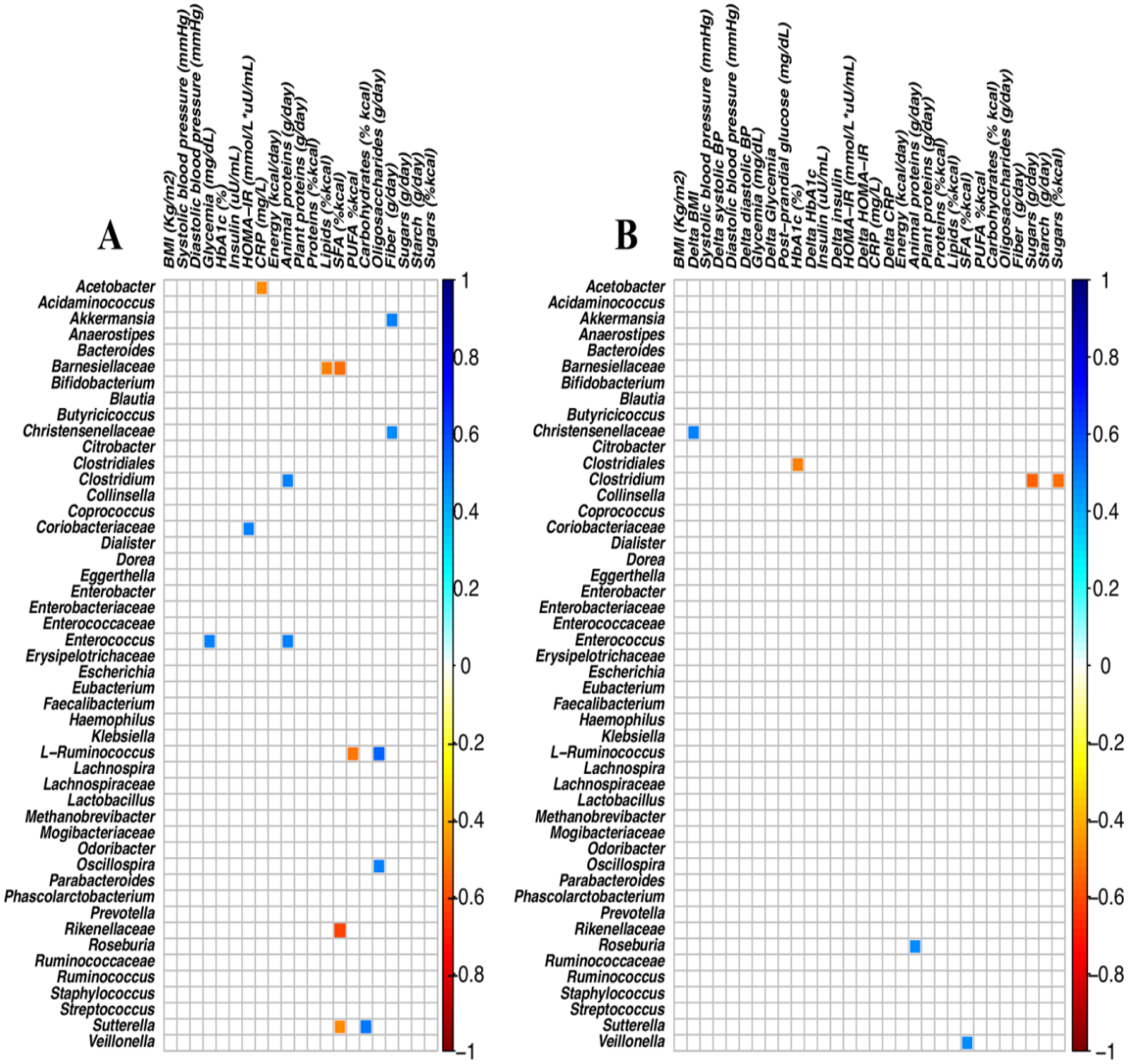
OTUs abundance is obtained from OTU table summarized at the higher taxonomy resolution and testing procedure based on Mann–Whitney tests

Figure 7. Principal Component Analysis (PCA) based on OTUs relative abundance of infant gut microbiota according to the maternal compliance to dietary recommendations.



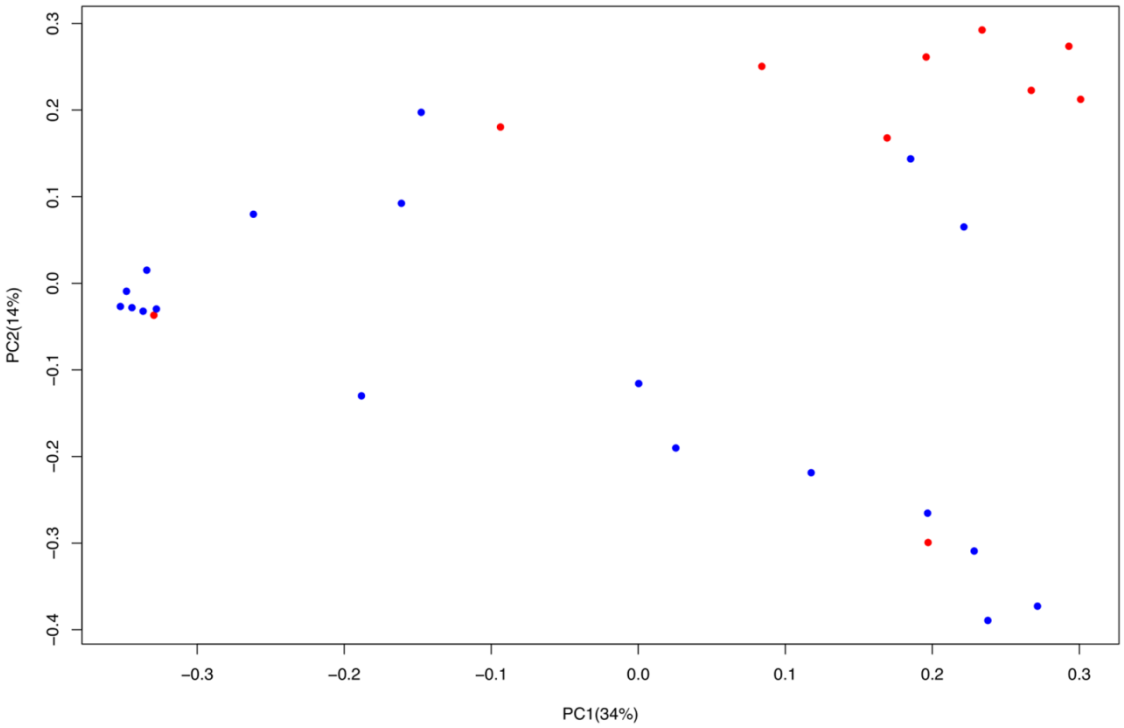
Samples are color-coded according to compliance to dietary recommendations (red) or non-compliance to dietary recommendations (blue).

Figure 8. Spearman's rank correlation of OTUs of offspring's samples and maternal dietary information and blood variables.



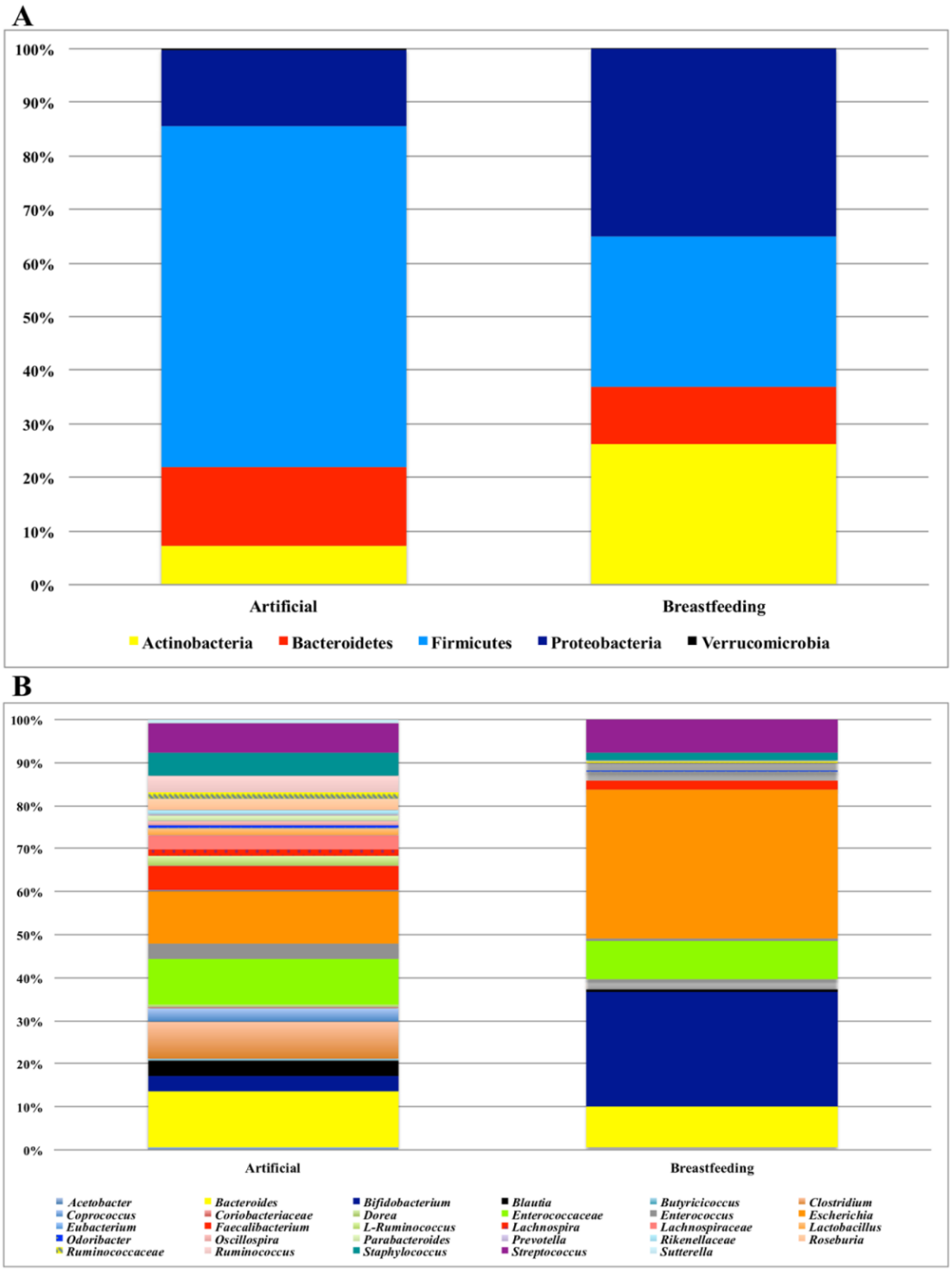
Spearman's rank correlation matrix of OTUs with > 0.2% abundance in at least 10 fecal samples, dietary information and blood variables. The colors of the scale bar denote the nature of the correlation, with 1 indicating a perfectly positive correlation (dark blue) and -1 indicating a perfectly negative correlation (dark red) between the two datasets. Only correlations with P-values <0.002 are shown. Data at enrolment (plot A) or at study-end (Plot B).

Figure 9. Principal coordinates analysis (PCoA) of unweighted UniFrac distances matrix for 16S rRNA gene sequence data.



Infant artificially-fed (blue dots) or breast-fed (red dots).

Figure 10. Relative phylum-level abundance profiles between breast-fed and artificially fed infants



Phylum-level abundance profiles of breastfed infants and artificially fed infants (Panel A); relative genus-level abundance of breastfed infants and artificially fed infants (Panel B).

References

1 Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. Diabetes Care 1998; 21(Suppl 2): B161–67.

2 Kaaja R, Rönnemaa T. Gestational diabetes: pathogenesis and consequences to mother and offspring. Rev Diabet Stud. 2008; 5(4): 194–202.

3 O'Sullivan J, Mahan C. Criteria for the oral glucose tolerance test in pregnancy. Diabetes 1964; 13: 278–285.

4 Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: World Health Organization; 1999.

5 Hoffman L, Nolan C, Wilson JD, Oats JJ, Simmons D. Gestational diabetes mellitus—management guidelines. The Australasian Diabetes in Pregnancy Society. Med J Aust 1998; 169(2): 93–97.

6 HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: associations with neonatal anthropometrics. Diabetes. 2009; 58(2): 453–459.

7 International Association of Diabetes and Pregnancy Study Groups Consensus Panel. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33: 676–682.

8 Chiefari E., Arcidiacono B., Foti D., Brunetti A. Gestational diabetes mellitus: An updated overview. J Endocrinol Investig 2017; 40: 899–909.

9 International Diabetes Federation . IDF Diabetes Atlas. 8th ed. IDF; Brussels, Belgium: 2017.

10 American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2011;34(Suppl 1):S62–S69.

11 Agarwal MM, Dhatt GS, Shah SM. Gestational Diabetes Mellitus Simplifying the International Association of Diabetes and Pregnancy diagnostic algorithm using fasting plasma glucose. Diabetes Care 2010; 33: 2018–2020.

12 Moses RG. Gestational diabetes mellitus: implications of an increased frequency with IADPSG criteria. Diabetes Care 2012; 35: 461–462.

13 Alfadhli EM. Gestational diabetes mellitus. Saudi Med J 2015; 36(4): 399–406.

14 Di Cianni G, Volpe L, Lencioni C, Miccoli R, Cuccuru I, Ghio A, et al. Prevalence and risk factors for gestational diabetes assessed by universal screening. Diabetes Res Clin Pract. 2003; 62(2): 131-137.

15 Lapolla A, Mazzon S, Marini S, Burattin G, Grella P, Fedele D. A screening programme for gestational diabetes in a north Mediterranean area. Diab Nutr Metab 1995; 8:33.

16 Lacaria E, Lencioni C, Russo L, Romano M, Lemmi P, Battini L, et al. Selective screening for GDM in Italy: application and effectiveness of National Guidelines. J Matern Fetal Neonatal Med 2015; 28(15): 1842-1844.

17 Durnwald C. Gestational diabetes: Linking epidemiology, excessive gestational weight gain, adverse pregnancy outcomes, and future metabolic syndrome. Semin Perinatol 2015; 39: 254–258.

18 Zhang C, Tobias DK, Chavarro JE, Bao W, Wang D, Ley SH, Hu FB. Adherence to healthy lifestyle and risk of gestational diabetes mellitus: prospective cohort study. BMJ 2014; 349: g5450.

19 Jenum AK, Mørkrid K, Sletner L, Vange S, Torper JL, Nakstad B, et al. Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: A population-based cohort study. Eur J Endocrinol 2012; 166: 317–324.

20 Anghebem-Oliveira MI, Martins BR, Alberton D, de Ramos EAS, Picheth G, de Rego FGM. Type 2 diabetes-associated genetic variants of FTO, LEPR, PPARg, and TCF7L2 in gestational diabetes in a Brazilian population. Arch Endocrinol 2017; 61: 238–248.

21 Lao TT, Ho LF, Chan BCP, Leung WC. Maternal Age and Prevalence of Gestational Diabetes Mellitus. Diabetes Care 2006; 29: 948–949.

22 Pettitt DJ, Jovanovic L. Low Birth Weight as a Risk Factor for Gestational Diabetes, Diabetes, and Impaired Glucose Tolerance During Pregnancy. Diabetes Care 2007; 30: S147–S149.

23 Levy A, Wiznitzer A, Holcberg G, Mazor M, Sheiner E. Family history of diabetes mellitus as an independent risk factor for macrosomia and cesarean delivery. J Matern Fetal Neonatal Med 2010; 23: 148–152.

24 Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabet Med 2004; 21: 103–113.

25 Bowers K, Tobias DK, Yeung E, Hu FB, Zhang C. A prospective study of prepregnancy dietary fat intake and risk of gestational diabetes. Am J Clin Nutr 2012; 95: 446–453.

26 Zhang C, Schulze MB, Solomon CG, Hu FB. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. Diabetologia 2006; 49: 2604–2613.

27 Taschereau-Charron A, Da Silva MS, Bilodeau JF, Morisset AS, Julien P, Rudkowska I. Alterations of fatty acid profiles in gestational diabetes and influence of the diet. Maturitas 2017; 99: 98–104.

28 Zhang C, Liu S, Solomon CG, Hu FB. Dietary Fiber Intake, Dietary Glycemic Load, and the Risk for Gestational Diabetes Mellitus. Diabetes Care 2006; 29: 2223–2230.

29 Bao W, Bowers K, Tobias DK, Olsen SF, Chavarro J, Vaag A, Kiely M, Zhang C. Prepregnancy low-carbohydrate dietary pattern and risk of gestational diabetes mellitus: A prospective cohort study. Am J Clin Nutr 2014; 99: 1378–1384.

30 Sivan E, Boden G. Free fatty acids, insulin resistance, and pregnancy. Curr Diabetes Rep 2003; 3: 319–322.

31 Fung TT, McCullough ML, Newby PK, Manson JE, Meigs JB, Rifai N, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005; 82: 163–173.

32 Zhang C. Gestational Diabetes during and after Pregnancy. Springer; London, UK: 2010. Risk Factors for Gestational Diabetes: From an Epidemiological Standpoint; pp. 71–81.

33 Lijinsky W. N-Nitroso compounds in the diet. Mutat Res 1999; 443: 129–138.

34 Pang WW, Colega M, Cai S, Chan YH, Padmapriya N, Chen LW, et al. Higher Maternal Dietary Protein Intake Is Associated with a Higher Risk of Gestational Diabetes Mellitus in a Multiethnic Asian Cohort J Nutr 2017; 147: 653–660.

35 Bao W, Bowers K, Tobias DK, Hu FB, Zhang C. Prepregnancy Dietary Protein Intake, Major Dietary Protein Sources, and the Risk of Gestational Diabetes Mellitus. Diabetes Care 2013; 36: 2001–2008.

36 Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr 2007; 27: 293–310.

37 Hezaveh ZS, Feizy Z, Dehghani F, Sarbakhsh P, Moini A, Vafa M. The Association between Maternal Dietary Protein Intake and Risk of Gestational Diabetes Mellitus. Int J Prev Med 2019; 10: 197.

38 Lee AJ, Hiscock RJ, Wein P, Walker SP, Permezel M. Gestational diabetes mellitus: clinical predictors and long-term risk of developing type 2 diabetes: a retrospective cohort study using survival analysis. Diabetes Care 2007; 30: 878–883.

39 Albareda M, Caballero A, Badell G, Piquer S, Ortiz A, de Leiva A, Corcoy R. Diabetes and abnormal glucose tolerance in women with previous gestational diabetes. Diabetes Care 2003; 26:1199–1205.

40 Lauenborg J, Hansen T, Jensen DM, Vestergaard H, Mølsted-Pedersen L, Hornnes P, et al. Increasing incidence of diabetes after gestational diabetes: a long-term follow-up in a Danish population. Diabetes Care 2004; 27:1194–9.

41 Bao W, Yeung E, Tobias DK, Hu FB, Vaag AA, Chavarro JE, et al. Long-term risk of type 2 diabetes mellitus in relation to BMI and weight change among women with a history of gestational diabetes mellitus: a prospective cohort study. Diabetologia 2015; 58: 1212–1219.

42 Peters RK, Kjos SL, Xiang A, Buchanan TA. Long-term diabetogenic effect of single pregnancy in women with previous gestational diabetes mellitus. Lancet Lon. Engl 1996; 347: 227–230.

43 Shostrom DCV, Sun Y, Oleson JJ, Snetselaar LG, Bao W. History of Gestational Diabetes Mellitus in Relation to Cardiovascular Disease and Cardiovascular Risk Factors in US Women. Front Endocrinol 2017; 8: 144.

44 Schwartz R, Gruppuso PA, Petzold K, Brambilla D, Hiilesmaa V, Teramo KA. Hyperinsulinemia and macrosomia in the fetus of the diabetic mother. Diabetes Care 1994; 17: 640–648.

45 Fetita LS, Sobngwi E, Serradas P, Calvo F, Gautier JF. Consequences of Fetal Exposure to Maternal Diabetes in Offspring. J Clin Endocrinol 2006; 91: 3718–3724.

46 Esakoff TF, Cheng YW, Sparks TN, Caughey AB. The association between birthweight 4000 g or greater and perinatal outcomes in patients with and without gestational diabetes mellitus. Am J Obstet Gynecol 2009; 200: 672.e1–672.e4.

47 Tam WH, Ma RCW, Ozaki R, Li AM, Chan MHM, Yuen LY, et al. In Utero Exposure to Maternal Hyperglycemia Increases Childhood Cardiometabolic Risk in Offspring. Diabetes Care 2017; 40: 679–686.

48 Petitt DJ, Bennett PH, Knowler WC, Baird HR, Aleck KA. Gestational diabetes mellitus and impaired glucose tolerance during pregnancy. Long-term effects on obesity and glucose tolerance in the offspring. Diabetes 1985; 34(2):119–122.

49 Lee SC, Pu YB, Chow CC, Yeung VT, Ko GT, So WY, et al. Diabetes in Hong Kong Chinese: Evidence for familial clustering and parental effects. Diabetes Care 2000; 23: 1365–1368.

50 Jovanovic-Peterson L, Peterson CM, Reed GF, Metzger BE, Mills JL, Knopp RH, et al. Maternal postprandial glucose levels and infant birth weight: the Diabetes in Early Pregnancy Study. The National Institute of Child Health and Human Development-Diabetes in Early Pregnancy Study. Am J Obstet Gynecol 1991; 164: 103–111.

51 de Veciana M, Major CA, Morgan MA, Asrat T, Toohey JS, Lien JM, et al. Postprandial versus preprandial blood glucose monitoring in women with gestational diabetes mellitus requiring insulin therapy. N Engl J Med 1995; 333: 1237–1241.

52 American Diabetes Association. Management of diabetes in pregnancy: Standards of medical care in diabetes-2018. Diabetes Care, 41 (suppl 1) (2018), pp. S137-S143

53 Jovanovic L. Role of diet and insulin treatment of diabetes in pregnancy. Clin Obstet Gynecol 2000; 43:46–55.

54 American Diabetes Association. Bantle JP, Wylie-Rosett J, Albright AL, Apovian CM, Clark NG, et al. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. Diabetes Care 2008; 31:S61–S78.

55 Cheng YW, Chung JH, Kurbisch-Block I, Inturrisi M, Shafer S, Caughey AB. Gestational weight gain and gestational diabetes mellitus: perinatal outcomes. Obstet Gynecol 2008; 112: 1015–1022.

56 Rasmussen KM, Catalano PM, Yaktine AL. New guidelines for weight gain during pregnancy: what obstetrician/gynecologists should know. Curr Opin Obstet Gynecol 2009; 21:521–526.

57 Han S, Middleton P, Shepherd E, Van Ryswyk E, Crowther CA. Different types of dietary advice for women with gestational diabetes mellitus. Cochrane Database Syst Rev. 2017; 2:CD009275.

58 Moreno-Castilla C, Mauricio D, Hernandez M. Role of Medical Nutrition Therapy in the Management of Gestational Diabetes Mellitus. Curr Diab Rep 2016; 16: 22.

59 Tsirou E. Guidelines for Medical Nutrition Therapy in Gestational Diabetes Mellitus: Systematic Review and Critical Appraisal 2018. J Acad Nutr Diet 2019; 119: 1320-1339.

60 Committee on Practice Bulletins–Obstetrics. ACOG Practice Bulletin No. 190: Gestational diabetes mellitus Obstet Gynecol, 131 (2) (2018), pp. e49-e64.

61 Duarte-Gardea MO, Gonzales-Pacheco DM, Reader DM, et al. Academy of Nutrition and Dietetics gestational diabetes evidence-based nutrition practice guideline. J Acad Nutr Diet 2018; 118(9): 1719-1742.

62 Blumer I, Hadar E, Hadden DR, Jovanovič L, Mestman JH, Murad MH, Yogev Y. Diabetes and pregnancy: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2013; 98 (11): 4227-4249.

63 Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, et al. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. Int J Gynecol Obstet 2015; 131: S173.

64 Italian Standards for Diabetes Mellitus, Italian Association of Diabetologists, Diabete Italia, and Italian Society of Diabetology, Torino, Italy (2007).

65 IDF Clinical Guidelines Task Force. Global Guideline on Pregnancy and Diabetes. Brussels: International Diabetes Federation 2009.

66 Jovanovic-Peterson L, Peterson CM. Dietary manipulation as a primary treatment strategy for pregnancies complicated by diabetes. J Am Coll Nutr. 1990; 9(4): 320–325.

67 Hernandez TL, Anderson MA, Chartier-Logan C, Friedman JE, Barbour LA. Strategies in the Nutritional Management of Gestational Diabetes. Clin Obstet Gynecol 2013; 56(4): 803–815.

68 American Diabetes Association. Nutritional management. Medical management of pregnancy complicated by diabetes. 2nd ed.. Virginia: American Diabetes Association, Inc.; 1995.

69 Major CA, Henry MJ, de Veciana M, Morgan MA. The effects of carbohydrate restriction in patients with diet-controlled gestational diabetes. Obstet Gynecol 1998;91:600–604.

70 Romon M, Nuttens MC, Vambergue A, Vérier-Mine O, Biausque S, Lemaire C, et al. Higher carbohydrate intake is associated with decreased incidence of newborn macrosomia in women with gestational diabetes. J Am Diet Assoc 2001; 101(8): 897–902.

71 Cypryk K, Kaminska P, Kosinski M, Pertynska-Marczewska M, Lewinski A. A comparison of the effectiveness, tolerability and safety of high and low carbohydrate diets in women with gestational diabetes. Endokrynol Pol 2007; 58(4): 314–319.

72 American Diabetes Association. Standards of Medical Care in Diabetes—2011. Diabetes Care 2011; 34(1):S11–S61.

73 Moreno-Castilla C, Hernandez M, Bergua M, Alvarez MC, Arce MA, Rodriguez K, et al. Low-Carbohydrate Diet for the Treatment of Gestational Diabetes Mellitus: A randomized controlled trial. Diabetes Care 2013; 36(8): 2233-2238.

74 Trout KK, Homko CJ, Wetzel-Effinger L, Mulla W, Mora R, McGrath J, Basel-Brown L, et al. Macronutrient Composition or Social Determinants? Impact on Infant Outcomes With Gestational Diabetes Mellitus. Diabetes Spectrum : A Publication of the American Diabetes Association 2016; 29(2): 71-78.

75 Sears B, Perry M. The role of fatty acids in insulin resistance. Lipids in Health and Disease. 2015; 14: 121.

76 Cortez M, Carmo LS, Rogero MM, Borelli P, Fock RA. A high-fat diet increases IL-1, IL-6, and TNF-α production by increasing NF-κB and attenuating PPAR-γ expression in bone marrow mesenchymal stem cells. Inflammation 2013; 36(2): 379-86.

77 Pendyala S, Walker JM, Holt PR. A High-Fat Diet Is Associated With Endotoxemia That Originates From the Gut. Gastroenterology 2012; 142(5): 1100-1101.e2.

78 Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, et al. Increased oxidative stress precedes the onset of high-fat diet induced insulin resistance and obesity. Metabolism 2008; 57(8): 1071-1077.

79 Lain KY, Catalano P. Metabolic changes in pregnancy. Clinical Obstet Gynecol 2007:938–48.

80 Diderholm B, Stridsberg M, Ewald U, Lindeberg-Nordén S, Gustafsson J. Increased lipolysis in non-obese pregnant women studied in the third trimester. BJOG-Int J Obstet Gy 2005; 112: 713-718.

81 Sivan E, Homko CJ, Whittaker PG, Reece EA, Chen X, Boden G. Free Fatty Acids and Insulin Resistance during Pregnancy. J Clin Endocr Metab 1998; 83: 2338–2342.

82 Barrett HL, Dekker NM, McIntyre HD, Callaway LK. Normalizing metabolism in diabetic pregnancy: is it time to target lipids? Diabetes Care 2014; 37: 1484–93.

83 Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, Herrera E. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care 2008; 31: 1858–1863.

84 Olmos PR, Rigotti A, Busso D, Berkowitz L, Santos JL, Borzone GR, et al. Maternal hypertriglyceridemia: a link between maternal overweight-obesity and macrosomia in gestational diabetes. Obesity 2014; 22(10): 2156-2163.

85 Hernandez TL, Anderson MA, Chartier-Logan C, Friedman JE, Barbour LA. Strategies in the Nutritional Management of Gestational Diabetes. Clin Obstet Gynecol 2013; 56(4): 803–815.

86 Reaven GM. Effects of differences in amount and kind of dietary carbohydrate on plasma glucose and insulin responses in man. Am J Clin Nutr 1979;32(12):2568-78.

87 Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981; 34:362–366.

88 Grant SM, Wolever TM, O'Connor DL, Nisenbaum R, Josse RG. Effect of a low glycaemic index diet on blood glucose in women with gestational hyperglycaemia. Diabetes Res Clin Pract 2011; 91(1): 15–22.

89 Moses RG, Barker M, Winter M, Petocz P, Brand-Miller JC. Can a low-glycemic index diet reduce the need for insulin in gestational diabetes mellitus? A randomized trial. Diabetes Care 2009; 32(6): 996–1000.

90 Perichart-Perera O, Balas-Nakash M, Rodríguez-Cano A, Legorreta-Legorreta J, Parra-Covarrubias A, Vadillo-Ortega F. Low glycemic index carbohydrates versus all types of carbohydrates for treating diabetes in pregnancy: a randomized clinical trial to evaluate the effect of glycemic control. Int J Endocrinol 2012; 2012: 296017.

91 Ma W, Huang Z, Huang B, Qi B, Zhang Y, Xiao B, et al. Intensive low-glycaemic-load dietary intervention for the management of glycaemia and serum lipids among women with gestational diabetes: A randomized control trial. Public Health Nutr 2015;18(8): 1506-1513.

92 Grant SM, Wolever TM, O'Connor DL, Nisenbaum R, Josse RG. Effect of a low glycaemic index diet on blood glucose in women with gestational hyperglycaemia. Diabetes Res Clin Pract 2011; 91(1): 15–22.

93 Louie JCY, Markovic TP, Perera N, Foote D, Petocz P, Ross GP, et al. Investigating the effects of a low-glycemic index diet on pregnancy outcomes in gestational diabetes mellitus. Diabetes Care 2011; 34: 2341–2346.

94 Perichart-Perera O, Balas-Nakash M, Rodríguez-Cano A, Legorreta-Legorreta J, Parra-Covarrubias A, Vadillo-Ortega F. Low glycemic index carbohydrates versus all types of carbohydrates for treating diabetes in pregnancy: a randomized clinical trial to evaluate the effect of glycemic control. Int J Endocrinol 2012; 2012: 296017.

95 Zhang R, Han S, Li ZN, Silva-Zolezzi I, Parés GV, et al. Effects of low-glycemic-index diets in pregnancy on maternal and newborn outcomes in pregnant women: a meta-analysis of randomized controlled trials. Eur J Nutr 2018; 57: 167–177.

96 Silva FM, Kramer CK, de Almeida JC, Steemburgo T, Gross JL, Azevedo MJ. Fiber intake and glycemic control in patients with type 2 diabetes mellitus: a systematic review with meta-analysis of randomized controlled trials. Nutr Rev 2013; 71: 790–801.

97 Hernandez TL, van Pelt RE, Anderson MA, Daniels LJ, West NA, Donahoo WT, et al. A higher-complex carbohydrate diet in gestational diabetes mellitus achieves glucose targets and lowers postprandial lipids: a randomized crossover study. Diabetes Care 2014; 37:1254–1262.

98 Hernandez TL, van Pelt RE, Anderson MA, Reece MS, Reynolds RM, de la Houssaye BA, et al. Women with gestational diabetes randomized to a higher-complex carbohydrate/low-fat diet manifest lower adipose tissue insulin resistance, inflammation, glucose, and free fatty acids: a pilot study. Diabetes Care 2016; 39: 39–42.

99 Hernandez TL. Nutrition therapy in gestational diabetes: the case for complex carbohydrates. Diabetes Spectrum 2016; 29(2): 82-88.

100 Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med. 2018; 378(25): e34.

101 Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Fito M, Chiva-Blanch G, et al. Effect of a high-fat Mediterranean diet on bodyweight and waist circumference: a prespecified secondary outcomes analysis of the PREDIMED randomised controlled trial. Lancet Diabetes Endocrinol. 2016;4(8): 666–676.

102 Assaf-Balut C, Garcia de la Torre N, Duran A, Fuentes M, Bordiu E, Del Valle L, et al. A Mediterranean diet with additional extra virgin olive oil and pistachios reduces the incidence of gestational diabetes mellitus (GDM): a randomized controlled trial: the St. Carlos GDM prevention study. PLoS One 2017; 12(10): e0185873.

103 Assaf-Balut C, Garcia de la Torre N, Duran A, Fuentes M, Bordiu E, Del Valle L, et al. Medical nutrition therapy for gestational diabetes mellitus based on Mediterranean diet principles: a subanalysis of the St Carlos GDM Prevention Study. BMJ Open Diabetes Res Care 2018;6(1):e000550.

104 Kunz C, Kuntz S, Rudloff S. Intestinal flora. Adv Exp Med Biol 2009; 639: 67–79.

105 Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J 2017; 474: 1823–1836.

106 Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ 2018; 361: 36–44.

107 Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2019; 7: 14.

108 Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 2012; 9: 577–589.

109 Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. Physiol Rev 2010; 90: 859–904.

110 Dieterich W, Schink M, Zopf Y. Microbiota in the Gastrointestinal Tract. Med Sci 2018; 6: 116.

111 Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: Mom matters. Trends Mol Med 2015; 21: 109–117.

112 Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA 2010; 107: 11971–11975.

113 Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006; 118: 511–521.

114 Turpin W, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, et al. Association of host genome with intestinal microbial composition in a large healthy cohort. Nat Genet 2016; 48: 1413–1417.

115 Bonder MJ, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, et al. The effect of host genetics on the gut microbiome. Nat Genet 2016; 48: 1407–1412.

116 David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505: 559–563.

117 Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature 2018; 555: 623–628.

118 Gilbert JA, Stephens B. Microbiology of the built environment. Nat Rev Microbiol 2018; 16: 661–670.

119 Ardissone AN, de la Cruz DM, Davis-Richardson AG, Rechcigl KT, Li N, Drew JC, et al. Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One. 2014; 9: e90784.

120 Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. Pediatrics 2012; 129(5): 950–960.

121 Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA. 2010; 107(26): 11971–11975.

122 Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. Cesarean Delivery May Affect the Early Biodiversity of Intestinal Bacteria. J Nutr 2008; 138: 1796S–1800S.

123 Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut 2014; 63: 559–566.

124 Martin R, Makino H, Yavuz AC, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLoS ONE 2016; 11: e0158498.

125 Stewart CJ, Embleton ND, Clements E, Luna PN, Smith DP, Fofanova TY, et al. Cesarean or vaginal birth does not impact the longitudinal development of the gut microbiome in a cohort of exclusively preterm infants. Front Microbiol 2017; 8: 1008.

126 Arboleya S, Binetti A, Salazar N, Fernández N, Solís G, Hernández-Barranco A, et al. Establishment and development of intestinal microbiota in preterm neonates. FEMS Microbiol Ecol 2012; 79: 763–772.

127 Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the Infant Microbiome Community Structure and Function Across Multiple Body Sites and in Relation to Mode of Delivery. Nat Med 2017; 23(3): 314–326.

128 Chong CYL, Bloomfield FH, O'Sullivan JM. Factors Affecting Gastrointestinal Microbiome Development in Neonates Nutrients 2018; 10; 274.

129 Le Huerou-Luron I, Blat S, Boudry G. Breast- v. formula- feeding: impacts on the digestive tract and immediate and long-term health effects. Nutr Res Rev 2010; 23(1): 23–36.

130 Fallani M, Amarri S, Uusijarvi A, Adam R, Khanna S, Aguilera M, et al. Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. Microbiology 2011; 157(5): 1385–1392.

131 Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. Pharmacol Res 2013; 69: 52–60.

132 Cummings JH, Englyst HN. What is dietary fibre? Trends Food Sci Tech 1991; 2: 99–103.

133 Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews 2001; 81: 1031–1064.

134 Keim NL, Martin RJ. Dietary whole grain–microbiota interactions: insights into mechanisms for human health. Adv Nutr 2014; 5: 556–557.

135 Brinkworth GD, Noakes M, Clifton PM, Bird AR. Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations. Brit J of Nutr 2009; 101: 1493–502.

136 Cummings JH. Carbohydrate and protein digestion: the substrate available for fermentation. In: The large intestine in nutrition and disease. Brussels, Belgium: Danone Institute; 1997. p. 15–42.

137 Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. J Appl Bact 1991; 70: 443–459.

138 Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med 2017; 15: 73.

139 Meijnikman AS, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. Endocrine Reviews 2018; 39: 133–153.

140 Sircana A, Framarin L, Leone N, Berrutti M, Castellino F, Parente R, et al. Altered Gut Microbiota in Type 2 Diabetes: Just a Coincidence? Curr Diab Rep 2018; 18: 98.

141 Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 2011; 94(1): 58–65.

142 den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 2013; 54(9): 2325-2340.

143 Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015; 11: 577–591.

144 Canfora EE, Blaak EE. Acetate: a diet-derived key metabolite in energy metabolism: good or bad in context of obesity and glucose homeostasis? Curr Opin Clin Nutr Metab Care 2017; 20(6): 477-483.

145 Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, Petersen KF, et al. Acetate mediates a microbiome–brain– b-cell axis to promote metabolic syndrome. Nature 2016; 534: 213–217.

146 Kim YA, Keogh JB, Clifton PM. Probiotics, prebiotics, synbiotics and insulin sensitivity. Nutr Res Rev 2018; 31: 35–51.

147 De Silva A, Bloom SR. Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity. Gut Liver 2012; 6: 10–20.

148 Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. J Nutr Biochem 2011; 22: 849–855.

149 Liu T, Li J, Liu Y, Xiao N, Suo H, Xie K, et al. Short-chain fatty acids suppress lipopolysaccharide-induced production of nitric oxide and proinflammatory cytokines through inhibition of NF-κB pathway in RAW264.7 cells. Inflammation 2012; 35: 1676–1684.

150 He M, Shi B. Gut microbiota as a potential target of metabolic syndrome: the role of probiotics and prebiotics. Cell Biosci 2017; 7: 54.

151 Hoyles L, Jiménez-Pranteda ML, Chilloux J, Brial F, Myridakis A, Aranias T, et al. Metabolic retroconversion of trimethylamine N-oxide and the gut microbiota. Microbiome 2018: 6(1): 73.

152 Brial F, Le Lay A, Dumas ME, Gauguier D. Implication of gut microbiota metabolites in cardiovascular and metabolic diseases. Cell Mol Life Sci 2018; 75(21): 3977-3990.

153 Li P, Zhong C, Li S, Sun T, Huang H, Chen X, et al. Plasma concentration of trimethylamine-N-oxide and risk of gestational diabetes mellitus. Am J Clin Nutr 2018; 108(3): 603-610.

154 Gao X, Liu X, Xu J, Xue C, Xue Y, Wang YJ. Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. J Biosci Bioeng 2014; 118(4): 476-481.

155 Rath S, Heidrich B, Pieper DH, Vital M. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. Microbiome 2017; 5(1): 54.

156 Ponzo V, Fedele D, Goitre I, Leone F, Lezo A, Monzeglio C, et al. Diet-Gut Microbiota Interactions and Gestational Diabetes Mellitus (GDM). Nutrients. 2019;11(2):E330.

157 Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012; 150: 470–80.

158 Liu J, Yang H, Yin Z, Jiang X, Zhong H, Qiu D, et al. Remodeling of the gut microbiota and structural shifts in preeclampsia patients in South China. Eur J Clin Microbiol Infect Dis 2017; 36: 713–719.

159 Crusell M, Hansen TH, Nielsen T, Allin KH, Rühlemann MC, Damm P, et al. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. Microbiome 2018; 6: 89.

160 Gohir W, Whelan FJ, Surette MG, Moore C, Schertzer JD, Sloboda DM. Pregnancy-related changes in the maternal gut microbiota are dependent upon the mother's periconceptional diet. Gut Microbes 2015; 6: 310–320.

161 DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci USA 2015; 112: 11060–11065.

162 Santacruz A, García-Valdés L, Segura MT, Martín-Lagos JA, Anjos T, Martí-Romero M, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 2010; 104: 82–92.

163 Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr 2008; 88: 894–899.

164 Stanislavski MA, Dabelea D, Wagner BD, Sontag MK, Lozupone CA, Eggesbø M. Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. Microbiome 2017; 5: 113.

165 Gomez-Arango LF, Barrett HL, McIntyre HD, Callaway LK, Morrison M, Dekker Nitert M; SPRING Trial Group. Connections between the gut microbiome and metabolic hormones in early pregnancy in overweight and obese women. Diabetes 2016; 65: 2214–2223.

166 Wang J, Zheng JM, Shi W, Xu X, Zhang Y, Ji P, et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. Gut 2018; 67: 1614–1625.

167 Serino M, Fernández-Real JM, García-Fuentes E, Queipo-Ortuño M, Moreno-Navarrete JM, Sánchez A, et al. The gut microbiota profile is associated with insulin action in humans. ActaDiabetol 2013; 50: 753-761.

168 Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 2005; 102: 11070–11075.

169 Wu Y, Bible PW, Long S, Ming WK, Ding W, Long Y, et al. Acta Diabetol 2019 doi: 10.1007/s00592-019-01434-2.

170 Xu Y, Zhang M, Zhang J, Sun Z, Ran L, Ban Y, et al. Differential Intestinal and Oral Microbiota Features Associated with Gestational Diabetes and Maternal Inflammation. Am J Physiol Endocrinol Met 2019. doi: 10.1152/ajpendo.00266.2019.

171 Kuang YS, Lu JH, Li SH, Li JH, Yuan MY, He JR et al. Connections between the human gut microbiome and gestational diabetes mellitus. Gigascience 2017; 6: 1–12.

172 Cortez RV, Taddei CR, Sparvoli LG, Angelo AGS, Padilha M, Mattar R, Daher S. Microbiome and its relation to gestational diabetes. Endocrine 2018; 64(2): 254-264.

173 Sircana A, Framarin L, Leone N, Berrutti M, Castellino F, Parente R, et al. Altered Gut Microbiota in Type 2 Diabetes: Just a Coincidence? Curr Diab Rep 2018; 18: 98.

174 Mokkala K, Houttu N, Vahlberg T, Munukka E, Rönnemaa T, Laitinen K. Gut microbiota aberrations precede diagnosis of gestational diabetes mellitus. Acta Diabetol 2017; 54: 1147–1149.

175 Fugmann M, Breier M, Rottenkolber M, Banning F, Ferrari U, Sacco V, et al. The stool microbiota of insulin resistant women with recent gestational diabetes, a high-risk group for type 2 diabetes. Sci Rep 2015; 5: 13212.

176 Hasan S, Aho V, Pereira P, Paulin L, Koivusalo SB, Auvinen P, Eriksson JG. Gut microbiome in gestational diabetes: A cross-sectional study of mothers and offspring 5 years postpartum. Acta Obstet Gynecol Scand 2018; 97: 38–46.

177 Hu J, Nomura Y, Bashir A, Fernandez-Hernandez H, Itzkowitz S, et al. Diversified Microbiota of Meconium Is Affected by Maternal Diabetes Status. PLoS ONE 2013; 8(11): e78257.

178 Su M, Nie Y, Shao R, Duan S, Jiang Y, Wang M, et al. Diversified gut microbiota in newborns of mothers with gestational diabetes mellitus. PLoS ONE 2018; 13(10): e0205695.

179 Röytiö H, Mokkala K, Vahlberg T, Laitinen K. Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women. Br J Nutr 2018; 120: 599–600.

180 Gomez-Arango LF, Barrett HL, Wilkinson SA, Callaway LK, McIntyre HD, Morrison M, et al. Low dietary fiber intake increases Collinsella abundance in the gut microbiota of overweight and obese pregnant women. Gut Microbes 2018; 9: 189–201.

181 Barrett HL, Gomez-Arango LF, Wilkinson SA, McIntyre HD, Callaway LK, Morrison M, et al. A Vegetarian Diet Is a Major Determinant of Gut Microbiota Composition in Early Pregnancy. Nutrients 2018; 10: 890.

182 Mandal S, Godfrey KM, McDonald D, Treuren WV, Bjørnholt JV, Midtvedt T, et al. Fat and vitamin intakes during pregnancy have stronger relations with a pro-inflammatory maternal microbiota than does carbohydrate intake. Microbiome 2016; 4: 55.

183 Mokkala K, Röytiö H, Munukka E, Pietilä S, Ekblad U, Rönnemaa T, et al. Gut Microbiota Richness and Composition and Dietary Intake of Overweight PregnantWomen Are Related to Serum Zonulin Concentration, a Marker for Intestinal Permeability. J Nutr 2016; 146: 1694–1700.

184 Lankelma JM, Nieuwdorp M, de Vos WM, Wiersinga WJ. The gut microbiota in internal medicine: implications for health and disease. Neth J Med 2015; 73(2): 61-68.

185 Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y, Sue A et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011; 334: 105–108.

186 Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, Morris MJ. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. PLoS One 2015; 10(5): e0126931.

187 Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, et al. Increase in Plasma Endotoxin Concentrations and the Expression of Toll-Like Receptors and Suppressor of Cytokine Signaling-3 in Mononuclear Cells After a High-Fat, High-Carbohydrate Meal: Implications for insulin resistance. Diabetes Care 2009; 32(12): 2281-2287.

188 Gohir W, Ratcliffe EM, Sloboda DM. Of the bugs that shape us: Maternal obesity, the gut microbiome, and long-term disease risk. Pediatr Re 2015; 77: 196–204.

189 Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev 2017; 81: e00036–17.

190 Soderborg TK, Clark SE, Mulligan CE, Janssen RC, Babcock L, Ir D, et al. The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD. Nat Commun 2018; 9: 4462.

191 Guzzardi MA, Ait Ali L, D'Aurizio R, Rizzo F, Saggese P, Sanguinetti E, Weisz A, et al. Fetal cardiac growth is associated with in utero gut colonization. Nutr Metab Cardiovasc Dis. 2019; 29: 170–176.

192 Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med. 2017; 23: 314–326.

193 Ferrocino I, Ponzo V, Gambino R, Zarovska A, Leone F, Monzeglio C, et al. Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM). Sci Rep 2018; 8: 122216.

194 Ponzo V, Ferrocino I, Zarovska A, Amenta MB, Leone F, Monzeglio C, et al. The microbiota composition of the offspring of patient with gestational diabetes mellitus (GDM). PLoS ONE 2019; 14(12): e0226545.

195 Taylor HL, Jacobs DR Jr, Schucker B, Knudsen J, Leon AS, Debacker G. et al. A questionnaire for the assessment of leisure time physical activities. J Chronic Dis 1978; 31: 741–755.

196 Carnovale E, Marletta P. Food Composition Table Milan, Istituto Nazionale della Nutrizione, EDRA, 1997.

197 Salvini S, Parpinel M, Gnagnarella P. Food composition database for epidemiological studies in Italy. European Oncology Institute, 1988 [in Italian]

198 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–336.

199 Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics 2011; 27: 863–864.

200 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011; 27: 2194–2200.

201Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010; 26: 2460–2461.

202 Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814–821.

203 Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics. 2009; 10: 161.

204 Brown J, Alwan NA, West J, Brown S, McKinlay CJ, Farrar D, Crowther CA. Lifestyle interventions for the treatment of women with gestational diabetes. Cochrane Database Syst Rev. 2017; 5: CD011970.

205 Caut C, Leach M, Steel A. Dietary guideline adherence during preconception and pregnancy: A systematic review. Matern Child Nutr. 2020; 16(2): e12916.

206 Portune KJ, Benítez-Páez A, Del Pulgar EMG, Cerrudo V, Sanz Y. Gut microbiota, diet, and obesity related disorders. The good, the bad, and the future challenges. Mol Nutr Food Res 2016; 252: 1–17.

207 Carrothers JM, York MA, Brooker SL, Lackey KA, Williams JE, Shafii B, et al. Fecal microbial community structure is stable over time and related to variation in macronutrient and micronutrient intakes in lactating women. J Nutr 2015; 145: 2379–2388.

208 Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013; 500: 541–546.

209 . Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, et al. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. Int. J. Gynecol. Obstet. 2015; 131: S173–211.

210 Cani PD. Gut microbiota and obesity: Lessons from the microbiome. Brief Funct Genomics 2013; 12: 381–387.

211 Coppa GV, Gabrielli O, Zampini L, Galeazzi T, Ficcadenti A, Padella L, et al. et al. Oligosaccharides in 4 different milk groups, Bifidobacteria, and Ruminococcus obeum. J Pediatr Gastroenterol Nutr 2011; 53: 80–87.

212 Su XL, Tian Q, Zhang J, Yuan XZ, Shi XS, Guo RB, et al. Acetobacteroides hydrogenigenes gen. nov., sp. nov., an anaerobic hydrogen-producing bacterium in the family Rikenellaceae isolated from a reed swamp. Int J Syst Evol Microbiol 2014; 64: 2986–2991.

213 Lippert K, Kedenko L, Antonielli L, Kedenko I, Gemeier C, Leitner M, et al. Gut microbiota dysbiosis associated with glucose metabolism disorders and the metabolic syndrome

in older adults. Benef Microbes 2017; 8: 545–556.

214 Bo S, Menato G, Lezo A, Signorile A, Bardelli C, De Michieli F, et al. Dietary fat and gestational hyperglycaemia. Diabetologia 2001; 44: 972–978.

215 Horan M K, Donnelly JM, McGowan CA, Gibney ER, McAuliffe FM. The association between maternal nutrition and lifestyle during pregnancy and 2-year-old offspring adiposity: analysis from the ROLO study. J. Public Health (Bangkok) 2016; 24: 427–436.

216 Murrin C, Shrivastava A, Kelleher CC. Maternal macronutrient intake during pregnancy and 5 years postpartum and associations with child weight status aged five. Eur J Clin Nutr 2013; 67: 670.

217 Chu DM, Antony KM, Ma J, Prince AL, Showalter L, Moller M, et al. The early infant gut microbiome varies in association with a maternal high-fat diet. Genome Med 2016; 8: 77.

218 Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, et al. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. JAMA Pediatr 2017; 171: 647–654.

219 Wang M, Li M, Wu S, Lebrilla CB, Chapkin RS, Ivanov I, et al. Fecal microbiota composition of breast-fed infants is correlated with human milk oligosaccharides consumed. J Pediatr Gastroenterol Nutr 2015; 60: 825–833.

220 Matsuyama M, Gomez-Arango LF, Fukuma NM, Morrison M, Davies PSW, Hill RJ. Breastfeeding: a key modulator of gut microbiota characteristics in late infancy. J Dev Orig Health Dis 2018; 1–8.

221 Yieh C, Chong L, Bloomfield FH. Factors Affecting Gastrointestinal Microbiome Development in Neonates. Nutrients 2018; 10: 274.

222 Makino H, Kushiro A, Ishikawa E, Kubota H, Gawad A, Sakai T, et al. Mother-to-infant transmission of intestinal bifidobacterial strains has an impact on the early development of vaginally delivered infant's microbiota. PLoS One 2013; 8: e78331.

223 Barile D, Rastall RA. Human milk and related oligosaccharides as prebiotics. Curr Opin

Biotechnol 2013; 24: 214–219.

224 Poslusna K, Ruprich J, de Vries JH, Jakubikova M, van't Veer P. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice Brit J Nutr 2009; 101(2): S73–S85.

225 Allin KH, Nielsen T, Pedersen O. Mechanisms in endocrinology: Gut microbiota in patients with type 2 diabetes mellitus. Eur J Endocrinol 2015; 172: R167–R177.

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