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Body composition and intervention with fish oil and probiotics

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To Sami and Sanni

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ABSTRACT

Every fifth pregnant woman is diagnosed with gestational diabetes in Finland. It predisposes both the mother and the child to several adverse short- and long-term consequences. The presence of obesity is one of the strongest risk factors for gestational diabetes. Nutritional factors mainly account for the obesity, and the ability to influence these factors could help to prevent gestational diabetes.

Previous studies suggest that fish oil and probiotic supplements could improve insulin sensitivity of the body by decreasing low-grade inflammation and thereby prevent gestational diabetes. The aim of this study was to determine whether these food supplements could improve glucose levels and reduce the risk of gestational diabetes in overweight and obese women. Further, the associations of diet, physical activity, and gestational diabetes with maternal body composition were evaluated.

In the trial, 439 overweight and obese pregnant women were randomized to consume probiotics and/or fish oil daily from 15 gestational weeks onwards. Weight, body composition, glucose and insulin concentrations were determined in early and in late pregnancy. Gestational diabetes was diagnosed with an oral glucose tolerance test. Information on diet and physical activity were collected.

As compared to placebo, intervention with fish oil and probiotics did not reduce the risk for gestational diabetes or affect weight or body composition. Three out of four women gained weight in excess of recommendations, which was related to increased fat mass accrual as compared to women with an ideal weight gain. Protein intake and the good quality of diet were positively correlated with fat free mass. Body composition in early pregnancy did not differ between healthy women and women developing gestational diabetes. Women with gestational diabetes gained weight and fat mass less than healthy women.

In conclusion, according to this study, supplementation with fish oil and/or probiotics does not prevent gestational diabetes or affect body fat mass in overweight and obese pregnant women. Body adiposity is reduced in women with gestational diabetes as compared with healthy overweight or obese women; adiposity increased in women with an excess weight gain in comparison with women with an ideal gestational weight gain.

KEYWORDS: adiposity, body composition, diet, fat mass, fish oil, gestational diabetes, obese, overweight, pregnancy, probiotics, supplement

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Synnytys- ja Naistentautioppi
OUTI PELLONPERÄ: Kehonkoostumus ja kalaöljy-/probiootti-interventio ylipainoisten naisten raskausdiabetesta määrittävinä tekijöinä
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TIIVISTELMÄ

Raskausdiabetes on raskaudenaikainen häiriö, joka todetaan Suomessa noin joka viidennellä raskaana olevalla naisella. Se lisää sekä äidin että lapsen sairastuvuutta. Lihavuus on raskausdiabeteksen tärkein riskitekijä, sillä se heikentää insuliinin vaikutusta kudoksissa. Aiempien tutkimusten perusteella kalaöljy- ja probiootti -ravintolisät saattavat parantaa kehon insuliiniherkkyyttä matala-asteista tulehdusta vähentämällä ja siten pienentää raskausdiabeteksen riskiä. Tämän väitöskirja-tutkimuksen tavoitteena oli selvittää, voidaanko näiden ravintolisien avulla ehkäistä raskausdiabetesta ja parantaa verensokeriarvoja, sekä kartoittaa, mitkä tekijät ovat yhteydessä raskaudenaikaiseen kehonkoostumukseen.

Tutkimuksessa 439 ylipainoista äitiä satunnaistettiin neljään ryhmään, jonka mukaisesti he käyttivät raskauden ajan päivittäin keskimäärin raskausviikolta 15 lähtien joko kalaöljyä, probiootteja, molempia tai lumevalmistetta. Äideiltä tutkittiin alku- ja loppuraskaudessa paino, kehonkoostumus, verensokeri- ja insuliinipitoisuudet ja kerättiin tiedot ravinnonsaannista. Raskausdiabeteksen ilmaantuvuus selvitettiin sokerirasituskokeella.

Äitien raskaudenaikainen kalaöljy- ja/tai probioottilisä ei vähentänyt raskausdiabeteksen puhkeamisen todennäköisyyttä tai vaikuttanut äitien kehonkoostumukseen lumevalmisteeseen verrattuna. Kolmella neljästä äidistä paino nousi raskausaikana suositukseen nähden liikaa, mikä oli yhteydessä rasvamassan lisääntymiseen. Proteiinin saanti ja hyvälaatuinen ruokavalio olivat positiivisesti yhteydessä kehon rasvattoman massan määrään. Alkuraskauden kehonkoostumus ei ennustanut raskausdiabeteksen puhkeamista ylipainoisilla ja lihavilla äideillä. Raskausdiabeetikkoäitien paino ja rasvamassa nousivat raskausaikana vähemmän kuin muiden äitien.

Kalaöljy- ja/tai probioottilisästä ei näyttäisi olevan hyötyä ylipainoisten ja lihavien äitien verensokeritasojen, painon tai kehonkoostumuksen hallinnassa. Raskausaikana kehonkoostumukseen yhteydessä olevia tekijöitä tämän tutkimuksen perusteella ovat tietyt ravintotekijät, raskausdiabetes sekä painonnousu.

AVAINSANAT: kalaöljy, kehonkoostumus, lihavuus, probiootti, raskaus, raskausdiabetes, rasvamassa, ravintolisä, ylipaino

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Abbreviations

ADP	air displacement plethysmography
BF%	body fat percentage
BMI	body mass index
DHA	docosahexaenoic acid
DXA	Dual-energy X-ray absorptiometry
EPA	eicosapentaenoic acid
FFM	fat free mass
FM	fat mass
GDM	gestational diabetes mellitus
GWG	gestational weight gain
HOMA-IR	homeostatic model assessment
IADPSG	International Association of Diabetes and Pregnancy Study Group
IOM	Institute of Medicine
LC-PUFA	long chain polyunsaturated fatty acid
MRI	magnetic resonance imaging
NFkB	nuclear factor kappa B
OGTT	oral glucose tolerance test
TBW	total body water
TGV	thoracic gas volume
TNF-α	tumor necrosis factor alpha

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Pellonperä O, Mokkala K, Houttu N, Vahlbeg T, Koivuniemi E, Tertti K, Rönnemaa T, Laitinen K. Efficacy of fish oil and/or probiotic intervention on the incidence of gestational diabetes mellitus in an at-risk group of overweight and obese women: A randomized, placebo-controlled, double-blind clinical trial. *Diabetes Care* 2019;42:1009–1017.

II Pellonperä, Vahlberg T, Mokkala K, Houttu N, Koivuniemi E, Tertti K, Rönnemaa T, Laitinen K. Body composition and gestational weight gain in women at risk of gestational diabetes: secondary analysis of a randomized, placebo controlled, double-blind clinical trial with probiotics and/or fish oil. *Submitted*.

III Pellonperä O, Koivuniemi E, Vahlberg T, Mokkala K, Tertti K, Rönnemaa T, Laitinen K. Dietary quality influences body composition in overweight and obese pregnant women. Clinical Nutrition 2019;38:1613–1619.

IV Pellonperä O, Koivuniemi E, Vahlberg T, Mokkala K, Tertti K, Rönnemaa T, Laitinen K. Body composition measurement by air displacement plethysmography in pregnancy: Comparison of predicted versus measured thoracic gas volume. Nutrition 2019;60:227–229.

1 Introduction

Gestational diabetes (GDM) is an increasingly common condition; around 14% of pregnancies are affected worldwide (Guariguata et al. 2014). In Finland in 2018, the incidence was 21% according to the statistics of the Finnish Institute of Health and Welfare. GDM predisposes both the mother and the child to several short-term and long-term complications. The mother is at increased risk of gestational hypertensive disorders and requiring a cesarean section, and in later life, the risk of type 2 diabetes is 7-fold higher than in women who have a normoglycemic pregnancy (Bellamy et al. 2009). The child is predisposed to macrosomia, hypoglycemia, and respiratory distress syndrome, and to the programming of obesity and diabetes i.e. fetal metabolic adaptation to the in-utero environment causing long-lasting consequences (Damm et al. 2016, Nicholas et al. 2016, Reece 2010).

In the light of this information and considering the 34 % higher costs of care related to GDM (Gillespie et al. 2013), prevention of GDM is of major importance. Interventions aimed at lowering the risk of GDM have typically focused on lifestyle changes. However, these interventions have yielded inconclusive results (Shepherd et al. 2017), emphasizing the need for new preventive approaches.

Insulin resistance plays a critical part in the pathogenesis of GDM (Catalano, P. M. 2010). Often the abnormally high insulin resistance is attributable to obesity (Buchanan and Xiang 2005). Pregnancy, obesity and GDM are all associated with an increase in inflammatory markers that contribute to insulin resistance, and thus a heightened inflammatory response has been proposed to play an important role in the development of GDM (Abell et al. 2015). Both probiotics and the n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFAs) present in fish oil have been demonstrated to possess anti-inflammatory properties and a capability to reduce insulin resistance (Calder 2017, Gomes et al. 2014, Lalia and Lanza 2016). Furthermore, there is previous experimental evidence indicating that a combination of these two active components might exert synergistic immunoregulatory effects (Kastel et al. 2007).

The increasing GDM prevalence is related to the growing prevalence of overweight and obesity, which enhance the risk of developing GDM by 2-10-fold (Chu et al. 2007, Gaillard et al. 2013). Approximately every third woman of

reproductive age in Europe and more than every second woman in the United States is overweight or obese (Ogden et al. 2014, Ovesen et al. 2011). Body mass index (BMI) is often used as a marker of adiposity. However, body composition reflects nutritional status and provides more precise information about the adiposity of the body than BMI (Lindsay, C. A. et al. 1997, Prentice and Jebb 2001). Further, it has been found that body composition and fat distribution correlate better with insulin sensitivity than BMI also in pregnant women (Gur et al. 2014, Sommer et al. 2014). However, little is known about the actual body composition of women diagnosed with GDM, or how body composition changes during pregnancy as compared to women without GDM.

Furthermore, the means to regulate body adiposity during pregnancy have been poorly studied, even if the degree of adiposity rather than mere weight gain is likely to be better associated with the onset of pregnancy complications. The consumption of n-3 LC-PUFA has been proposed to modestly reduce weight and body fat percentage (BF%) in non-pregnant individuals (Bender et al. 2014). Additionally, the previous literature suggests that also probiotics may help weight loss and fat mass loss in overweight adults, especially if provided as certain strains of Lactobacillus and Bifidobacterium (Borgeraas et al. 2018, Crovesy et al. 2017). The effects of these supplements on body composition during pregnancy are unknown.

The purpose of the studies in this thesis was to examine whether an intervention with n-3 LC-PUFA and/or probiotics could exert favorable effects on maternal glucose control and body composition. Furthermore, the relationship of lifestyle factors and the presence of GDM on maternal body composition was evaluated.

2 Review of the Literature

2.1 Gestational diabetes mellitus (GDM)

2.1.1 Glucose metabolism in uncomplicated pregnancy

During a normal pregnancy, hormone-regulated metabolic changes occur to ensure the adequate transfer of nutrients to the fetus. Pregnancy induces a progressive insulin resistance that begins in mid-pregnancy and increases gradually as pregnancy progresses (Catalano, P. M. et al. 1991, Catalano, P. M. et al. 1999). The placenta produces tumor necrosis factor α (TNF-α), placental lactogen, and growth hormone, which in combination with enhanced cortisol and progesterone levels are thought to result in increased insulin resistance (Catalano, P. M. 2010, Di Cianni et al. 2003). In order to maintain normoglycemia in the mother, hypertrophy and hyperplasia of pancreatic beta cells occur and the production of insulin increases (Baeyens et al. 2016). The enhanced maternal basal endogenous hepatic glucose production further augments glucose transport to the fetus through the placenta (Catalano, P. M. et al. 1992). By the end of the third trimester, insulin sensitivity is halved, hepatic glucose production increased by 30%, and insulin secretion by 200% (Catalano, P. M. et al. 1999).

2.1.2 Pathogenesis of GDM

Gestational diabetes is defined as hyperglycemia during pregnancy that is not overt diabetes (American Diabetes Association 2018). Women unable to adapt to the normal pregnancy-induced physiological changes, develop GDM. The pathogenesis of GDM consists of two main factors: high insulin resistance and a decreased ability of pancreatic beta cells to produce enough insulin (Buchanan 2001, Catalano, P. M. 2010) (Figure 1). In women with GDM, β-cell dysfunction and increased insulin resistance are present already before pregnancy, although insulin secretion is still sufficient to maintain normoglycemia (Buchanan et al. 2007, Catalano, P. M. et al. 1993). During pregnancy, hyperglycemia emerges when pancreatic β-cells become too exhausted to produce enough insulin to overcome the increasing insulin resistance caused by placental hormones and the possible excessive gestational

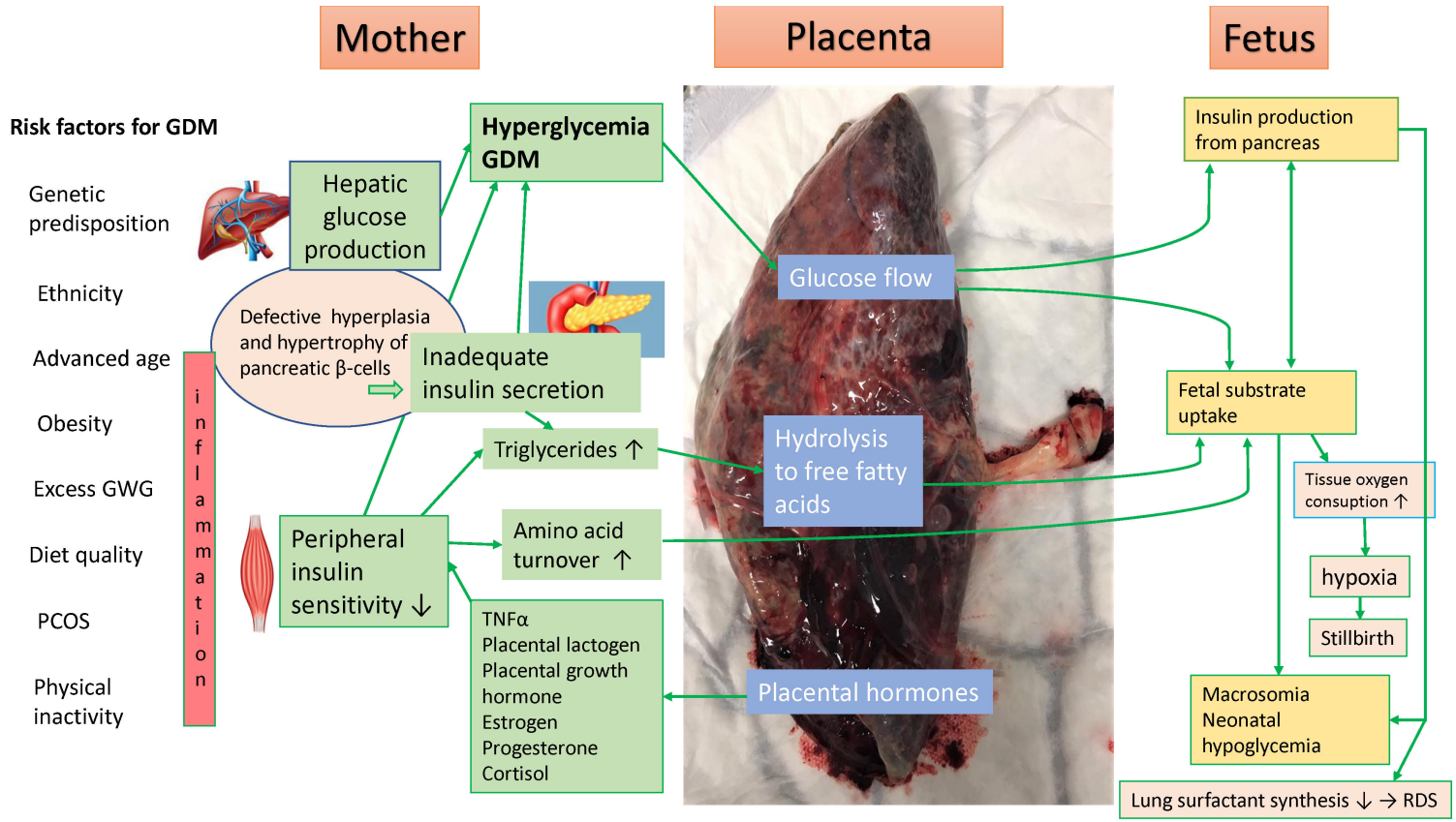

Figure 1. Factors contributing to maternal glucose metabolism and fetal complications. GDM=gestational diabetes, GWG= gestational weight gain, PCOS=polycystic ovary syndrome, TNF-α= tumor necrosis factor alpha, RDS=respiratory distress syndrome. Modified from reviews by Agha-Jaffar et al. 2016, Hod et al. 2015, Plows et al. 2018.

weight gain (GWG). Some women with GDM suffer predominantly from an insulin secretion disorder, while in others, the primary underlying cause is insulin resistance (Powe et al. 2016). This makes GDM a heterogenous condition e.g. it might reflect the different genetic background of the women. Women with a predominantly insulin secretion disorder have more commonly BMI and the risk of pregnancy complications resembling normoglycemic women (Powe et al. 2016). However, most women have primarily obesity related enhanced insulin resistance underlying GDM, and they are more prone to complications related to the obesity (Powe et al. 2016).

2.1.2.1 Insulin resistance related to obesity

Obesity related excessive insulin resistance is suggested to be partly mediated via inflammatory processes in the adipose tissue. The immune cells within adipose tissue are regulated by different stimuli such as diet, body weight, and the ingestion of excess calories that can cause metabolic distress. As a result, immune cells can switch from anti-inflammatory subtypes towards more the pro-inflammatory subtypes (Grant and Dixit 2015). Although uncomplicated pregnancy is characterized as a state of mild maternal systemic inflammation, excessive adipose tissue and placental inflammation are associated with several gestational disorders including GDM (Kuzmicki et al. 2009). The inflammation is demonstrated by higher circulating levels of pro-inflammatory cytokines in women with GDM as compared to normoglycemic women, e.g. increased levels of TNF-α, interleukin-6, and interleukin-1β have been detected (Ategbo et al. 2006, Fasshauer et al. 2014). Plasma TNF-α, especially, is clearly related to insulin resistance (Kirwan et al. 2002). Likewise, the placental gene expressions of TNF-α, interleukin-1β and their receptors have been shown to be enhanced in GDM (Kirwan et al. 2002, Radaelli et al. 2003). These cytokines have been found to both reduce insulin release from β-cells, and impair insulin signaling, thus inducing insulin resistance (Barbour et al. 2007, Kim et al. 2009).

Adiponectin, a hormone that is mainly secreted by adipocytes, has also been proposed to play a role in the pathogenesis of GDM. Obesity and GDM are both associated with reduced adiponectin levels (Williams et al. 2004), but there is a greater inverse correlation of adiponectin with insulin resistance than with adiposity (Retnakaran et al. 2004). This could indicate that adiponectin has a part in the pathogenesis of GDM, independent of obesity. Adiponectin has been found to increase insulin signaling and fatty acid oxidation, and hinder gluconeogenesis (Yamauchi et al. 2002). Moreover, it promotes insulin secretion by augmenting insulin gene expression and exocytosis of insulin granules from β-cells (Kishida et al. 2012).

Systemic insulin resistance can also be promoted by inflammation that is mediated by an obesogenic diet and dysbiosis of microbiota, which may enhance gut permeability and enable the passage of inflammatory mediators from the gut into the circulation (Backhed et al. 2005, Jayashree et al. 2014). Gut permeability is controlled by tight junction proteins, such as zonulin, and enhanced zonulin concentrations have been detected in early pregnancy in women that later develop GDM (Mokkala, Tertti et al. 2017).

Finally, excessive accretion of fat mass (FM) in obesity results in inadequate storage capacity of adipose tissue for lipids and, hence lipotoxic damage of insulin signaling in peripheral tissues and low-grade inflammation (Herrero et al. 2010). This lipolysis, with the increased release of free fatty acids and hepatic glucose production, has also been suggested to induce insulin resistance during pregnancy (Barbour et al. 2007, Buchanan et al. 1990, Catalano, P. M. et al. 1991). All in all, placental hormones, chronic inflammation, and excess lipolysis are essential in the pathogenesis of insulin resistance in GDM. After the delivery, pregnancy-induced insulin resistance resolves within a few days and the woman returns to her prepregnancy glycemic stage (Ryan et al. 1985).

2.1.3 Risk factors

Several factors predispose a woman to GDM (Figure 1). They are generally related to either pre-existing insulin resistance, decreased insulin secretory capacity, or enhanced insulin resistance caused by pregnancy (Ben-Haroush et al. 2004). Genetic characteristics play an important role in the background of these features.

Well established risk factors for GDM include being overweight or obese, as a linear increase in the risk of GDM with an increase in maternal BMI has been detected. The GDM risk has been shown to rise by 4% per unit of increase in BMI, which translates to odds ratios 2.0 for overweight women and 4.0 for obese women as compared to normal weight women (Najafi et al. 2019). Gestational weight gain is also an important risk factor for GDM, as over 40% of women who gain weight in excess of the recommendations of Institute of Medicine (IOM) during the first trimester of pregnancy, develop GDM (Carreno et al. 2012, Hedderson et al. 2010). Furthermore, parity is a risk factor independent of age (Ben-Haroush et al. 2004). Other predisposing factors include ethnicity (Caughey et al. 2010), genetic polymorphisms (Huopio et al. 2013), advanced maternal age (Lao et al. 2006), prior macrosomic newborn (Pintaudi et al. 2013), systemic corticosteroid medication (Yildirim et al. 2006), family history of type 2 diabetes (Levy et al. 2010, Mustaniemi et al. 2018, Ogonowski et al. 2007), personal history of GDM (Mustaniemi et al. 2018), and other conditions of insulin resistance, such as polycystic ovary syndrome (Ben-Haroush et al. 2004).

Although less thoroughly studied, there are some indications that a distinct metabolomic profile (Mokkala, Vahlberg et al. 2019), and high triglyceride levels (Li et al. 2015) in early pregnancy are a characteristic feature in women who develop GDM. Additionally, consumption of diets that have abundantly saturated fats, refined sugars, and red and processed meats have been suggested to be related to an enhanced risk of GDM (Bowers et al. 2012, Zhang, C. et al. 2006). It has been proposed that saturated fats affect insulin signaling and provoke inflammation (Sivan and Boden 2003).

2.1.4 Implications to the mother and child

As GDM can cause several consequences for both the mother and the fetus (Table 1), it is important to understand and effectively prevent or treat GDM. The magnitude of the risk for each outcome varies according to the diagnostic criteria applied and the study population.

GDM affects the health of mother acutely and over the long term. The risk of gestational hypertensive disorders and pre-eclampsia is higher than in normoglycemic women (Yogev et al. 2004). In Finland, about 20% of GDM women develop either gestational hypertension or pre-eclampsia (Suhonen and Teramo 1993). There is also an increased risk of macrosomic infant, preterm birth and need for cesarean section (HAPO Study Cooperative Research Group et al. 2008). Additionally, maternal glycemic status has been found to display positive linear associations with the induction of labor, large for gestational age infant, and with shoulder dystocia, with no obvious sign of a threshold effect. It seems that the fasting concentration is better correlated with these outcomes than the post-load concentration (Farrar et al. 2016).

In later life, these women have a higher risk of type 2 diabetes, metabolic syndrome and cardiovascular disease. According to a recent large observational study examining over four thousand Danish and North American women, the progression from GDM to type 2 diabetes was 23% in US women over 23 years and 27% in Danish women within 9 years (Zhang, C. et al. 2019). Furthermore, it was shown that the risks of hypertension and cardiovascular diseases, as well as early stages of renal damage were clearly higher in women with GDM. Similarly, in Finland, women with two or more abnormal values in the oral glucose tolerance test (OGTT) during pregnancy had a 25% risk of later type 2 diabetes and a 62.5% risk of metabolic syndrome after 10 years of follow-up, as compared to 0.8% and 24.2% of normoglycemic women, respectively (Hakkarainen 2019).

Table 1. Short- and long-term risks for the mother and the child in pregnancies complicated by gestational diabetes.

Short-term risks	OR (95% CI)	Long-term risks	OR (95%CI)
Pre-eclampsia or PIH[1] [a]	3.8 (1.5-9.9)	**Mother**	
Macrosomia[b]	2.7 (1.9-3.7)	Type 2 diabetes[c]	5.4 (3.7-7.9)
Large for gestational age[b]	3.3 (2.5-4.6)	Hypertension[d]	1.26 (1.1-1.4)[2]
Cesarean delivery[b]	1.9 (1.5-2.4)	Metabolic syndrome[e]	3.4 (2.5-4.8)
Shoulder dystocia[b]	4.1 (1.6-10.2)		
Neonatal hypoglycemia[b]	10.4 (6.5-16.6)	**Child**	
Neonatal respiratory complication[b]	4.4 (2.9-6.8)	Type 2 diabetes/prediabetes[f]	7.8 (2.6-23.4)
Neonatal hyperbilirubinemia[b]	3.9 (2.6-5.7)	Obesity[c]	1.6 (1.2-2.0)

[1] Pregnancy induced hypertension.[2]Hazard ratio. Adapted from [a]Suhonen et al. 1993, [b]Langer et al. 2005, [c]Lowe et al. 2018, [d]Tobias et al 2011, [e]Lauenborg et al. 2005, [f]Clausen et al. 2008.

GDM also affects the short- and long-term health of the infant (Table 1). The enhanced placental delivery of glucose, amino acids, and fatty acids stimulate the fetus's endogenous production of insulin and insulin-like growth factor 1 (Figure 1). This can result in fetal macrosomia at birth (Schwartz et al. 1994), which is a risk factor for shoulder dystocia. Due to the fetal hyperinsulinemia, these infants are at an increased risk of hypoglycemia. There is also evidence that GDM increases the risk of respiratory distress syndrome, hyperbilirubinemia and stillbirth (Langer et al. 2005, Reece 2010).

In the long term, the offspring of women with GDM display higher weights, BMI, systolic blood pressure, fasting blood glucose, insulin, insulin resistance (assessed by homeostatic model assessment, HOMA-IR) and triglycerides, as revealed by the results of the Danish National Birth Cohort that examined offspring at a mean twelve years of age (Grunnet et al. 2017). Even after adjustment for maternal prepregnancy BMI, the offspring of mothers with GDM had significantly higher BMI, fasting glucose, and HOMA-IR levels. In another large study, it was found that maternal GDM was independently associated with the offspring's risk of abnormal glucose tolerance, obesity, and higher blood pressure at 7 years of age (Tam, W. H. et al. 2017). Females are hence more prone to develop GDM in their own pregnancies, contributing to a vicious intergenerational cycle of GDM (Lee et al. 2000). These long-term risks could be partially due to the similar genetic factors,

dietary patterns, and physical activity levels in their families (Najafi et al. 2019). However, the findings may also be explained by the programming effects of hyperglycemia in pregnancy (Nicholas et al. 2016).

2.1.5 Diagnosis

GDM can be diagnosed with a one-phase 2-hour 75 g OGTT or a two-phase 3-h 100-g OGTT. Since the first introduction of diagnostic criteria for GDM in 1960s by O'Sullivan and Mahan (O'Sullivan and Mahan 1964), the type of OGTT and criteria for diagnosing GDM have varied over the decades and around the world. Furthermore, some countries prefer risk-based screening, while others endorse universal screening. These variations have complicated the interpretation of research results. Therefore, during the past decade, the scientific community has sought to uniform the screening of GDM, although with limited progress. Recently, the most widely accepted diagnostic criteria internationally have been the criteria recommended by the International Association of Diabetes and Pregnancy Study Group (IADPSG) (International Association of Diabetes and Pregnancy Study Groups Consensus Panel et al. 2010), which are also endorsed by the American Diabetes Association and the World Health Organization. These criteria were developed based on the findings of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) -study demonstrating a direct association between glucose concentrations in the 75 g OGTT and adverse pregnancy and neonatal outcomes (HAPO Study Cooperative Research Group et al. 2008). According to the IADPSG recommendation, all women not known to have diabetes should undergo a one-phase 75-g OGTT at 24–28 gestational weeks and be diagnosed with GDM if one or more values meet or exceed the cut-off values of 5.1mmol/l (0h) -10.0 mmol/l (1h) -8.5 mmol/l (2h). One important finding of the HAPO Study and another large study by Farrar et al. 2016, was that there was a continuous risk of maternal and fetal complications with increasing maternal glycaemia, which makes all diagnostic thresholds somewhat arbitrary. Nonetheless, the lower cut-off values with IADPSG criteria find more women and children at risk of GDM related complications as compared to more traditional higher cut-off values (International Association of Diabetes and Pregnancy Study Groups Consensus Panel et al. 2010, Lowe et al. 2018), and these women and children could benefit from the treatment of GDM. As risk-based screening is known to miss a significant proportion of women with GDM, universal screening is recommended (Farrar et al. 2017).

In Finland, GDM is diagnosed with the criteria issued by Finnish Current Care Guidelines (Working group established by the Finnish Medical Society Duodecim 2008). All women, except women at very low risk, i.e. women 1) <25 years of age with normal BMI, and no family history of diabetes or 2) <40 years of age, with

normal BMI and a previous normoglycemic pregnancy without fetal macrosomia, are screened for GDM at 24-28 weeks of gestation with one-phase 75 g OGTT. Thresholds in the OGTT are ≥5.3, ≥10.0, and ≥8.6 mmol/l for 0, 1, and 2 hours, respectively, and one value meeting or exceeding the cut-off is enough for a diagnosis. With these criteria, approximately every fifth woman is diagnosed with GDM (Perinatal statistics, Finnish Institute of Health and Welfare 2018, www.thl.fi). Moreover, women at a high risk of GDM are recommended to be screened with the same threshold values already between 12 and 16 gestational weeks. This early OGTT is recommended for women with a BMI ≥ 35 kg/m^2, previous GDM, early glucosuria during pregnancy, polycystic ovary syndrome, oral glucocorticoid medication, or a family history of diabetes. Women with an early diagnosis of GDM represent a high-risk subgroup, with an increased incidence of obstetric complications, recurrent GDM in subsequent pregnancies, and the future development of Type 2 diabetes (Ben-Haroush et al. 2004). The identification and treatment of GDM in these women already in early pregnancy may reduce the risks of obstetric complications.

2.1.6 Treatment

The treatment of GDM, including medication when needed, has been shown to reduce pre-eclampsia, infant macrosomia, shoulder dystocia and cesarean delivery (Hartling et al. 2013, Landon et al. 2009). In Finland, all women with GDM are offered lifestyle counselling and equipment for self-monitoring of glucose concentrations at local maternal welfare clinics. Lifestyle counselling consists of dietary advice and recommendations for physical activity, as instructed in the Finnish Current Care Guidelines (Working group established by the Finnish Medical Society Duodecim 2008). If the target values (<5.5 mmol/l for fasting glucose and <7.8mmol/l for 1h postprandial glucose) are repeatedly exceeded despite conservative treatment, medication is offered. Depending on gestational weeks, maternal characteristics, the severity of GDM and whether fasting or postprandial glucose concentrations are the main issue, either insulin or metformin medication is initiated.

2.2 Prevention of gestational diabetes mellitus

2.2.1 Lifestyle interventions

Physical activity during pregnancy has been found to be safe and might even reduce the risk of preterm birth (Beetham et al. 2019, Magro-Malosso et al. 2017). Interventions to prevent GDM by enhancing physical activity have yielded positive

results, as several recent reviews/meta-analyses conclude that exercise can reduce the incidence of GDM by 20–40% (Davenport et al. 2018, Dipietro et al. 2019, Magro-Malosso et al. 2017, Russo et al. 2015). Most of the interventions in these RCT studies seem to have involved mainly aerobic activity of moderate intensity, such as walking, swimming, and aerobic exercise, taking place at least three times a week for a duration of 30 - 60 minutes per session (Dipietro et al. 2019).

Diet interventions to reduce the incidence of GDM have generated conflicting results. According to Cochrane systematic review (Tieu et al. 2017), low quality evidence suggests that the GDM risk of women could be reduced by dietary advice versus standard care. Regarding the diets that have been investigated, a DASH-style diet (The Dietary Approach to Stop Hypertension) compared to other diets, was found to improve fasting glucose (Ha et al. 2017). Similarly, the risk of GDM was reduced by a high adherence to a Mediterranean diet as compared with women with low adherence (Assaf-Balut et al. 2018). Meanwhile, several studies have not found any consistent correlation between diet quality and glycaemia (Simmons 2019). All in all, it is evident that better nutritional approaches are needed to prevent GDM.

Multiple intervention trials combining diet and exercise have been conducted in an attempt to prevent GDM, such as the LIMIT, UPBEAT and DALI trials, with no reduction in the risk of GDM or insulin sensitivity (Dodd et al. 2014, Poston et al. 2015, Simmons et al. 2017). Recent meta-analyses have found more promising results, indicating that there could be a reduced risk of GDM in the diet and exercise intervention groups as compared with the standard care groups (Guo et al. 2019, Shepherd et al. 2017). Furthermore, the Finnish RADIEL trial showed that women with a high risk for GDM (history of GDM or BMI $\geq$30kg/m^2) could benefit from combined diet and physical activity counselling in terms of reducing GWG and the incidence of GDM (Koivusalo et al. 2017). The reason for the discrepant results between studies has been proposed to be the substantial heterogeneity between the interventions, the differing characteristics of the trial participants, the settings of trials, and differences in the screening of GDM and diagnostic criteria. In addition, the adherence to the intervention (which has been reported to be low in many studies), and the fact that pregestational glucose intolerance is not screened before randomization, may be confounding factors. The genetic background can also affect the responsiveness to the intervention, as suggested by the results of the RADIEL study (Grotenfelt et al. 2016).

It has been proposed that the risk of GDM can only be reduced if interventions are started in or before the first trimester and the women are willing to adhere to the intervention (Egan and Simmons 2019). Furthermore, due to the heterogeneity of GDM, it is likely that interventions to prevent GDM will need to be individualized, according to the subject's characteristics. Other key aspects in the improvement of

lifestyle intervention outcomes could include targeting other than BMI-based high-risk populations, determining the correct intensity and frequency of exercise, and GWG management (Guo et al. 2019).

All in all, it seems that lifestyle approaches to reduce the risk of GDM are not effective for many women at risk and new means to prevent GDM are clearly needed.

2.2.2 N-3 long chain polyunsaturated fatty acids

N-3 (or omega-3) fatty acids belong to a family of long chain poly-unsaturated fatty acids (LC-PUFA), which are vital for the functioning of the body (Simopoulos 2002, Surette 2008). As opposed to saturated fatty acids that are more solid and have no double bonds, n-3 fatty acids are soft and contain three or more double bonds. The term 'n' or omega (ω), refers to the position of the first double bond, i.e. n-3 LC-PUFA possesses the first double bond between the third and fourth carbon atoms (Anderson, B. M. and Ma 2009). The major n-3 LC-PUFAs are α-linolenic acid (ALA 18:3n-3), eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Anderson, B. M. and Ma 2009). PUFAs are present in all cell membranes and contribute to membrane fluidity and the function of membrane-bound enzymes and receptors, and thus influence many metabolic processes (Surette 2008, Wall et al. 2010). Because n-3 LC-PUFA ALA cannot be synthesized by the body, it is termed 'essential' and must be obtained from the diet (Sprecher et al. 1995). Important sources of ALA include different kinds of plant oils. To a small degree, ALA can be converted in the body to biologically active derivatives DHA and EPA (Burdge et al. 2002, Emken et al. 1994). EPA and DHA can be found in some species of fish and seafood (Ellulu et al. 2015, Surette 2008). They can also be consumed as nutritional supplements, such as fish oil capsules.

DHA and EPA are precursors to a range of compounds that are known to reduce and help resolve inflammatory responses and oxidative stress, as has been shown in animal and in vitro studies (Calder 2006, Chapkin et al. 2009, Ellulu et al. 2015, Wall et al. 2010). Several mechanisms have been suggested to underpin the anti-inflammatory properties of the n-3 LC-PUFAs, although the precise molecular mechanisms are not completely understood. First, they can interact with the major inflammatory signaling pathways and reduce pro-inflammatory cytokine levels, as has been shown in animal and human studies (Calder 2011, Lorente-Cebrian et al. 2015). Second, inflammation can be reduced by n-3 LC-PUFA supplementation via disruption of pro-inflammatory eicosanoid production or augmenting the production of anti-inflammatory forms (Calder 2003). Third, it has been suggested that an essential part of the anti-inflammatory effect of n-3 LC-PUFAs is their ability to alter inflammatory gene expression through influencing transcription factors such as

nuclear factor kappa B (NFkB) and peroxisome proliferator-activated receptors (PPARs)(Calder 2006, de Castro et al. 2015). Furthermore, one of the important causes in increasing the obesity-related inflammatory response has been recognized to be the Toll-like receptor 4 signaling pathway, which can be attenuated by DHA and EPA (Rogero and Calder 2018). This attenuation in macrophages in muscle has been shown to improve markers of inflammation (TNF-α, CRP, interleukin-6) and insulin resistance (Liu et al. 2013, Smith et al. 2010). Finally, n-3 LC-PUFA supplementation can modify gut microbiota composition and thereby enhance intestinal integrity and reduce LPS-mediated metabolic endotoxemia and inflammation (Mokkala, Houttu et al. 2019).

However, despite solid evidence in favor of DHA and EPA being able to reduce insulin resistance from animal and mechanistic studies (Flachs et al. 2014, Lamping et al. 2013), findings from human clinical trials on glycemic control and insulin resistance have been equivocal (Coelho et al. 2017). In a recent meta-analysis, which evaluated the effects of fish oil supplementation in human RCTs, fish oil supplementation was found to have no effects on insulin sensitivity as compared with the placebo (Gao et al. 2018). Nevertheless, in a subgroup analysis, it was observed that short-term fish oil supplementation was related to improved insulin sensitivity among people with metabolic disorders (Gao et al. 2018). In another meta-analysis in type 2 diabetic patients, it was concluded that although n-3 LC-PUFA supplementation did not affect the glucose control, the level of triglycerides was reduced and a high ratio of EPA/DHA led to an almost statistically significant decrease in plasma insulin and HbA1c levels (Chen, C. et al. 2015). Still, caution with high doses (≥4g daily) of n-3 LC-PUFA supplementation is warranted, as they could even worsen glycemic control (Puhakainen et al. 1995, Woodman et al. 2002). Although the causes of the effects of dietary interventions can be complex, and thus must be separated from interventions with supplementation, it has been postulated that replacing carbohydrates or saturated fatty acids with PUFA in human diet would be one way to induce improvements in glycaemia, insulin resistance and insulin secretion capacity (Imamura et al. 2016).

The pregnancy is a period of increased risk for n-3 LC-PUFA deficit as these compounds are used by the developing fetus (Gil-Sanchez et al. 2010, Makrides and Gibson 2000, Otto et al. 1997). Thus, it is typical that the maternal serum DHA levels decrease during the pregnancy (Al et al. 2000). It has been stated that pregnant women should increase their DHA intake to 200 mg/day (Koletzko et al. 2007). The same is recommended in Finland (Finnish Institute of Health and Welfare, https://thl.fi/fi/web/elintavat-ja-ravitsemus/ravitsemus/syodaan-yhdessa-ruokasuositukset-lapsiperheille). However, it has been proposed that only about one in four women meets this recommendation (Jia et al. 2015), although it has been demonstrated that regular consumption of fish oil results in enhanced concentrations

of n-3 LC-PUFA in the maternal circulation and in cord blood (Miles et al. 2011, Min et al. 2014). Additionally, placental and fetal tissue concentrations of n-3 LC-PUFAs have been found to correlate positively with their respective concentrations in maternal blood during late pregnancy (Jones et al. 2013). However, fish intake is low in many countries, and pregnant women may be unwilling to increase their fish intake due to perceptions that the presence of mercury and other pollutants in fish may affect their unborn child (Bloomingdale et al. 2010).

It has been suggested in a few observational studies that there is an association with low dietary PUFA intake and gestational hyperglycemia (Bo et al. 2001, Wang, Y. et al. 2000). In addition, DHA concentrations in red blood cell membranes have been shown to be lower in women with GDM as compared to the concentrations in women with normoglycemic pregnancies (Taschereau-Charron et al. 2017). Additionally, alterations in the molecular mechanisms regulating placental fatty acid transport have been reported in patients with GDM (Magnusson et al. 2004). As is the case in a non-pregnant population, the n-3 LC-PUFA have been detected to exert both antioxidant and anti-inflammatory properties also in pregnant women (Haghiac et al. 2015, Jones et al. 2014, Leghi and Muhlhausler 2016). The increase in placental membrane DHA after DHA supplementation was associated with a reduction in inflammatory markers, along with an increase in fatty acid transport capacity (Lager et al. 2017). Similar results were found in another study, as supplementation of overweight/obese pregnant women with DHA and EPA reduced inflammation in maternal adipose and the placental tissue (Haghiac et al. 2015).

Despite these promising mechanistic studies, human trials with n-3 LC-PUFA supplementation have not succeeded in preventing GDM, as summarized in Table 2. In addition, a meta-analysis of randomized controlled studies, primarily with the main outcomes other than GDM, have failed to demonstrate any benefit of consuming n-3 LC-PUFA on the incidence of GDM (Chen, B. et al. 2015). A number of studies have also been conducted in women already diagnosed with GDM; a recent meta-analysis of 7 randomized controlled trials reported evidence of a benefit on glucose metabolism and insulin resistance associated with n-3 LC-PUFA consumption (Gao et al. 2018). In contrast, a Cochrane review on Omega-3 fatty acid addition during pregnancy did not detect improvements in insulin resistance (Middleton et al. 2018).

Table 2. Randomized controlled trials on n-3 LC-PUFA with information on gestational diabetes (GDM).

Author	Country	Year	Sample size	Intervention and daily dose	Start/End	Main outcome	Result
Harper et al.	USA	2010	852	DHA 800mg, EPA 1200 mg + 17-α hydroxyprogesterone caproate	16–21+6 gw/ delivery or 36+6 gw	Preterm birth < 37 gw	No difference in the incidence of GDM
Zhou et al.	Australia	2012	2399	DHA 800 mg EPA 100 mg	<21gw (mean 19.0 gw) / delivery	GDM (OGTT ~ 28 gw)	No difference in the incidence of GDM
Hauner et al.	Germany	2012	208	DHA 1020 mg EPA 180 mg + dietary advice	<h15 / 4 months postpartum	Offspring body composition	No difference in the incidence of GDM
Carlson et al.	USA	2013	301	DHA 600 mg	<20 gw/ delivery	Duration of gestation	No difference in the incidence of GDM
Mozurkewich et al.	USA	2013	118	DHA 274 mg +EPA 1060 mg OR DHA 900 mg + EPA 180 mg	12-20 gw/ 6–8 wk postpartum	Depression	No difference in the incidence of GDM
Bisgaard et al.	Denmark	2016	695	DHA 890 mg EPA 1320 mg	24 gw/1 wk after delivery	Wheeze, asthma	No difference in the incidence of GDM

DHA= docosahexaenoic acid, EPA= eicosapentaenoic acid, gw= gestational weeks, OGTT= oral glucose tolerance test, wk= weeks.

2.2.3 Probiotics

The gut microbiota has been shown to have an influence on several pathological conditions in rodents and humans, such as obesity and insulin resistance (Backhed et al. 2004, Le Chatelier et al. 2013, Vrieze et al. 2012). In animals, it has been shown that germ-free mice are resistant to obesity, and after transplantation of gut microbes, the animals are likely to increase their energy intake and develop fat deposition and insulin resistance (Backhed et al. 2004, Backhed et al. 2007). Moreover, mice that were colonized with gut microbiota from obese animals harvested energy more efficiently and accumulated body fat more quickly (Turnbaugh et al. 2006). Clinical studies in humans also support the connection between gut microbiota and obesity (Duncan et al. 2008, Ley et al. 2006). Therefore, it has been proposed that interventions that modify the gut microbiota and its functions could also impact on the metabolic health of the host.

Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host', as defined by World Health Organization (Joint FAO WHO Working Group 2002). The *Lactobacillus, Bifidobacterium* and *Bacteroides* are the genera that have often been associated with a reduction of FM, adipose tissue inflammation or improved glycemic control (Gauffin Cano et al. 2012, Plovier and Cani 2017). However, it is likely that the

benefits induced by probiotics are dependent on the specific species or even which strains are used (Torres et al. 2019).

The potential effects of probiotics are mediated in several mechanisms, which are still not completely understood. For example, probiotics can break down ingested complex polysaccharides into short-chain fatty acids. There is evidence that these fatty acids act at various levels to reduce the inflammatory state which in turn, decreases insulin resistance and improves β-cell function (Puddu et al. 2014, Yadav et al. 2013). An additional suggested mechanism of probiotics is their ability to enhance the gut epithelial barrier integrity (Anderson, R. C. et al. 2010, Dai et al. 2012). Intestinal permeability is enhanced by dysbiosis of the microbiota, which leads to adipose tissue inflammation through a process where lipopolysaccharide from the cell walls of gram-negative bacteria, binds to the Toll-like receptor 4, which in turn induces the activation of NFkB (Wu et al. 2012). The favorable effects of probiotics can also be induced by the competitive exclusion of pathogenic bacteria in the gut (Mazloom et al. 2019). Furthermore, an essential mechanism of probiotics is their ability to attenuate chronic inflammation by modulation of immune responses of the host. Numerous probiotic strains, such as *Lactobacillus rhamnosus* GG, have been recognized as inhibitors of NFkB mediated chronic systemic inflammation (Tien et al. 2006, Zhang, L. et al. 2005). Other proposed anti-inflammatory effects of probiotics include their abilities to 1) regulate the maturation of dendritic cells in the gut 2) induce the generation of anti-inflammatory rather than inflammatory cytokines and 3) induce the differentiation of T-helper cells into Th2 cells (Torres et al. 2019). Probiotics might also be able to modulate the secretion of adipokines in the adipose tissue, leading to the suppression of pro-inflammatory cytokines and/or enhanced production of adiponectin in adipocytes (Fabersani et al. 2017, Novotny Nunez et al. 2015).

According to human clinical trials, probiotics can help in weight loss, reduction of BMI or FM, as several recent meta-analyses have described (Borgeraas et al. 2018, Dror et al. 2017, John et al. 2018, Koutnikova et al. 2019). However, the effect sizes were relatively small. The benefits were seen especially among overweight subjects and the mean duration of intervention was around 8 weeks. Different strains of probiotics were used, and the duration of intervention has varied, and additionally, a variety of effects on the gut microbiota was discovered. The benefits were primarily detected with mixtures containing *bifidobacteria* (*B. breve and B. longum*), *Streptococcus salivarius* subsp. *thermophilus* and *lactobacilli* (*L. acidophilus, L. casei, L. delbrueckii*) with most of the trials yielding benefits being conducted in Iran. Probiotic consumption may also modestly improve glycemic control in humans (Koutnikova et al. 2019, Nikbakht et al. 2018, Ruan et al. 2015). This was detected especially in subjects with type 2 diabetes (Koutnikova et al. 2019, Nikbakht et al. 2018). The probiotic species and the dose used have varied between studies.

In an uncomplicated pregnancy, the maternal gut microbiota changes from first to third trimester (Gomez-Arango et al. 2016, Koren et al. 2012). The first trimester gut microbiotas are like each other and comparable to that found in non-pregnant healthy women. By the third trimester, the between-subject diversity has significantly increased, even though within-subject diversity is decreased (Koren et al. 2012). Maternal stools show strong signs of inflammation in the third trimester, as concentrations of the pro-inflammatory cytokines are significantly higher than in the first trimester. When delivered into germ-free mice, third trimester microbiota caused a greater adiposity and insulin resistance than first trimester stools (Koren et al. 2012). Thus, the features common in the metabolic syndrome and type 2 diabetes, such as weight gain, dysbiosis and inflammation, are also central to normal pregnancy. Nevertheless, these features may be appropriate during pregnancy, as they promote energy storage in fat tissue and the growth of the fetus. However, the bacterial alterations in microbiota might also influence the mother's metabolic profile and thus play a part in the development of pregnancy complications (Musso et al. 2010, Thum et al. 2012). For example, genera of *Collinsella* has been linked to obesity and insulin resistance (Gomez-Arango et al. 2016).

There is some evidence which indicates that the microbiota composition differs between women who later develop GDM and those who remain healthy (Mokkala, Houttu et al. 2017). For example, the abundance of bacteria belonging to Ruminococcaceae family has been found to correlate with markers of glucose metabolism and GDM (Gomez-Arango et al. 2016, Mokkala, Houttu et al. 2017). Additionally, in women already diagnosed with GDM, the microbiota has been shown to be markedly altered in comparison to that in normoglycemic pregnant women in the third trimester (Crusell et al. 2018, Wang, J. et al. 2018). However, the conducted trials are relatively small and not all studies have detected differences in the microbiota between healthy women and women with GDM (Koren et al. 2012).

Several randomized controlled trials have been conducted on administration of probiotics to prevent GDM, and these are presented in Table 3. Meta-analyses of these trials have yielded conflicting results on glucose metabolism, as one of them found that probiotics reduced fasting blood glucose, as well as serum insulin levels, HOMA-IR and triglyceride levels (Han et al. 2019), while other meta-analyses showed only a significant reduction in serum insulin and HOMA-IR. (Jarde et al. 2018, Zheng et al. 2018). In patients already diagnosed with GDM, several studies, mainly conducted on Asian populations, have reported benefits on glucose metabolism with a range of different probiotics (Dolatkhah et al. 2015, Karamali et al. 2016, Kijmanawat et al. 2018), although there are also some trials detecting no benefits (Jafarnejad et al. 2016, Lindsay, K. L. et al. 2015). Meta-analyses have concluded that administration of probiotics in women with GDM can reduce insulin resistance but does not affect fasting blood glucose levels (Taylor et al. 2017, Zheng et al. 2018).

Table 3. Randomized controlled trials on probiotics with information on glycemic control.

Author	Country	Year	Sample size	Intervention	Start/End	Main outcome	Result
Laitinen et al., Luoto et al.	Finland	2009	256	*Lactobacillus rhamnosus* GG (10^{10} cfu), *Bifidobacterium lactis* BB12 (10^{10} cfu) +/- dietary counselling	I st trimester/ postpartum	FPG	FPG, Ins, HOMA-IR ↓ in probiotics vs. placebo. Incidence of GDM↓.
Asemi et al.	Iran	2013	70	*Lactobacillus acidophilus* LA5, *Bifidobacterium animalis* Bb12 (total of min 10^7 cfu)	3 rd trimester → 9 weeks	FPG, Insulin, HOMA-IR	No difference in FPG. ∆Ins and ∆HOMA-IR ↓ in probiotics vs placebo.
Lindsay et al.	Ireland	2014	175 obese women	*Lactobacillus salivarius* UCC118 (10^9 cfu)	24 gw/28 gw, 4 weeks	FPG	No difference in FPG, Ins, HOMA-IR or incidence of GDM
Jamilian et al.	Iran	2016	60	*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum* (2×10^9cfu)	9 gw /21 gw	∆Ins	No difference in FPG. ∆Ins, ∆HOMA-IR, ∆trig., ∆hs-CRP ↓ in probiotics vs. placebo.
Wickens et al.	New Zealand (NZ)	2017	373	*L. rhamnosus* HN001 6×10^9 cfu	14-16 gw / postpartum	incidence of GDM	GDM↓ in probiotics vs placebo with NZ OGTT criteria (no difference with IADPSG criteria except >35y and history of GDM).
Okesene-Gafa et al.	New Zealand	2019	230 obese women	*Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* BB12 min 6.5×10^9 cfu	12-17+6 gw / birth	GWG, birthweight	No difference in FPG, OGTT, HbA1c or risk of GDM.
Callaway et al.	Australia	2019	411 overweight and obese	*Lactobacillus rhamnosus* (LGG), *Bifidobacterium animalis* subsp. *lactis* (BB-12) $>1\times10^9$ cfu	<20 gw / birth	incidence of GDM	No difference in incidence of GDM or OGTT 1h and 2 h. FPG ↑in probiotics vs placebo,
Asgharian et al.	Iran	2019	128 overweight and obese	*Lactobacillus acidophilus* La5, *Bifidobacterium lactis* BB12 5×10^8 cfu/g	24 gw / birth	FPG, OGTT at 28 gw	FPG and 2h-OGTT ↓ in probiotics vs placebo. No difference in the incidence of GDM.

Gestational diabetes (GDM), FPG= fasting plasma glucose, Ins=insulin, OGTT=oral glucose tolerance test, GWG=gestational weight gain, gw= gestational weeks.

2.3 Gestational weight gain (GWG)

Although weight gain during gestation is an essential physiologic adaptation that promotes the development and growth of a fetus, both inadequate and excess GWG have been linked to adverse outcomes (Gilmore et al. 2015). Excessive GWG has been found to increase the risks of hypertensive disorders during pregnancy, GDM, Caesarean delivery, large for gestational age at birth, and overweight in the offspring (Gaillard et al. 2013, Santos et al. 2019). The preterm birth risk is higher in women gaining weight either inadequately or in excess (Santos et al. 2019). In a large epidemiological study, both extremities of GWG were discovered to have a higher association with infant death except that in women with BMI >40 kg/m2, GWG was no longer related to infant demise (Bodnar et al. 2016).

To reduce the risks for maternal and offspring complications related to pregnancy, IOM has issued guidelines for GWG, which have been revised in 2009 (Institute of Medicine 2009). These guidelines recommend less GWG for women in the higher BMI categories prior to pregnancy. Normal weight women are advised to gain weight 11.5–16 kg, overweight women 7–11.5 kg and obese women 5–9 kg (Figure 2).

Over the last decades, GWG has markedly increased independent of prepregnancy BMI (Kinnunen et al. 2003). Only one third of pregnant women gain weight in accordance to IOM recommendations (Deputy et al. 2015, Devlieger et al. 2016). Although overweight and obese women usually gain less weight than normal weight women, they are more often likely to exceed the GWG recommendations (Deputy et al. 2015). As obesity among reproductive women is very common, and obese mothers who gain weight in excess have a particularly high risk of different kinds of pregnancy complications (Santos et al. 2019), it is essential find means to maintain healthy GWG in pregnant women.

Risk factors for excess GWG include maternal European ethnicity, nulliparity, high total energy intake, smoking during pregnancy, and high paternal BMI (Gaillard et al. 2013). Excess GWG occurring in the first trimester has been demonstrated to strongly predict excess GWG during the entire pregnancy in both normal weight and overweight/obese women (Knabl et al. 2014).

Interventions to reduce excess GWG have yielded inconclusive results although these could be related to the intervention in question. The UPBEAT trial succeeded in reducing GWG with a combined lifestyle intervention (Poston et al. 2015), and two reviews have suggested that exercise-only interventions can reduce both total and excess GWG (Dipietro et al. 2019, Ruchat et al. 2018). In contrast, a very recent German multicenter trial found that lifestyle advice given by trained healthcare providers in the setting of routine prenatal care was not successful in reducing GWG or pregnancy complications (Kunath et al. 2019).

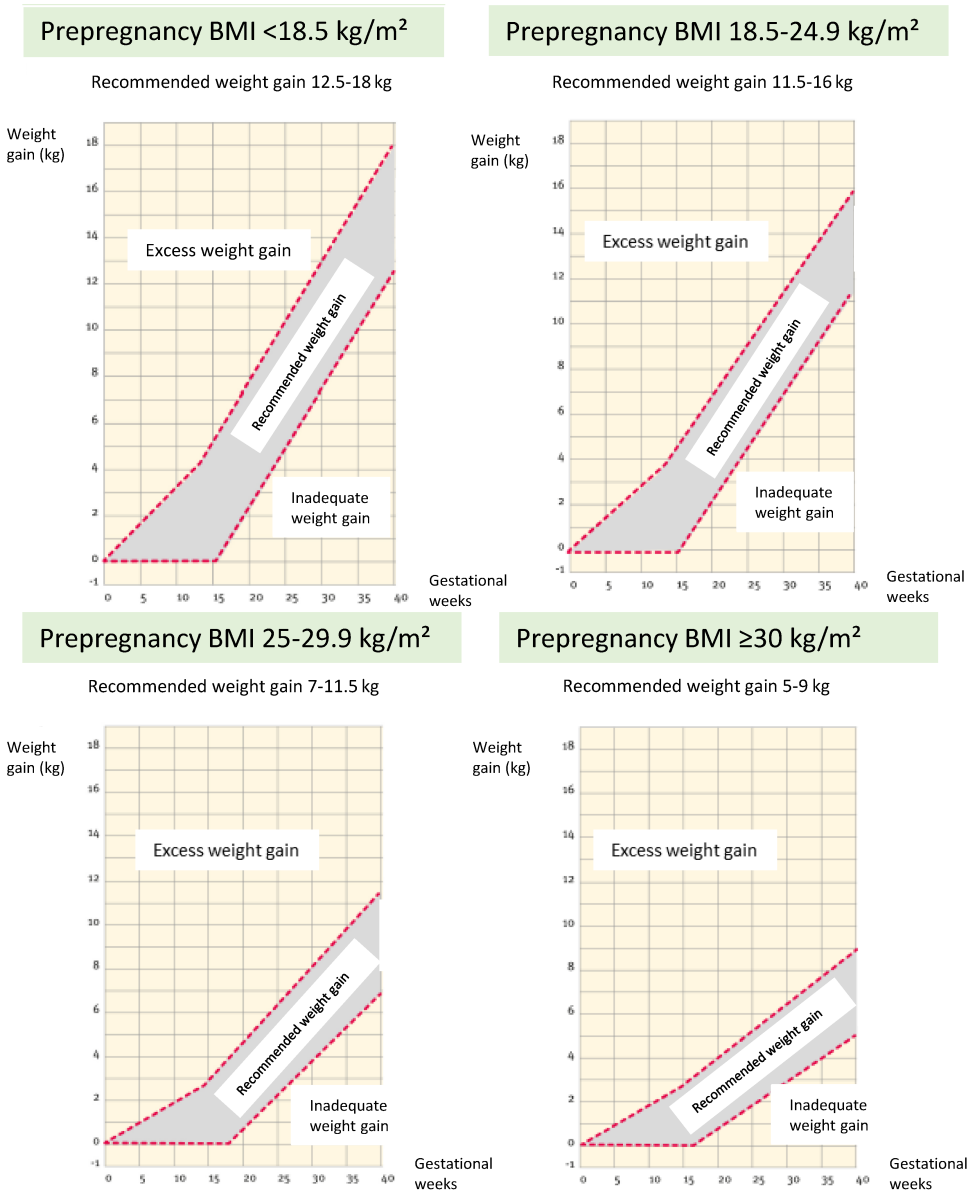

Figure 2. Gestational weight gain recommendations based on prepregnancy BMI (Nelli study, UKK Institute for Health Promotion Research, Tampere, Finland). Adapted from recommendations issued by Institute of Medicine 2009.

The impacts of excess GWG extend well beyond pregnancy as women who gain weight in excess, have more fat retention postpartum (Butte et al. 2003) and a greater risk of metabolic syndrome (Mamun et al. 2010, McClure et al. 2013). In summary,

GWG represents a major public health concern and more information is needed about ways to normalize GWG, especially in overweight and obese women.

2.4 Body composition during pregnancy

2.4.1 General principles

Body composition provides much more precise information about the adiposity of the body than the more widely used BMI and reflects also nutritional intakes, losses and expenses over time (Lindsay, C. A. et al. 1997, Prentice and Jebb 2001). Obesity denotes accrual of excess body fat, and it is the amount of this excess fat that relates to health problems (Prentice and Jebb 2001). In contrast to body weight and BMI, methods for the assessment of body composition permit a measurement of tissue gains and losses.

The human body can be divided into compartments at five different levels: atomic, molecular, cellular, tissue and whole-body levels (Wang, Z. M. et al. 1992). At the molecular level, body weight consists of FM and fat free mass (FFM), where FM is composed of nonessential lipids (90 % of total body lipids) and FFM is almost entirely constructed of water, protein, mineral and essential lipids (10 % of total body lipids) (Wang, Z. M. et al. 1992).

In order to support the growth and development of the fetus and to prepare the mother for breastfeeding, the maternal body composition changes markedly during gestation. The GWG that reflects these changes, includes gains in maternal and fetal FM and FFM, as well as the placenta and amniotic fluid. There is marked inter-individual variation especially in the FM gain during pregnancy, addressing the importance of measuring body composition (Marshall et al. 2016, Sewell et al. 2007). During late pregnancy, BMI has been found to explain only 40% of the variance in BF% (Lindsay, C. A. et al. 1997).

The measurement of body composition during pregnancy is challenging. The methods which have traditionally been used, cannot distinguish fetal from maternal tissues and handle the maternal-fetal dyad as a single unit. Another complicating factor is the enhanced hydration of FFM, which involves enlargement of the body water compartments. For example, total body water is enhanced during pregnancy by about 5–8 liters (Forsum et al. 1988, Kopp-Hoolihan et al. 1999, Lederman et al. 1997). Thus, the composition of lean tissue changes across the pregnancy, a fact that must be accounted for in all body composition measurements based on FFM (Catalano, P. M. et al. 1995).

It is acknowledged that age, diet, physical activity and chronic illness may influence body composition in humans that are not pregnant (Thibault et al. 2012). These same factors are probably also associated with body composition during

pregnancy, although less is known about features affecting gestational body composition.

2.4.2 Measurement of body composition

Body composition can be estimated at the same five levels from which the body is composed (Wang, Z. M. et al. 1992). Different components of the body can be measured either directly or indirectly, often applying the laws of physics in the calculations and exploiting the fact that certain stable proportions among the different components exist at the same level. There are no practical methods to measure body fat directly, thus indirect methods must be applied. This usually means that FFM is determined by measuring directly some component of it that forms a known proportion of FFM, and then FFM is subtracted from the total body weight to estimate the amount of fat (Wang, Z. M. et al. 1992).

The measurement techniques of body composition can be categorized by the number of body compartments they determine (Elia 1992). Two compartment models (2C) divide the body into FM and FFM, where FFM constitutes of the combined mass of total body water (TBW), bone, protein, and mineral mass other than bone. In these models, a single measurement of one compartment permits calculation of the other by subtraction from body weight. More advanced models of the assessment of body composition that are also used in pregnant women, include 3-compartment (3C) and 4-compartment (4C) models. They subdivide the FFM component into TBW and a combination of mineral and protein (3C), or into mineral, water and protein (4C-models). In addition, the 2C, 3C or 4C models cannot disentangle the maternal-fetal unit (Widen and Gallagher 2014). The 3C and 4C models are often composed by uniting parts of methods used in 2C models. Thus, although these models are often considered more precise than 2C models, they could bring about the propagation of any error created by its sub-methods.

The IOM has stated that 2C, 3C and 4C models estimate gestational body composition changes with acceptable precision. The precondition is the use of corrected values for hydration and density of FFM (2C models), and for 3C and 4C models in which FFM hydration or density are measured (Institute of Medicine 2009). Imaging techniques like magnetic resonance imaging (MRI) can assess specific organ volumes and may therefore allow an estimation of maternal and fetal deposits separately in the future (Most et al. 2018). A comparison of methods that measure body composition also during pregnancy is presented in table 4.

Table 4. Methods that measure body composition. 2C/3C/4C= 2-, 3-, and 4 compartment models. Modified from Widen and Gallagher 2014.

Method	technique	Advantages	Disadvantages	Suitability in pregnancy
Anthropometrics -skinfold thickness	2C: FM and FFM estimated using validated equations	Simple, rapid, cheap, information on regional distribution of fat Suitable for larger sample sizes	Measurement subjective - technician expertise required, less precise	-Safe -Equations apply only for certain gestational ages -Pregnancy can alter compressibility of skinfold -Overestimates subcutaneous fat vs MRI -Edema can disturb measurements
Total body water (TBW)	2C: Stable isotope of water is ingested and it equilibrates within the TBW pool of the body. The size of the TBW pool determines the maximal concentration of the isotope. FFM is calculated with assumptions for the TBW: FFM ratio.	Portable devices, can be used in 3C and 4C models	Laborious, takes 4-5h, no information on regional distribution of fat	-Safe -Hydration of FFM must be accounted for -Unable to disentangle maternal-fetal unit -requires fasting overnight + the measurement time
Total body potassium (TBK)	2C: the ratio of radioactive isotope potassium 40 (K40) found in human tissues and TBK is constant. Measurement of K40 allows calculation of TBK. The known ratio of total body potassium to FFM allows determination of body composition.	Can be used in 3C and 4C to estimate protein	Potassium concentration in FFM can vary.	-Safe -Hydration of FFM must be accounted for -Unable to disentangle maternal-fetal unit -When used on 3C model (with TBW), no significant difference was detected compared to 4C model
Underwater weighing	2C: body volume is defined with Archimedes principle and used to calculate body density (weight/volume). Known densities of FM and FFM allows calculation of body composition.	Noninvasive, precise, can be used in 3C/4C models	Immersion under water can cause discomfort, no information on regional distribution of fat	-Safe -Hydration of FFM must be accounted for -Unable to disentangle maternal-fetal unit
Air displacement plethysmograhy	2C: body volume is assessed with plethysmograph and used to calculate body density. Known densities of FM and FFM allows calculation of body composition.	Rapid, well tolerated, can be used in 3C/4C models, suitable for larger sample sizes	No information on regional distribution of fat	-Safe -Hydration of FFM must be accounted for -Unable to disentangle maternal-fetal unit -Performs well compared to 3C/4C models

(continued)

Table 4. Continued.

Method	technique	Advantages	Disadvantages	Suitability in pregnancy
Bloimpedance	2C: The opposition of the electrical flow by tissues allows for estimation of total body water from which estimates of FM and FFM can be derived.	rapid, cheap, noninvasive, well tolerated, suitable for larger sample sizes	Accuracy Not validated for regional distribution of fat	-Safe -Unable to disentangle maternal-fetal unit -BIA may underestimate total body water -Additional validation studies needed
Dual-energy X-ray absorptiometry (DXA)	3C/4C: Two X-ray beams, with different energy levels, are aimed at the patient's bones to measure bone mineral content, FFM and FM.	Well tolerated, gives information on regional fat distribution, practical, precise, suitable for larger sample sizes	Hydration of tissues affects results	-Not suitable due to radiation exposure -Used just before/after pregnancy as a part of 3C/4C models - Bone mineral density is decreased during late gestation and during lactation and this could affect the accuracy.
ultrasound	Based on production of sound waves at changing frequencies to measure adipose tissue depot	Well tolerated, cheap, information on regional fat distribution, suitable for larger sample sizes	Measures only regional- not whole-body fat distribution, compressibility of the skin may vary and cause error	-Safe -Standardized measurement locations difficult to achieve -Uterine contents can be separated from maternal tissues -Not validated against MRI
MRI	Area estimates of fat can be achieved. These can be transformed into volume measures if the distance between slices is accounted for.	Precise, well tolerated, noninvasive, gives information on regional fat distribution. validated against phantoms and cadaver body composition	Expensive, complicated, laborious, requires technician expertise and time	- Safety at 1st trim. has not been sufficiently evaluated - Standardized measurement locations difficult to achieve when regional fat is evaluated -Uterine contents can be separated from maternal tissues -Discomfort possible especially for overweight and obese women - No publications reporting the use of MRI to evaluate changes in body composition throughout pregnancy

2.4.2.1 Air displacement plethysmography

Air displacement plethysmography (ADP), a 2C method, utilizes densitometric principles of lean soft-tissues and fat to estimate FM and FFM.

Plethysmography refers to the measurement of size, usually volume. In ADP, body volume is measured indirectly by determining the volume of air that the body displaces inside an enclosed chamber (plethysmograph). Body volume can be calculated with subtraction of the volume of air outside the body in chamber from the air volume of an empty chamber (Fields et al. 2002). ADP device constitutes of two chambers, one 450-liter chamber where the subject sits, and a reference chamber of 300 liters (Dempster and Aitkens 1995, McCrory et al. 1995) (Figure 3). A diaphragm oscillates between the chambers, creating volume changes that cause tiny pressure alterations, which are monitored by transducers and analyzed for pressure. The test chamber volume is measured as a ratio of these pressures (Fields et al. 2002), as is described by Poisson's Law.

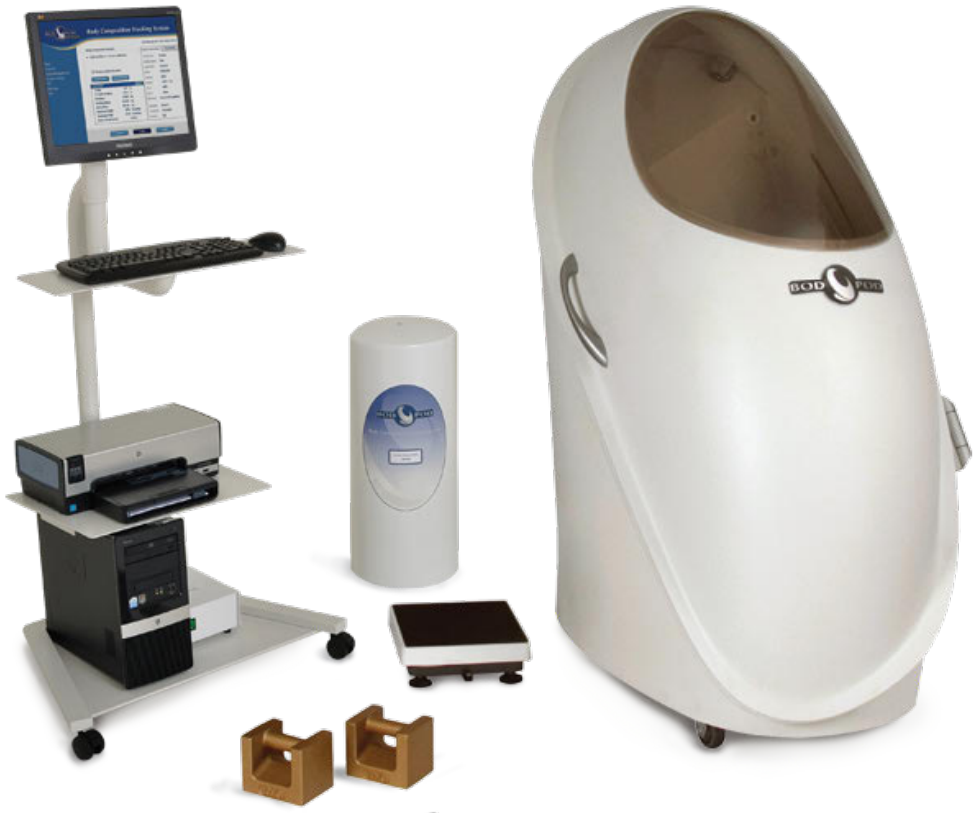

Figure 3. Air displacement plethysmography for measuring body composition (the Bod Pod system, COSMED, Inc., Concord, CA, USA).

However, there is some volume of isothermal air that is contained in the lungs, near to skin or hair, and in clothing that must be accounted for in the calculations of body composition with ADP (Fields et al. 2002). To minimize the error, the subjects are asked to wear tight underwear and a cap, and additionally, a surface area artifact is calculated. The isothermal air in the lungs is considered by either measurement or prediction of thoracic gas volume (TGV).

The results of a previous small study in normal-weight women indicate that the prediction of TGV compared to its measurement leads to an overestimation of BF% by 0.5% at 32 weeks of gestation (Henriksson et al. 2013). Loss and gain of weight have also been discovered to affect the measured TGV in non-pregnant overweight women (Minderico et al. 2008). Nevertheless, recent trials have used predicted TGV in defining FM during pregnancy (Henriksson et al. 2015, Svensson et al. 2016). As both the growing uterus and high BMI might influence the estimation of TGV during gestation, the measurement of TGV could provide more precise estimations of body composition, particularly in pregnant women in upper BMI categories. As explained by Fields et al. (2002), it is important to correct the body volume estimate for the average amount of air in the lungs during normal breathing. Thus, the Bod Pod device determines TGV at mid-tidal exhalation. When the TGV is measured, the subject breathes through a disposable tube while wearing a nose clip. The subject is asked to first breathe normally and later to make a couple of gentle puffs by interchangeably contracting and relaxing the diaphragm. This causes slight changes in the gas volume of the airways, simultaneously with alterations in body volume that are equal but opposite. These volume alterations create pressure changes that are monitored. A comparison of the magnitudes of the changes in airway and chamber pressure allows calculation of TGV (Fields et al. 2002).

Once the body volume and weight have been measured, body density is determined from the ratio of body mass to volume, from which FM and FFM can be estimated combining the assumed respective densities for FM and FFM. In the calculations, it is important to consider the enhanced hydration of FFM caused by pregnancy. Van Raaij et al. (1988) have provided estimates of FFM densities over the course of pregnancy that consider the physiological changes and contribution of the products of conception to the composition of the pregnant body. As a result, the IOM has stated that 2C, 3C and 4C models estimate gestational body composition changes with acceptable precision (Institute of Medicine 2009).

ADP has been considered as a good method to measure adiposity in overweight and obese non-pregnant women (Wingfield et al. 2014). In the study of Marshall et al. (2016), body composition of pregnant women at term was determined with 2-, 3- and 4-compartment models and compared with measurements with dual-energy X-ray absorptiometry (DXA) 2 weeks postpartum. It was discovered that ADP was well accepted in late gestation and, with the use of the Van Raaij equation based on

gestational age (van Raaij et al. 1988), provided the most precise estimate of maternal FM compared with postpartum DXA. The limitations of ADP, like many other methods except imaging techniques, include the failure of measuring the intra-abdominal and visceral fat separately and the inability to differentiate fetal tissues from maternal tissues (Widen and Gallagher 2014). These limitations can be overcome, at least to some extent, by assessing change in sufficiently many subjects and thus minimizing the effect of bias due to the measurement method and the effect of possible individual variation related to results.

For these reasons, and the fact that ADP is rapid, suitable for larger sample sizes, and well accepted by the pregnant women, ADP was chosen to measure maternal body composition in the studies included in this thesis.

2.4.3 Physiological changes during pregnancy

Maternal body composition changes are reflected in the accrual of protein, fat, water, and minerals that are deposited in the fetus, placenta, amniotic fluid, uterus, mammary gland, blood, and adipose tissue (Pitkin 1976). In early pregnancy, smaller changes in maternal FM and FFM occur compared to the larger and variable changes in FM and FFM detected in late pregnancy caused by the growing fetal unit (Institute of Medicine 2009). The IOM also states that it is not completely understood the extent to which the changes in body composition, and particularly in FM, are critical for normal fetal development or are incidental to pregnancy.

The uterine contents (the placenta, fetus and amniotic fluid) constitute about one third of the total GWG at term (Pitkin 1976). At birth, the BF% of human fetus is roughly 12–16%, corresponding to around average 350g of FM (Catalano, P. M. et al. 2003). Most of this fat is accrued in late pregnancy (Eriksson et al. 2011). The rest (84–88%) of fetus is FFM, of which approximately 80% is water. The placenta consists of 88% water, 11% protein, and only 1% fat (Widdowson and Spray 1951). The volume of amniotic fluid varies widely between pregnancies but is rather stable from 22 to 39 gestational weeks with the mean of 800 ml (Brace and Wolf 1989). Thus, it contributes markedly to the increased water accumulation during pregnancy.

Less than 1 kg of protein is accrued in the body during gestation, and predominantly in late pregnancy (Butte et al. 2003, Forsum et al. 1988, Pipe et al. 1979). Protein is deposited mainly in the fetus (42%), but also in the uterus (17%), blood (14%), placenta (10%), and breasts (8%) (Hytten, F. and Chamberlain 1991).

TBW accrual in FFM is largely hormonally regulated during pregnancy (Institute of Medicine 2009). The accumulation has been found to average about 7-8 liters in uncomplicated pregnancies (Hytten, F. E. et al. 1966) and it has not been found to differ systematically among the underweight, normal weight or obese women (Lederman et al. 1997). The large proportion of water accrued relative to GWG leads

to enhanced hydration of FFM throughout pregnancy (Hytten, F. E. et al. 1966, van Raaij et al. 1988).

On average, GWG is 42% FM and 58% FFM (Butte et al. 2003), although there is a marked individual variation. Whereas GWG is positively associated with the gain in FM, FFM gain is rather constant between pregnancies. As with GWG, the FM gain during pregnancy is inversely correlated with prepregnancy BMI. Among women who gain weight in accordance to IOM recommendations, changes in FFM seem to be rather consistent and independent of BMI. Thus, lesser GWG among obese women is almost completely accounted for by a lesser gain in FM (Lederman et al. 1997).

In studies conducted with MRI, Sohlstrom and Forsum (1995) discovered that three fourths of FM gain were deposited subcutaneously during gestation, resembling the fat distribution before pregnancy. During gestation, 68% of the increased adipose tissue volume was in the trunk and 16% in the thighs. However, there is inter-individual variation in the site where fat is stored (Larciprete et al. 2003). Obese women with larger deposits of subcutaneous fat prior to pregnancy tend to accrue FM in visceral depots more than normal weight women over pregnancy (Ehrenberg et al. 2003, Sohlstrom et al. 1993). FM gain has also been shown to correlate positively with postpartum weight retention (Butte et al. 2003). Moreover, it is known that FM accrual in visceral depots and around the central organs is correlated with insulin resistance, cardiovascular disease risk, and metabolic syndrome (Despres 2006).

It has to be recognized that in most studies on gestational body composition, the participating women have had normal prepregnancy BMI, and thus studies with longitudinal changes in FM in overweight and obese women are lacking (Institute of Medicine 2009, Most et al. 2018).

2.4.4 Impact of lifestyle on maternal body composition

Physical activity has been found to reduce GWG and in some studies, also improve blood glucose concentration and prevent GDM (Davenport et al. 2018, Ruchat et al. 2018). However, the impact of physical activity on body composition of pregnant women is largely unknown. It is known that women reduce physical activity markedly during pregnancy (Amezcua-Prieto et al. 2013, Evenson and Wen 2011) and less than half of the women adhere to the physical activity guidelines (Most, Dervis et al. 2019). Along with energy consumed, the reduction of physical activity could have an influence on maternal FM accrual.

Although the relationship between energy intake and GWG has been studied, there is little information in the literature concerning the association of body composition with energy intake during pregnancy. It has been found that pregnant

women may increase their BF% without any significant change in energy intake and even with the increase in total energy expenditure (Abeysekera et al. 2016). The authors suggested that pregnancy alters homeostatic mechanisms, allowing for a more efficient fat storing state mediated by factors additional to those controlling metabolism in the non-pregnant state. However, the reliability of methods assessing energy intake (self-reported food diaries) have been questioned, suggesting that there could be inconsistencies between reported and actual energy intake (Dhurandhar et al. 2015).

Regarding energy intake, Most et al. (2019) have stated that in order to stay within IOM GWG recommendations, obese women must lose 2.5 kg FM and their energy intake should be less than energy expenditure. In their study, the ideal GWG was accomplished by the gain of FFM alone. The consumption of energy of obese pregnant women staying within IOM recommendations did not increase after early pregnancy. Most et al. suggested that the energy demands of the pregnant body with the fetal unit is compensated for by the utilization of maternal FM. It was also proposed that it is the energy imbalance that regulates GWG, and not the diet quality or physical activity. No adverse effects on maternal or fetal outcomes were detected in the group of women with inadequate GWG but it was acknowledged that the sample size was too small to reliably assess these outcomes. In two previous studies, weight loss or GWG< 5kg has been associated with significantly increased risks of small for gestational age infants (Blomberg 2011, Catalano, P. M. et al. 2014). These neonates had also less FM, FFM and a smaller head circumference (Catalano, P. M. et al. 2014), which could be associated with further increases in the metabolic and cognitive risk for the offspring through adaptive processes in utero and programming of health (Catalano, P. M. et al. 2014, Dewey and Begum 2011).

Other lifestyle factors that could affect body composition include some nutritional supplements. The consumption of n-3 LC-PUFA has been suggested to modestly reduce weight and BF% in non-pregnant individuals (Bender et al. 2014). In animal models, evidence indicates a role for n-3 LC-PUFAs in reducing FM (Albracht-Schulte et al. 2018). The literature also indicates that probiotics may help to reduce weight and FM in overweight adults, especially certain strains of Lactobacillus and Bifidobacterium (Borgeraas et al. 2018, Crovesy et al. 2017). The effects of both n-3 LC-PUFA and probiotics on GWG and body composition during pregnancy are largely unknown.

All in all, more information is needed regarding the association of lifestyle factors such as physical activity, dietary intake and diet quality with body composition in pregnant women especially in the upper BMI categories.

2.4.5 Body composition of women with GDM

Although GDM is associated with adiposity, mainly defined as high prepregnancy BMI, little is known about the actual body composition of women diagnosed with GDM. A few studies have evaluated the possibility of early pregnancy body composition to predict the development of GDM. These studies suggest that ultrasonographically measured visceral fat thickness is associated with GDM or higher glucose values (Bartha et al. 2007, De Souza et al. 2016, Gur et al. 2014, Martin, A. et al. 2001). As this is an area with a paucity of literature, further studies are needed to elucidate the impact of GDM on maternal body composition.

2.5 Summary of the literature

As the prevalences of obesity and GDM are rising, it is essential to find new and more efficient means to prevent GDM, reduce maternal adiposity and to enable GWG within recommendations. There is some indication that probiotics and fish oil could exert beneficial effects on glucose control and insulin resistance, but the literature in pregnant women regarding this topic is scanty. As n-3 LC-PUFA and probiotics have immunomodulatory effects and both improve gut barrier integrity, they could also potentially have synergetic effects on the morbidities related to chronic low-grade inflammation and insulin resistance, such as GDM. However, no studies on this subject exist in pregnant women. Further, knowledge related to the factors that influence body composition of overweight and obese pregnant women is limited, and more information is needed to enable development of more efficient means to improve maternal body composition and possibly prevent the pregnancy complications associated with increased adiposity. Surprisingly, as GDM is a common condition, the literature concerning the body composition of women with GDM is almost nonexistent. The studies in this thesis were targeted to fill in these gaps in the literature and to provide new means to prevent GDM.

3 Aims

The objective of this study was to evaluate in a randomized, placebo-controlled trial, the effects of fish oil and probiotic intervention on glucose metabolism and body composition in overweight and obese pregnant women. Additionally, the purpose of this thesis was to characterize the determinants of maternal body composition.

The specific aims were to investigate in overweight and obese women:

1. If an intervention with fish oil and/or probiotics decreases the risk of GDM, fasting glucose concentrations, and insulin resistance, and if it influences GWG and maternal body composition (Studies I and II).
2. Whether a GDM diagnosis affects the accrual of FM during pregnancy, and whether body composition in early pregnancy predicts the development of GDM at approximately 24–28 gestational weeks (Study II).
3. If energy intake, energy yielding nutrients, diet quality and physical activity associate with maternal body composition, and how inadequate, appropriate and excess GWG relate to body composition changes during pregnancy (Study III).
4. Whether the measured TGV differs significantly from predicted TGV in pregnant women and whether the possible difference affects to any significant extent the calculations of body composition (Study IV).

4 Materials and Methods

4.1 Study design, randomization and subject characteristics

4.1.1 Study design

A double-blind, randomized trial on the effects of fish oil and/or probiotic food supplements on maternal and child health was conducted. This single-center trial was executed in the University of Turku and Turku University Hospital in Finland with recruitment between October 2013 and July 2017 (ClinicalTrials.gov Identifier: NCT01922791). The study complies with the Declaration of Helsinki. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol and all participants provided written informed consent.

4.1.2 Recruitment and randomization

Leaflets with the study information were distributed in maternal welfare clinics. Furthermore, media and social media were used to inform about the study. Those women willing to participate in the study contacted the project coordinator for further information and to schedule their first study visit. Eligible women were randomly assigned to one of the four parallel groups at the first study visit in early pregnancy: fish oil+placebo (i.e. placebo for probiotics), probiotics+placebo (i.e. placebo for fish oil), fish oil+probiotics or placebo+placebo (placebo for probiotics and placebo for fish oil). Subjects were allocated into intervention groups according to the mother's parity and their history of GDM (primipara; multipara; multipara with previous GDM). The stratified randomization was performed with random permuted blocks of 4, and randomization lists of the three blocks were generated by a statistician who was not involved in either study recruitment or its execution. Women were assigned to the intervention groups according to the randomization list in their order of recruitment on the first study visit. The staff responsible for enrollment of participants, study visits and assessing outcomes remained blinded to the intervention, as were the participants.

4.1.3 Participants

A total of 988 women from Southwest Finland were screened for eligibility and 439 women were randomized to the intervention (Figure 4). The study was discontinued by 39 (8.9%) women before the OGTT and altogether by 59 (13.5%) women before the late pregnancy study visit. A few test results were unavailable because of failure to fast before blood tests (n=4) or interruption of the OGTT (n=2) or giving birth prematurely (n=6).

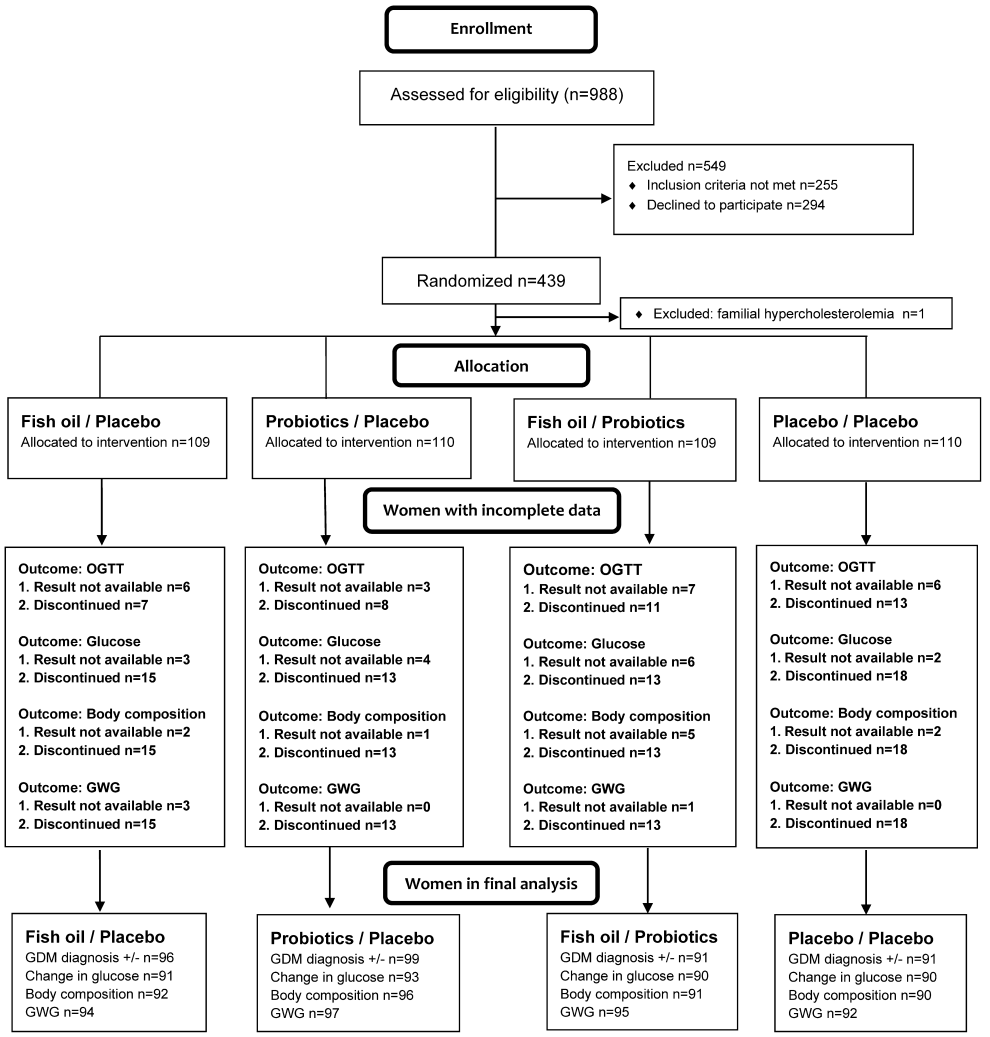

Figure 4. Flow diagram of the study. Modified from Pellonperä et al., studies I and II.

In studies III and IV, the data from 110 women that were the first to recruit and whose body composition was measured at the two visits during the pregnancy was utilized. In studies I and II, the available data collected from all the recruited women was utilized. The inclusion criteria of the study were: self-reported prepregnancy BMI≥25 kg/m^2, less than 18 gestational weeks and absence of chronic diseases (asthma and allergies were allowed). Exclusion criteria were: pregestational diabetes (HbA1c ≥6.5% [48 mmol/mol] or fasting glucose ≥7.0 mmol/l at randomization); multifetal pregnancy; chronic diseases impacting on metabolic and gastrointestinal health including inflammatory bowel diseases; refusal to terminate the intake of other probiotic or fish oil supplements; diagnosis or history of coagulopathy; use of anticoagulants.

4.2 Study conduct

Women attended two study visits during gestation (mean 13.9±2.1 and 35.2±0.9 gestational weeks). On the first study visit, height was measured with a wall stadiometer to the nearest 0.1cm. Prepregnancy BMI was calculated using height and self-reported prepregnancy weight obtained from the maternal welfare clinic records. Weight, body composition and blood pressure were measured on both visits and blood samples were obtained.

Women filled in questionnaires and were interviewed concerning their health, education, smoking habits, obstetric medical history and family history of diabetes. Questionnaires concerning physical activity and dietary quality were filled in. Furthermore, three-day food diaries were recorded during the week preceding the study visits. During the intervention period, women were asked to keep a diary on a weekly basis to report possible adverse effects related to supplement consumption. Data on pregnancy and delivery were collected from hospital medical records.

4.3 Food supplements

Food supplements were provided from the first study visit, throughout the pregnancy and until 6 months postpartum. Women were advised to take two fish oil capsules and one probiotic capsule daily. The stability of the supplements was monitored by both manufacturers regularly during the trial. All capsules were identically packaged and identified by trial codes. Women were asked not to consume any other probiotic and n-3 LC-PUFA or other oil products during the study.

4.3.1 Fish oil supplements

The fish oil capsules (Croda Europe Ltd., Leek, UK) contained a total of 2.4 g of n-3 fatty acids, of which 79% (1.9 g) DHA (22:6 n-3,) and 9.4% (0.22 g) EPA (20:5 n-3,), the rest being other n-3 fatty acids including docosapentaenoic acid. Placebo capsules for fish oil contained an equal amount of medium-chain fatty acids (capric acid C8 54.6% and caprylic acid C10 40.3%) and were of the same size, shape, color and lemon flavor as the fish oil capsules. The oil capsules were stored at room temperature.

4.3.2 Probiotic supplements

Probiotic capsules contained *Lactobacillus rhamnosus* HN001 (ATCC SD5675; Dupont, Niebüll, Germany) and *Bifidobacterium animalis* ssp. *lactis* 420 (DSM 22089; Dupont, Niebüll, Germany), each 10^{10} CFU per capsule. Placebo for the probiotics consisted of microcrystalline cellulose; the capsules were identical to the probiotic capsules in size, shape, and color. Capsules were stored at -20°C until provided to the subjects, who were instructed to store the capsules in a refrigerator.

4.3.3 Compliance

Compliance with the consumption of capsules was assessed first by a phone call at mean 28 gestational weeks, subsequently by interview at the second study visit (good compliance being defined as taking study capsules ≥5 days/week reported at both time points), and thirdly, by counting the numbers of consumed fish oil capsules i.e. subtracting the capsules returned to the study unit from the total provided by a random sample of 62 women (14% of participants).

4.4 Diagnosis and management of GDM

GDM was diagnosed based on a 2-hour 75 g OGTT if one or more values were at or above the threshold level: 0h ≥5.3, 1h ≥10.0, 2h ≥8.6mmol/l according to the Finnish Current Care guidelines (Finnish Current Care guidelines for gestational diabetes 2013). OGTT was offered by maternal welfare clinics to all women between 24–28 gestational weeks and to high-risk women also at 12–16 gestational weeks (BMI≥35 kg/m^2, previous GDM, glucosuria, polycystic ovary syndrome or family risk of diabetes). We used also the diagnostic criteria from the IADPSG with the following diagnostic thresholds: 0h ≥5.1, 1h ≥10.0, 2h ≥8.5 mmol/l. Regardless of the timing of OGTT, treatment for GDM was offered soon after diagnosis by health care services independent of the research protocol and in accordance with the national guidelines.

4.5 Blood sampling and analysis

On the morning of each study visit, after at least 9-hours of overnight fasting, blood samples were drawn from an antecubital vein. A certified laboratory (TYKSLAB, the Hospital District of Southwest Finland) was used for the sampling, with analyses of glucose and insulin conducted by an enzymatic method utilizing hexokinase and by immunoelectrochemiluminometric assay, respectively. Insulin resistance was assessed by calculating HOMA2-IR from fasting glucose and insulin concentrations (Wallace et al. 2004).

4.6 Body composition and gestational weight gain

4.6.1 Determination of maternal body composition

On both study visits, ADP and an electronic scale (the Bod Pod system, software version 5.4.0, COSMED, Inc., Concord, CA, USA) were applied to define the weight and the volume of the body according to the manufacturer's instructions. FM and FFM in kilograms were calculated from density using the formulas devised by van Raaij et al. (van Raaij et al. 1989), which consider the gestational age and the presence of marked general swelling if necessary. When possible, TGV was measured (n=385/438 in early pregnancy and n=341/369 in late pregnancy) and used in the calculations of FM and FFM, otherwise predicted TGV was used. Women were asked to fast overnight, wear a tight cap and tight underwear and empty their bladder before the measurement of body composition. They were also advised to avoid exercise and showering in the morning of measurements.

4.6.2 GWG

Women were classified into groups of excess, appropriate and inadequate GWG according to the recommendations issued by the IOM (Institute of Medicine 2009). We assessed 1) the weight gain during the pregnancy and 2) weekly weight gain rate.

1) Weight gain during the whole pregnancy was calculated as a difference of the last measured weight in the third trimester (either at maternity welfare clinic or at the second study visit, whichever visit was the latest) and self-reported prepregnancy weight. This weight gain was compared to IOM recommendations issued for overweight and obese women.

2) In study III, the actual measured weight gain between the visits was compared to recommended minimum and maximum weight gains considering the gestational age and the recommended weight gain between the two visits. The IOM recommendation assumes a weight gain of 0.5–2 kg in the first trimester for all

women, and during the 2nd and the 3rd trimesters, overweight women (pre-pregnancy BMI 25.0–29.9 kg m^{-2}) are recommended to gain 0.23–0.33 kg per week and obese women (pre-pregnancy BMI≥30.0 kg m^{-2}) 0.17–0.27 kg per week (Institute of Medicine 2009). Enrollment of some women in the first trimester (i.e. ≤ 13+0 gestational weeks) was considered by multiplying the proportion of gestational weeks that was left from the first trimester with either 0.5 kg (recommended minimum) or 2.0 kg (recommended maximum) and by adding these first trimester weight gains respectively to recommended minimum or maximum weight gains during the 2nd and 3rd trimesters of pregnancy.

In study II, we used the same method as described above to assess the weight gain, but additionally, we also categorized the weekly GWG rate between the first study visit and the last gestational weight measurement.

4.7 Lifestyle

4.7.1 Total energy and energy yielding nutrients

Women were asked to keep a food diary over 2 weekdays and 1 weekend day in three consecutive days during the week preceding the study visits. They were given oral and written instructions on how to fill in the food diary and diaries were checked by the research personnel for completeness and accuracy with the help of an illustrated portion booklet. Mean daily intakes of energy (megajoules) and energy yielding nutrients (grams and percentage of energy intake, E%) were calculated by using computerized software (AivoDiet 2.0.2.3;Aivo, Turku, Finland) utilizing the food composition database provided by the Finnish National Institute for Health and Welfare (www.fineli.fi).

4.7.2 The quality of diet

IDQ (index of diet quality) questionnaire was used to evaluate the quality of overall diet on both study visits (Leppala et al. 2010). This validated questionnaire consists of several questions concentrating on the frequency and amount of consumption of certain food products during the preceding week. The health promoting criteria included the following: use of wholegrain bread (≥4 slices/per day), saturated/unsaturated fatty acids (vegetable oil-based margarine on bread, fish at least twice a week, use of low-fat ≤1% dairy products), dairy products (at least 4 dl/ day), consumption of vegetables, fruits and berries (≥400 g/day), and use of sugar containing drinks and sweets, and less than 2 skipped meals/week. The quality of the diet was defined as poor when index points were <10/15 and good when points were ≥ 10/15 (Leppala et al. 2010).

4.7.3 Physical activity

Physical activity was evaluated with a questionnaire (Mansikkaniemi et al. 2012). Subjects were requested to report the intensity, frequency and duration of their habitual leisure-time physical activity during the preceding week. A metabolic equivalent index for leisure-time physical activity (MET-index) was calculated from the product of intensity x frequency x duration of activity (MET h/wk) on both study visits. The coefficients for the intensity of physical activity were estimated from the existing tables (Ainsworth et al. 1993).

4.8 Fetal weight and the amount of amniotic fluid

Fetal weight was estimated ultrasonographically (Medison Accuvix XQ, Seoul, South Korea) in late pregnancy (35.3±1.1 gestational weeks) by determining fetal abdominal circumference, femur diaphysis length and biparietal diameter and applying the Hadlock II formula (Hadlock et al. 1985). The amount of amniotic fluid was estimated by measuring the amniotic fluid index (AFI), which involves summing the deepest vertical pockets in each of four quadrants of the uterus (Phelan et al. 1987).

4.9 Power calculations and statistical analyses

4.9.1 Power calculations

In this trial, power calculations were executed for the primary outcomes of study I, i.e. the incidence of GDM based on the OGTT result targeted at 24–28 weeks of gestation and the change in fasting plasma glucose between the early and late pregnancy study visits. The sample size was calculated on the basis of the main outcome variables (power of 80% and significance level $p<0.05$). Based on a 20% reduction in the incidence of GDM in the fish oil or probiotic group from 50% to 30% (Chu et al. 2007, Luoto et al. 2010) and a further 5% decrease in the combined intervention group (from 50% to 25%), a sample size of 93 per group was estimated. For fasting plasma glucose levels, a sample size of 50 subjects per group was calculated in order to detect a treatment effect of -0.2 mmol/l in glucose assuming that the standard deviation was 0.35 (Laitinen et al. 2009). We aimed to recruit 440 volunteers to the study (110 in each intervention group), allowing for 20% dropout.

Prespecified secondary outcomes without power calculations included the change in insulin and HOMA2-IR-values, the need for medication in the management of GDM (insulin or metformin), maternal body composition and GWG.

4.9.2 Statistical analyses

In all studies, the normality of the data was checked visually from histograms. The data were summarized as frequencies and percentages for categorical variables and as means and standard deviations for normally distributed continuous variables. For non-normally distributed variables, medians and interquartile ranges were utilized. In cases where differences were reported, 95% confidence intervals were calculated. Statistical significance was set at p-value<0.05. Analyses were performed using either SAS software (version 9.4, SAS Institute Inc., Cary, NC, studies I and II) or with IBM SPSS statistics version 22.0 for Windows (IBM SPSS Inc. USA, Chicago, IL, USA, studies III and IV).

4.9.2.1 Study I

Our analysis was by intention to treat. The comparisons of baseline characteristics, OGTT-test result, GDM diagnosis and maternal/neonatal outcomes among the intervention groups were conducted by one-way ANOVA for continuous variables and $\chi 2$ test or Fisher's exact test for categorical variables, when applicable. Differences in the change of glucose, insulin and HOMA2-IR were also compared with one-way ANOVA. General Linear models with binomial distribution and log link function was used to compare the relative risk of GDM in each intervention group with the placebo+placebo group. The modifying effect of potential confounding factors on the effect of the intervention (confounding factor×group interaction effect) on the incidence of new GDM diagnoses was analyzed using the generalized linear model. Two-way ANOVA was used to analyze the modifying effect of potential confounding factors on the effect of the intervention (confounding factor×group interaction effect) on the change in fasting plasma glucose, insulin and HOMA2-IR. Again, we used $\chi 2$ test or Fisher's exact test to compare differences among the intervention groups with respect to compliance, number of women discontinuing the study and adverse effects. The comparison of duration of the side effects and postpartum hemorrhage between the intervention groups was conducted with the nonparametric Kruskal-Wallis test.

4.9.2.2 Study II

The comparisons of baseline characteristics among the intervention groups as well as the effect of the intervention on GWG and body composition were conducted by one-way ANOVA for continuous variables and χ^2 test or Fisher's exact test for categorical variables, when applicable.

The energy consumption among the intervention groups was compared with one-way ANOVA. Physical activity was measured with the MET-index, which was not

normally distributed and hence, the median with interquartile range was calculated and Kruskal-Wallis test applied when the intervention groups were compared.

GDM and non-GDM women were compared at baseline with two-sample t-test, χ^2 test or Fisher's exact test, when applicable. When GWG and body composition between GDM women and non-GDM women were compared, for continuous variables we used two-sample t-test and a linear model adjusted for variables that differed between the groups significantly at baseline and that were significantly associated with the measured outcome. Likewise, categorical variables were analyzed with χ^2 test and, in addition, with logistic regression adjusted for confounding variables. As a result, adjustments were made for age, prepregnancy BMI, previous GDM, intervention group and for the GWG analyses, also for gestational weeks at the last weight measurement.

Possible correlations between lifestyle variables and changes in body composition measures in all women, in women with GDM and in normoglycemic women, were assessed using partial Pearson's correlation test. Correlations of at least a medium effect size ($r \geq 0.3$) were considered meaningful (Cohen 1992).

4.9.2.3 Study III

Paired samples t-test was applied to calculate the change between early and late gestation in body composition variables or daily energy/macronutrient intakes. Wilcoxon signed ranks test was used to assess the potential changes in physical activity (MET-index). When comparing the differences in body composition between women with inadequate, appropriate or excess weight gain, we used one-way ANOVA with Games-Howell post-hoc tests. When comparisons of energy intake, IDQ- or MET-indices were made between subgroups of different GWG and BMI categories, one-way ANOVA and independent samples t-test were applied for normally distributed data and Mann Whitney and Kruskall-Wallis tests for non-normally distributed data.

Possible correlations between lifestyle variables or ultrasound measurements and body composition measures (outcome variables) were assessed using partial Pearson's correlation test adjusting for gestational age and pre-pregnancy BMI. In adjustments, gestational age at the point of measurement of each variable were used and when the change was evaluated, we also adjusted for weeks between early and late gestation visits. Associations between MET-index and body composition (outcome variable) were assessed with Spearman's correlation test without adjustments, since no correlation between body composition variables and gestational weeks or pre-pregnancy BMI existed. Correlations of at least medium effect size ($r \geq 0.3$) were reported (Cohen 1992). We also conducted linear models assessing the relationship between explanatory lifestyle variables (physical activity,

dietary quality and macronutrient intake) and the change in either FM, FFM or BF% (outcome variables). We adjusted all models for intervention group, gestational weeks at enrollment, age, height, and for each early gestation body composition variable.

4.9.2.4 Study I V

In the comparisons, paired samples t-test was used to calculate the difference in measured TGV between early and late gestation and to estimate differences between measured and predicted TGV in body composition results at both time points.

5 Results

5.1 Characteristics of women

A high compliance rate related to the intervention was reported by 88.4% of the women with this number being similar in the four intervention groups (p>0.98, data not shown). The compliance calculated from the returned fish oil capsules indicated that a mean of 91.8% (SD 15.9) of the capsules had been consumed.

The clinical characteristics of the women at baseline are presented in Table 5. Baseline characteristics did not differ among the intervention groups, except for a family history of diabetes that was more common in women in the fish oil+placebo group as compared to the probiotics+placebo and placebo+placebo groups (p=0.012). Women participating in the study were generally in good health, although some mild medical conditions including allergy and/or atopy (20.5% of the women), asthma (8.8%), migraine (8.8%) and hypothyroidism (7.0%) were reported (all non-significant between the intervention groups). OGTT, scheduled for all women, was performed at mean±SD 26.4±2.2 gestational weeks, when the duration of the intervention was a mean of 12.5±3.1 weeks. At any stage of pregnancy, 119 (30.0%) and 175 (43.8%) of the participants were diagnosed with GDM with Finnish and IADPSG criteria, respectively. At 24–28 gestational weeks, 94 (24.9%) women developed GDM according to the Finnish criteria and 145 (38.3%) with the IADPSG criteria.

5.2 Intervention with fish oil and probiotics

5.2.1 Incidence of GDM in the intervention groups

No significant difference in the incidence of GDM at mean 26 gestational weeks was found between the intervention groups (p>0.76 with both criteria) (Figure 5). Furthermore, after excluding the early OGTT positive women from the analysis, no differences in the incidence of new GDM diagnosis or OGTT values were detected between the groups (p> 0.58 with both criteria).

Table 5. Characteristics of the women.

	n*	Mean ± SD or n (%)
Age	110/109/109/110	30.6 ± 4.6
Prepregnancy weight (kg)	110/109/109/110	82.8 ± 13.5
Prepregnancy BMI (kg/m²)	110/109/109/110	29.7 ± 4.2
overweight		266 (60.7)
obese		172 (39.3)
Primipara	110/109/109/110	210 (47.9)
Ethnic region	110/109/109/110	
European		430 (98.2)
Asian		2 (0.5)
Middle Eastern		3 (0.7)
other/mixed		3 (0.7)
College or university education	100/94/99/98	239 (61.1)
Previous gestational diabetes	110/109/109/110	40 (9.1)
Family history of diabetes	93/89/94/86	61 (15.6)
Smoking during pregnancy	100/95/98/98	19 (4.9)
Gestational weeks at 1st visit	110/109/109/110	13.8 ± 2.1
Gestational weeks at 2nd visit	93/95/92/92	35.2 ± 0.9
Gestational weeks at last weight measurement	95/96/95/92	38.1 ± 2.1

Modified from Pellonperä et al. 2019, studies I and II. *order of the groups: fish oil + placebo, probiotics + placebo, fish oil + probiotics, placebo + placebo

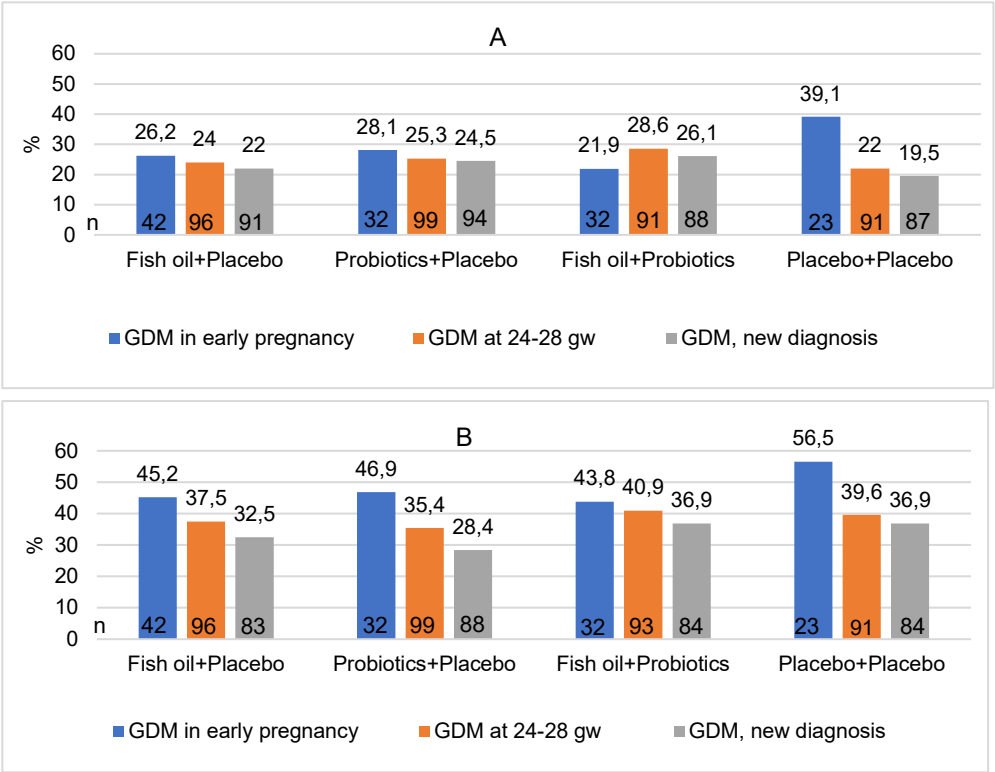

Figure 5. Incidence of GDM (%) according to the intervention groups. New diagnosis= the early OGTT positive women excluded from the analysis A) Finnish criteria B) IADPSG criteria. No significant differences between the intervention groups were detected (p>0.55 for all comparisons, χ^2 test).

In early gestation, 132 women at high risk for GDM were referred to OGTT (after randomization) at a mean 14.7±2.0 gestational weeks and 36 (27.9%) were diagnosed with GDM according to the Finnish criteria, and 61 (47.3%) according to the IADPSG criteria.

Every fifth (24/119, 20.2%) woman diagnosed with GDM (Finnish criteria) needed insulin or metformin for the management of GDM (p=0.33 between the intervention groups). The effect of the intervention on the incidence of new GDM diagnoses at 24–28 weeks of gestation was not influenced by confounding factors including compliance or duration of the intervention, maternal age <35 or ≥35 years, prepregnancy BMI, gestational weight gain between study visits, consumption of fish oil or probiotic supplements before randomization, family history of diabetes, or previous GDM (p>0.05 for confounding factor × group interactions in all comparisons).

5.2.2 Glucose and insulin concentrations

Fasting glucose concentrations decreased, and the insulin concentrations and HOMA2-IR rose significantly in all intervention groups from early to late gestation (Figure 6). However, no significant differences were discovered in the change of glucose or insulin concentrations or HOMA2-IR between the intervention groups (p>0.1).

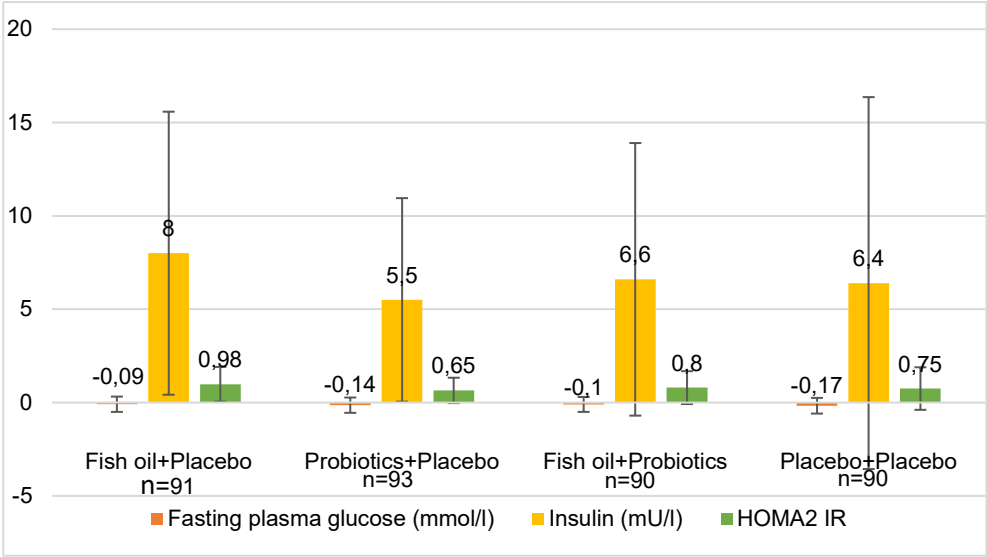

Figure 6. Change in fasting glucose, insulin and HOMA2-IR concentrations between early and late pregnancy study visits according to the intervention groups (mean and sd). No significant differences between the intervention groups were found (p>0.11 for all comparisons, One-way ANOVA).

5.2.3 GWG and body composition in intervention groups

GWG was not influenced by the fish oil and/or probiotic intervention (Table 6). The proportions of women either exceeding, falling below or adhering to the GWG recommendations were also essentially similar between the groups. Additionally, we found no differences among the intervention groups in body composition at the first or the second study visit, or in the change of body composition between the visits (Table 6).

The change in physical activity or the dietary intake of energy did not differ among the intervention groups (p>0.3 in both comparisons).

Table 6. Gestational weight gain (GWG) and body composition in the intervention groups.

	n	Fish oil + Placebo	Probiotics + Placebo	Fish oil + Probiotics	Placebo + Placebo	p
GWG						
GWG between 1st and 2nd visit (kg)	94/95/92/92	9.3 ± 3.5	9.3 ± 3.8	9.2 ± 4.0	9.2 ± 4.3	1.00*
GWG from randomization to the end of pregnancy (kg)	95/96/95/92	12.2 ± 4.3	11.7 ± 4.7	11.8 ± 5.4	11.7 ± 5.4	0.93*
Ideal weekly GWG rate from randomization n (%)	95/96/95/92	3 (3.2)	14 (14.6)	9 (9.5)	11 (12.0)	0.17†
Excess weekly GWG rate from randomization n (%)	95/96/95/92	87 (91.6)	78 (81.3)	79 (83.2)	74 (80.4)	
Inadequate weekly GWG rate from randomization n (%)	95/96/95/92	5 (5.3)	4 (4.2)	7 (7.4)	7 (7.6)	
Body composition						
Δ Body fat percentage (% points)	93/95/91/90	-2.4 ± 2.2	-2.6 ± 2.7	-2.2 ± 2.5	-2.5 ± 2.9	0.80*
Δ Fat mass (kg)	93/95/91/90	1.7 ± 3.1	1.5 ± 3.5	1.8 ± 3.4	1.7 ± 4.0	0.96*
Δ Fat free mass (kg)	93/95/91/90	7.5 ± 2.0	7.8 ± 2.4	7.4 ± 2.2	7.6 ± 2.2	0.67*

*one-way ANOVA, † χ^2 test. Women were divided into different GWG classes according to the recommendations issued by Institute of Medicine (2009). Modified from Pellonperä et al., study II.

5.2.4 Maternal and neonatal outcomes

There were no differences in maternal pregnancy outcomes among the intervention groups, including numbers of miscarriages, hypertensive complications, preterm or post term deliveries, or mode of delivery (p>0.23 in all comparisons). In addition, there was no difference in the postpartum hemorrhage (ml) or in the number of women bleeding over 1000ml among the intervention groups (p>0.82 in both comparisons). Furthermore, the neonatal outcomes did not differ among the intervention groups (including numbers of 5 min Apgar scores and birth weight,

macrosomia, congenital malformations, hypoglycemia, or admittance to neonatal intensive care unit, p>0.05 in all comparisons).

5.3 Body composition

5.3.1 GWG and body composition

The mean GWG between prepregnancy and the last measurement (1.6±1.6 weeks before delivery) was 13.0 ± 6.3 kg (n=378). With respect to the weight gain between the study visits, 18% was FM and 82% FFM. On average, FM and FFM increased 1.7 ± 3.5 kg and 7.6 ± 2.2 kg between the study visits respectively, thus BF% decreased by 2.4 ± 2.6 percentage points. Overweight women gained significantly more weight and FM than obese women (p<0.001 in both comparisons), while there was no difference in terms of FFM gain (p=0.34) (Figure 7). Consequently, the proportion of body fat decreased in both overweight and obese women, but significantly more in obese women (-1.8 ± 2.6 vs. -3.4 ± 2.3 percentage points, p<0.001).

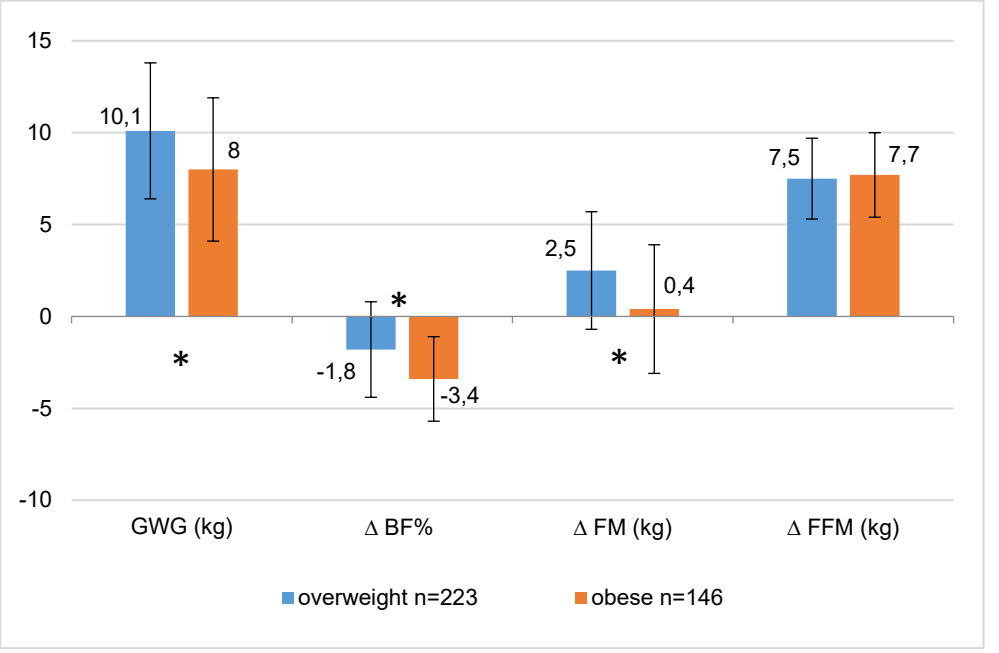

Figure 7. Weight and body composition changes in overweight and obese women (mean and sd). GWG= gestational weight gain, ΔBF%= change in body fat percentage, ΔFM=change in fat mass, ΔFFM=change in fat free mass. *p< 0.001 (One-way ANOVA).

5.3.2 Thoracic gas volume (TGV)

The measurement of TGV was successful in 100/110 women in early gestation and 106/110 women in late gestation. From early to late pregnancy, measured TGV reduced significantly (p=0.0002). The use of predicted instead of measured TGV led to a significant overestimation of 0.37 kg and 0.75 kg of FM in early and late pregnancy, respectively (Figure 8). As predicted TGV was higher in both early and late pregnancy (6.3% and 12.6% respectively), FM and BF% values calculated with the predicted TGV were 0.8% and 1.1% higher in early pregnancy, and 2.6% and 2.0% higher in late pregnancy, respectively (p≤0.002 for all comparisons). In studies I and II, measured TGV was used in the calculations of body composition whenever possible (385/438 in early gestation and n=341/369 in late gestation).

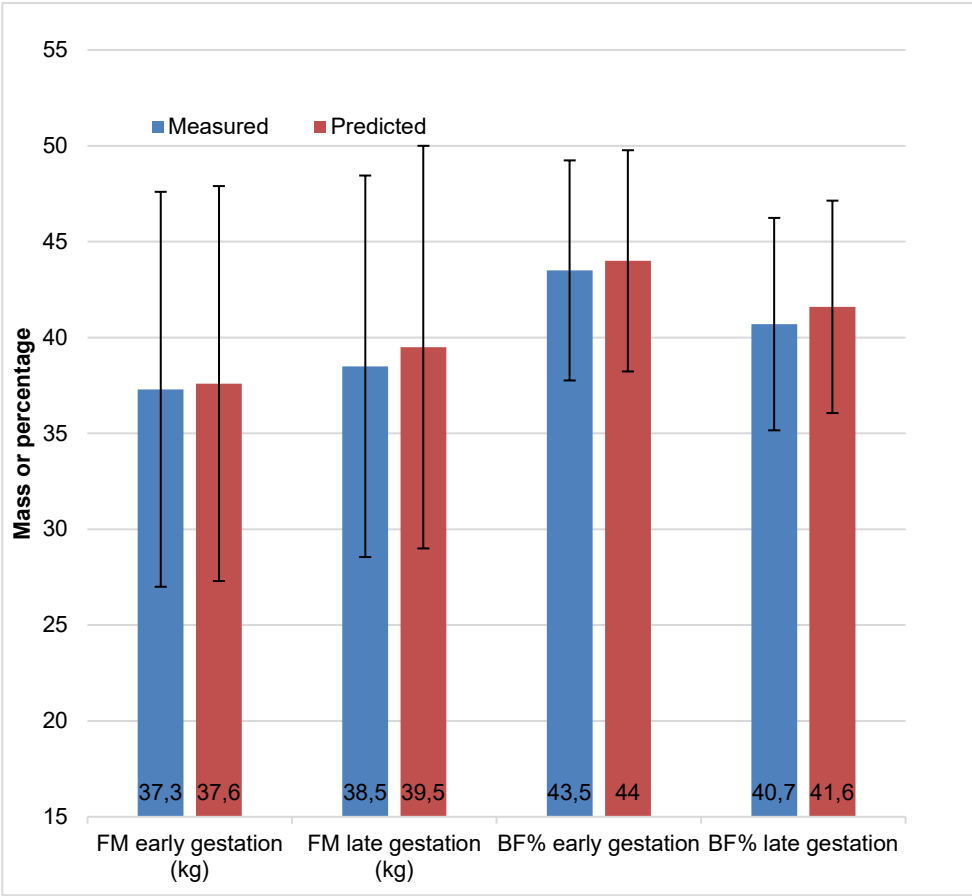

Figure 8. Mean fat mass (FM) and body fat percentage (BF%) calculated with measured and predicted thoracic gas volume (significant difference in all comparisons, p≤0.002, paired samples t-test). Data were available for 100 and 106 participants at the first and second study visits, respectively.

5.3.3 Weight gain according to the recommendations of the Institute of Medicine

Most women, 77% (85/110), exceeded the recommended weight gain rate whereas 11% gained weight inadequately. Only women with excess GWG gained FM on average (Figure 9). Although subjects with excessive GWG rate also gained more FFM, BF% diminished significantly less than in subjects with appropriate GWG.

Women with inadequate GWG lost significantly more FM than women with appropriate weight gain, whereas no difference in the change of FFM was detected.

No significant difference was detected in the energy intake, dietary quality or physical activity among the groups of appropriate, inadequate and excess GWG ($p \geq 0.092$ in all comparisons).

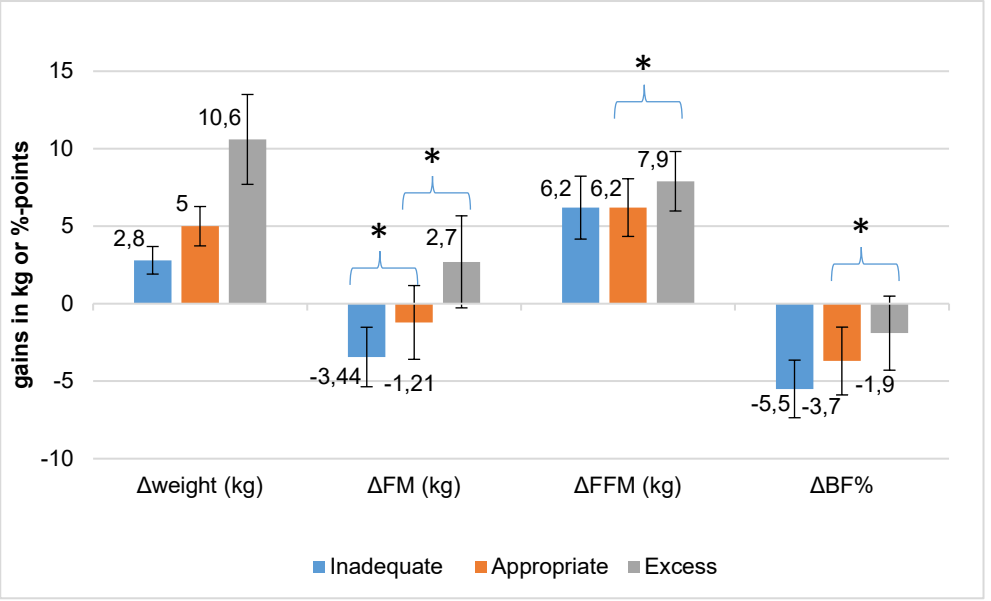

Figure 9. Adherence to the IOM GWG recommendations between the study visits vs. change in body composition (means and standard deviations). GWG= gestational weight gain, ΔBF%= change in body fat percentage, ΔFM=change in fat mass, ΔFFM=change in fat free mass. Inadequate GWG n=12 (10.9%), appropriate GWG n= 13 (11.8%), excess GWG n=85 (77.3%). * $p<0.05$. Inadequate and excess weight gains are compared with appropriate weight gain (one-way ANOVA with Games-Howell post-hoc tests).

5.3.4 Lifestyle

5.3.4.1 Diet

The daily consumption of energy did not change significantly between the early and late gestation (p=0.4). Nonetheless, the daily consumption of fat enhanced and consumption of carbohydrates as a proportion of energy intake (E%) reduced significantly over the follow-up (Figure 10).

No significant change was discovered in the dietary quality (IDQ points) between early and late gestation (9.8±2.2 vs. 9.8±2.1, p=0.81). The quality of diet was assessed as poor in 50 % of the women in early gestation and 42% of the women in late gestation.

The poor quality of diet was reflected in maternal body composition, as the amount of FFM was reduced in subjects with a poor quality of diet as compared to those subjects with good dietary quality (in early gestation 45.8±4.7 kg vs. 48.8±5.8 kg, p=0.004 and in late gestation 53.4±5.6 kg vs. 56.1±6.4 kg, p=0.025).

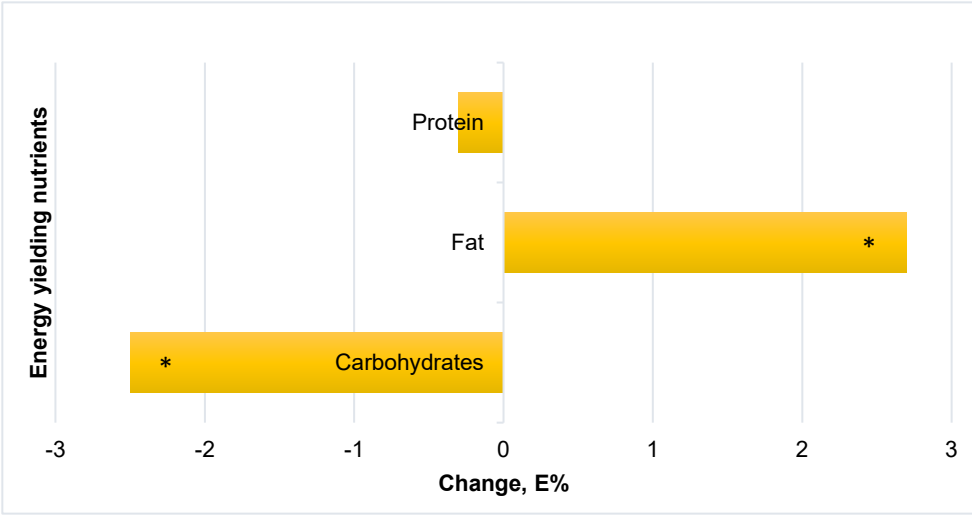

Figure 10. Change in the intakes of energy yielding nutrients from early to late pregnancy. *p= 0.001(fat), p= 0.005 (carbohydrates), Paired samples t-test. Data obtained from 3-day food diaries filled in by 99 women in both early and late gestation.

Regarding the energy yielding nutrients, protein intake (g) was found to correlate positively with FFM in early and late pregnancy (r=0.31, p=0.002 and r=0.39, p<0.001 respectively). No other correlations were discovered between body composition measures and intakes of other macronutrients, total energy intake or dietary quality.

5.3.4.2 Physical activity

Between the two study visits, physical activity of the women was reduced from the median of 5.0 h/week (IQR 2.0–12.0) to 3.0 h/week (IQR 0.2–11.0), $p<0.001$.

Body composition variables did not correlate with physical activity or the change in physical activity in either early or late gestation (data not shown). Furthermore, no significant associations were discovered in a linear model assessing the relation of physical activity with a change in body composition (data presented in Pellonperä et al. 2019, study III, Supplementary Table 2).

5.3.5 GDM

Compared to the women without GDM, the women diagnosed with GDM at any stage of pregnancy were significantly older, less educated, had higher prepregnancy weight and BMI, had more often a history of GDM and parents with diabetes ($p<0.034$ for all comparisons). The women with GDM also had their last weight measurement before delivery earlier in gestation than non-diabetic women. These factors were accounted for in the adjustments of the results, when the weight and body composition of healthy women and women with GDM were compared. As knowledge of GDM diagnosis potentially affects the lifestyle behavior of the women, only Finnish criteria were applied, when weight and body composition were analyzed.

Women with GDM gained less weight than women without GDM between the study visits ($p<0.001$, Figure 11). The proportion of women with excessive weekly weight gain rate was greater and the proportion of inadequate weekly weight gain rate lower in women without GDM than in women with GDM (adjusted $p=0.004$).

In early pregnancy, women diagnosed with GDM at any stage of pregnancy had more FM and greater BF% than women without GDM ($p<0.02$ in both comparisons). However, after adjusting for confounding factors, especially with prepregnancy BMI, the differences were no longer statistically significant. At the second visit in late gestation, women with an established GDM had less FM and lower BF% than women without GDM (adjusted $p=0.003$ and $p= 0.026$ respectively). The change in body composition from early to late pregnancy was also significantly different between women with GDM and non-diabetic women: less FM was gained and BF% was reduced more in women with GDM ($p<0.001$ and $p=0.011$, respectively), while no difference between the groups was found in the change of FFM (Figure 11).

When the early gestation OGTT positive women were removed from the analyses (n=27), the adjusted results remained essentially same, i.e. the body composition at the first visit did not differ between non-diabetic women and women who later developed GDM.

Correlations between lifestyle variables (change in energy intake, carbohydrates, fat, protein and physical activity assessed by MET-index) and change in body composition were not detected in all women, or in women with or without GDM ($r<0.21$).

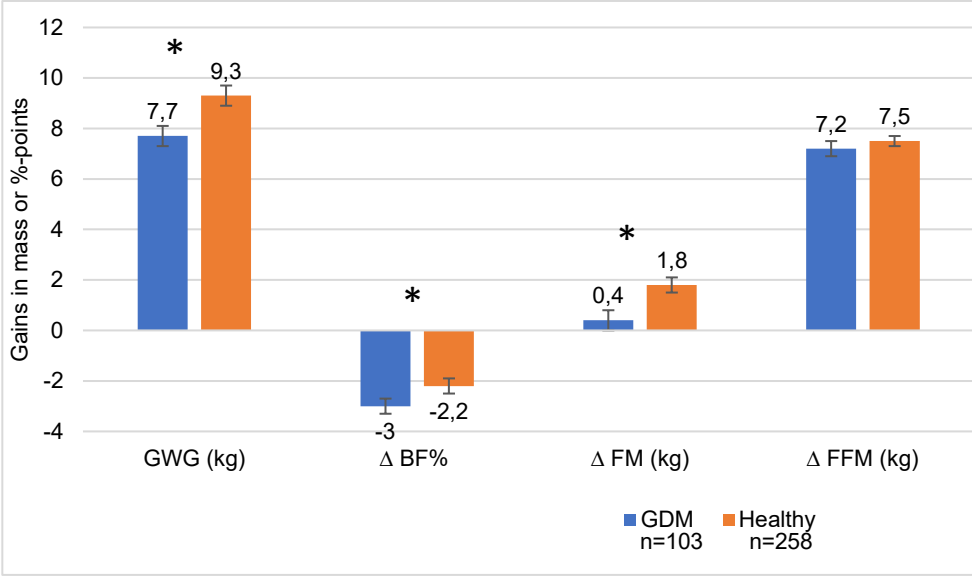

Figure 11. GWG and the change in body composition in women with and without GDM (adjusted means and standard errors). GWG and body composition variables were assessed as gains between the two study visits. GWG= gestational weight gain, ΔBF%= change in body fat percentage, ΔFM=change in fat mass, ΔFFM=change in fat free mass. *Significant difference analyzed with linear model adjusted for age, prepregnancy BMI, previous GDM and intervention group.

5.3.6 Fetal weight and amniotic fluid

The average estimated fetal weight was 2623 g (± 327 g) and mean amniotic fluid index (AFI) was 12.7 cm (±3.75 cm) at mean 35 gestational weeks (n=103). Estimated fetal weight correlated with maternal FFM in early and late pregnancy ($r=0.31$, $p=0.003$ and $r=0.36$, $p<0.001$ respectively), and with the change of maternal FFM ($r=0.34$, $p<0.001$). In contrast, there was no correlation between AFI and body composition measures ($r<0.16$, $p>0.1$).

6 Discussion

Obesity and GDM are worldwide problems that challenge the care of pregnant women. The perception that maternal physiology, body composition, and lifestyle exerts a profound and lasting influence on their offspring's long-term health through metabolic programming, has been supported by solid data from epidemiological, medical and basic science studies (Barker and Thornburg 2013, Fleming et al. 2018). This theory of ´Developmental Origins of Health and Disease', presents a vicious cycle as offspring of the obese mothers with GDM are more prone to obesity, hypertension and disturbances in glucose metabolism. Thus, it would be most desirable to break this cycle, as well as reduce maternal short- and long-term complications. However, the prevention of GDM has proven to be very difficult.

Interventions to prevent or take care of these conditions are an area of intensive research, and several new studies emerged also during the conductance of our trial. Different interventions have been tried, but with limited or no success. Unfortunately, the trial presented in this thesis with fish oil and probiotics falls within this category of ineffectual interventions. As also proposed by others (Egan and Simmons 2018, Fleming et al. 2018), GDM and the resulting programming of offspring health may only be prevented if interventions are initiated before conception or at the latest, in the first trimester. However, novel information of the effects of lifestyle, GWG and GDM on maternal body composition was provided in the studies presented here.

6.1 Intervention with fish oil and probiotics

Previous studies with n-3 LC-PUFA and probiotics indicate that these food supplements may help in the management of glucose levels in women with GDM (Gao et al. 2018, Karamali et al. 2016, Kijmanawat et al. 2018), and in the case of probiotics, also prevent GDM (Laitinen et al. 2009, Luoto et al. 2010, Wickens et al. 2017). In the present trial, no benefit was detected on glucose metabolism or on the incidence of GDM with these food supplements as compared to placebo. Only one large study has been published on the effect of n-3 LC-PUFA on the prevention of GDM as a main outcome, this appearing after the planning of present trial with fish oil and probiotics had been started. The results were similar as presented here,

although the dose of DHA and EPA were considerably smaller (Zhou et al. 2012). With regard to probiotics, the positive findings mentioned above have been challenged by two trials (Callaway et al. 2019, Lindsay, K. L. et al. 2014). Resembling the results presented here, these studies showed that a) *L. Salivarius* and b) *L. Rhamnosus* and *Bifidobacterium lactis* consumed by overweight/obese women do not improve glucose metabolism or prevent GDM.

With respect to loss of weight and FM, several recent systematic reviews and meta-analyses focusing on overweight or obese non-pregnant adults have found that probiotics are beneficial (Borgeraas et al. 2018, Crovesy et al. 2017). Similarly, the consumption of fish oil has been suggested to reduce weight and BF% in non-pregnant individuals and in animals (Albracht-Schulte et al. 2018, Bender et al. 2014). In pregnant women, studies measuring body composition in a probiotic or fish oil intervention are almost non-existent. Therefore, new information was generated in this trial. Unfortunately, no improvements in maternal weight or body composition with these food supplements were detected compared to placebo.

This is the first time that the potential synergistic benefits of combining n-3 LC PUFA with probiotics have been investigated in pregnant women. The published literature on this topic is scanty; one previous study demonstrated promising synergistic results on insulin sensitivity in a population of healthy overweight adults (Rajkumar et al. 2014). Furthermore, there is previous experimental evidence indicating that a combination of these two active components might exert synergistic immunoregulatory effects (Kastel et al. 2007).

The reason why no intervention effect was detected, separately or in combination, remains unknown, although several explanations can be speculated. It may be that the metabolic burden of obesity in our recruited women was so severe that it could not be overcome by the potential interventional effect in regulating glucose metabolism. Indeed, a previous study demonstrated more pronounced changes in both microbiota and markers of low-grade inflammation with increasing BMI (Houttu et al. 2018). Future analyses of inflammatory markers (hs-CRP and α1-acid glycoprotein) of this study population will reveal if the intervention had any effect on low-grade inflammation. With regard to n-3 LC-PUFA, genetic variation may influence responsiveness to EPA and DHA, although the available evidence is relatively limited (Madden et al. 2011). Furthermore, the baseline concentrations of DHA and EPA in this study population are yet to be determined. If the concentrations were sufficiently high at the beginning, it could explain why n-3 LC-PUFA supplementation had very little influence on the measured outcomes. It has also been suggested that the ratio of EPA and DHA could influence the effects of an n-3 LC-PUFA intervention, as a meta-analysis in patients with type 2 diabetes revealed that a high ratio of EPA to DHA could be beneficial in terms of glucose control (Chen, C. et al. 2015). This may be one reason why the fish oil intervention failed to exert

any glucose regulating benefit, as DHA was the dominant fatty acid in the fish oil consumed by the women. Other causes for the absence of an intervention effect could be related to the dose, timing and duration of intervention, compliance and the probiotic strains used.

6.2 Factors affecting maternal body composition

6.2.1 TGV

According to previous studies conducted with ADP in non-pregnant individuals, TGV can be predicted, instead of measured, without significantly altering the body composition results (Collins and McCarthy 2003, Otterstetter et al. 2015), although a weight change may influence the measured TGV (Minderico et al. 2008).

In the present study, compared to measured TGV, the usage of predicted TGV in body composition calculations led to an overestimation of FM and BF% that increased from around 1% to 2% during pregnancy. These results are in line with a former study pointing out that the prediction of TGV results in a relatively small but significant overestimation of BF% by 0.5% at 32 gestational weeks in subjects with normal BMI (Henriksson et al. 2013). The greater overestimation in the present study could be caused by the obesity of the women participating in this study, and also the more advanced gestational age at the time when the late pregnancy body composition measurements were made. These results also indicate that the overestimation increases during the pregnancy, which can be due to the growth of the uterus that might influence TGV by elevating the diaphragm. The finding of Most et al. (2018) suggesting that measurement of TGV by BodPod is difficult in pregnant women, was not supported by this study, as successful measurement was attained in 96% of women in late pregnancy.

Numerous factors, such as fasting state, clothing and emptiness of the bladder must be considered when body composition is measured with ADP. Pregnancy poses further challenges to the measurement of body composition, as specific gestational age and the increased hydration of FFM related to it have to be taken into account. As every attempt should be made to standardize and control all the factors possibly causing inaccuracies in the measurements, also the measurement of TGV can be recommended.

6.2.2 Dietary intake and quality

When the lifestyle determinants of body composition in this study are considered, good quality of diet and daily consumption of protein were found to positively correlate with FFM in early and in late gestation. The role of FFM during pregnancy

is not well defined, although it is suggested that maternal FFM correlates with offspring birth weight and FFM (Henriksson et al. 2015, Kent et al. 2013). Indeed, as 85-90% of fetal weight is FFM (Catalano, P. M. et al. 2003, Koo et al. 2000), fetal tissues comprise a significant proportion of maternal FFM gain, which averaged 7.6 kilograms in late pregnancy in this study.

FFM consists mainly of water and protein, and is the main determinant of the basal metabolic rate, which in turn, is an essential component of total energy expenditure (Hronek et al. 2011, Lof et al. 2005, Pipe et al. 1979). Protein intake has been suggested to decrease GWG as a result of higher energy expenditure because the thermogenesis of protein is larger than that of carbohydrate or fat (Raben et al. 2003). In the current study, the consumption of fat was elevated above the recommended gestational dietary reference intakes issued by the IOM (Institute of Medicine 2002/2005). Meanwhile, the consumption of carbohydrates decreased, and although the consumption of protein stayed constant, it was not at a particularly high level. The protein requirement is increased during pregnancy in order to deliver sufficient amino acids for protein synthesis in maternal, fetal, and placental tissues. In a study with rodents, a maternal diet with a low protein intake has been shown to be related to changes in the pancreatic islets of Langerhans of the offspring, possibly resulting in glucose intolerance in later life (King et al. 2019). Furthermore, the replacement of carbohydrates with fat may increase lipolysis, free fatty acid concentrations, and maternal insulin resistance (Hernandez et al. 2018). All things considered, instead of increasing fat intake, sufficient protein consumption and intake of high-quality nutrient-dense carbohydrates could exert health benefits for both mother and child.

6.2.3 GWG

Compared to the previous literature also involving overweight and obese women (Berggren et al. 2016, Deputy et al. 2015), in the present study, even a greater proportion of subjects exceeded the GWG recommendations. The excess GWG led to increased body adiposity, which is in line with previous research (Berggren et al. 2016, Widen et al. 2015). Furthermore, the results of the present study are also in agreement with the concept that both the GWG and the increase in FM are inversely proportional to the pre-pregnancy BMI, as overweight women gained significantly more weight and FM than obese women. Interestingly, the recommended ideal GWG was reached by the gain of FFM alone, as also reported by Most et al. (2019). In women with ideal and inadequate GWG, the energy demands for fetal growth were likely to be met by the mobilization of maternal FM.

While the negative impact of excess GWG on pregnancy complications and offspring health is clear, the harmfulness of inadequate weight gain among the

overweight and obese women is still under debate (Catalano, P. 2018, Oken et al. 2009). However, there is an indication that in obese women, the weight loss or gain <5 kg is associated with an increased risk of small for gestational age infants and decreased neonatal FM, FFM, head circumference, and, also with a higher risk of hypertension and insulin resistance in future life (Catalano, P. M. et al. 2014, Tam, C. H. T. et al. 2018). In present study, 11% of women gained weight inadequately and lost significantly more FM (3.4 vs 1.2 kg) as compared with women with an appropriate weight gain, which could affect the future growth and cardiometabolic health of their children.

6.2.4 GDM

The body composition of women with GDM has not been widely examined. A few studies have evaluated the possibility of early pregnancy body composition as a way of predicting the development of GDM. These studies have indicated that ultrasonographically measured visceral fat thickness is associated with GDM or higher glucose values (Bartha et al. 2007, De Souza et al. 2016, Martin, A. M. et al. 2009). Likewise, a few studies conducted with bioimpedance analysis have reported that truncal fat gains between 15–28 gestational weeks or FM and BF% measured at 21–24 gestational weeks are related to the onset of GDM (Sommer et al. 2014, Xu et al. 2016). Our results indicate that after adjustment for BMI and other confounding factors, body composition measured with ADP in early pregnancy is not different between women who later develop GDM and women without GDM.

Regarding late pregnancy, the data presented here on body composition of women with GDM is novel. Our results indicate that in late pregnancy, the adiposity of diabetic women falls below that of women without GDM. The reason for this finding remains unclear. The most logical explanation would be that the treatment of GDM would result in positive lifestyle changes and improvements in body composition. However, we could not find any correlation between lifestyle changes and body composition changes in those women who developed GDM as compared to those not displaying this condition. Previously, a small study has been conducted to investigate the relationship of GDM and changes of body composition over pregnancy (Ehrenberg et al. 2003). These researchers used hydrodensitometry to measure body composition before conception, at 12–14 gestational weeks and at 33–36 gestational weeks. Although the trial included only 19 patients with GDM and 33 controls, the gains of FM tended to be smaller and BF% reduced in diabetic women ($p=0.08$ and $p= 0.07$ respectively), which is in line with our results. While it is unclear what causes the greater weight gain in healthy women as compared to diabetic women, it does seem clear that the excess weight gained is mainly FM and not FFM.

6.2.5 Fetal weight and amniotic fluid

In this study, fetal weight estimation correlated positively with maternal FFM explaining around 13% of the variation in FFM. This indicates that fetal tissues, which are mainly FFM (Catalano, P. M. et al. 2003, Koo et al. 2000), should be considered when interpreting the FFM results. Instead, the amount of amniotic fluid was not related with maternal FFM. This could be attributable to an inaccuracy in the AFI-method reflecting the true amniotic fluid volume (Phelan et al. 1987), a variation in hydration of maternal tissues and small variation in AFI values, as 99% of subjects had an AFI within the normal range.

6.3 Strengths and limitations

This was a well conducted prospective trial with a large sample size and a small drop-out rate. Regarding the nutritional intervention, this was also a double-blind, placebo-controlled trial. Nonetheless, some limitations are acknowledged. The study was only powered for primary outcomes of the food supplement intervention (incidence of GDM and fasting glucose in late pregnancy), and not for secondary outcomes such as body composition or GWG. However, when inspecting the results of body composition among the intervention groups with high p-values, and the great variance in body composition, it is unlikely that even a considerably larger sample size would have yielded statistically significant or clinically meaningful differences. Regarding the relation of GDM to body composition, further research is needed to confirm the findings of this study.

Limitations relate also to the method of measuring body composition. ADP does not allow for the estimation of alterations in intra-abdominal or visceral fat, which could have provided meaningful information especially regarding the comparisons of diabetic and non-diabetic women. Furthermore, fetal tissues cannot be distinguished from maternal tissues. This might influence the findings, as fetal growth is heterogeneous especially in late gestation and, additionally, there are variations in the amount of amniotic fluid and the size of the placenta. The same challenges apply in general for the analysis of body composition during pregnancy, but nevertheless ADP has been proposed to be the preferred method for the determination of maternal FM in late gestation and provides more accurate information on body adiposity than BMI (Marshall et al. 2016).

In addition, the compliance with the intervention could have been more precisely determined; especially the biomarkers related to the consumption of fish oil supplements could have been measured. The concentrations of plasma phospholipid fatty acids of the women participating in this trial are still to be analyzed.

With regard to the evaluation of the lifestyle, diet and physical activity were evaluated only twice during the pregnancy which could be a study limitation; more

frequent exploration could improve the accuracy in evaluating the associations with the body composition, although, the compliance in recordings could also be hampered. Moreover, the reliability of methods assessing energy intake (self-reported food diaries) has been questioned, suggesting that there could be inconsistencies between reported and actual energy intake (Dhurandhar et al. 2015). However, the reliability should be increased by the fact that repeated measurements were conducted on the same women and we had a relatively large sample size.

Another limitation may be attributable to the recruitment procedure in which the women contacted the study personnel, and consequently it is likely that these women were more motivated to pay attention to their lifestyle than the average overweight and obese women. Nonetheless, the majority of our subjects gained weight in excess of the IOM guidelines, and even more often than indicated in the previous literature, which may partly contradict this argument. Additionally, a control group of normal weight women could also have provided important information in comparisons related to the intervention, as previous positive effects on glucose metabolism were detected in women with a predominantly normal BMI. However, considerably more women would have been required to be recruited to maintain the statistical power.

Interventions to reduce the risk of GDM are proposed to be more efficient, if initiated in the first trimester or even before (Egan and Simmons 2018). The recruitment of overweight and obese women only planning a pregnancy was also considered when this trial was designed, but the execution of this design was thought to be both difficult and impractical. However, many steps were taken to recruit women as early as possible, which resulted in a mean duration of intervention of over 12 weeks, which must be considered as reasonably good as compared to most of the trials conducted with these food supplements.

As the effects of probiotics can be strain specific and the effects of n-3 LC-PUFA dose dependent, the use of other strains and different doses could have yielded different results. However, there was indication from the previous study that these probiotics, namely *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12, could exert favorable effects on glucose concentrations (Laitinen et al. 2009).

Since there was little knowledge regarding the role of fish oil and probiotics in pregnant women, and the body composition determinants of overweight and obese women with, or without GDM, new information was generated in the studies included in this thesis.

6.4 Clinical implications

According to the results of this study, the utilization of the strains of probiotics and the dose and ratio of n-3 LC-PUFA used in this trial cannot be recommended to be consumed to improve glycemic status or to reduce adiposity in overweight and obese

pregnant women. If the previous literature (Callaway et al. 2019, Zhou et al. 2012) about this issue is considered alongside the results of this study, the evidence clearly implies that these interventions do not appear to yield benefits in terms of glucose metabolism, at least in overweight and obese pregnant women. Fortunately, no safety concerns were raised in this study, which is important when the possible use of these food supplements are considered for other indications, particularly to improve child health.

In this study, fat intake increased above recommendations alongside with excess GWG and FM accrual, while the carbohydrate intake decreased. Therefore, it is proposed that overweight and obese women are counselled to avoid augmentation of fat, especially saturated fat, while sufficient intakes of high-quality nutrient-dense carbohydrates and protein should be ensured. These adjustments to the diet are already possible within current nutritional recommendations (Institute of Medicine 2002/2005, Nordic Council of Ministers 2014), and thus may be implemented by counseling the pregnant women. However, to achieve appropriate GWG, obese women should gain no FM, and the energy intake of obese women should not increase during pregnancy (Most et al. 2019).

6.5 Future aspects

The intervention trial with fish oil and probiotics was designed to investigate the effects of the intervention on maternal and child health. Probiotics have been proposed to reduce maternal postpartum depression and eczema of the child, and fish oil supplementation may reduce preterm birth and sensitization to certain foods in the child (Garcia-Larsen et al. 2018, Middleton et al. 2018, Slykerman et al. 2017). The possible effects of this on-going trial on outcomes other than GDM and maternal body composition, remain to be examined in further analyses of the data. Particularly, the potential benefits on child health over both the short and the long term need to be evaluated.

The literature concerning the body composition of women diagnosed with GDM is almost non-existent. Future studies will be required to confirm our discovery of reduced adiposity of women with GDM as compared to women without GDM and to investigate the role of treatment and other probable causes behind this finding. Another area of interest that needs further clarification is to identify the factors that contributed to either the appropriate or the inadequate GWG in the overweight and obese women participating in this trial.

The harmful role of excess maternal fat on pregnancy outcomes has been clearly demonstrated. The results of this study raised a question of the role of maternal FFM on pregnancy outcomes. Further studies are necessary to assess the impact of maternal FFM on outcomes other than birth weight.

It seems clear that there are no easy ways to reduce maternal adiposity and the pregnancy complications related to it, when women are already pregnant. Therefore, interventions to normalize body composition should be introduced earlier, probably already during childhood and as a part of health education in school. Enlightened health-policy decisions will be required to promote this goal, in conjunction with inexpensive healthy eating and sufficient physical activity.

7 Conclusions

1. Although safe, the administration of n-3 LC PUFA (1.9g DHA and 0.22g EPA) and/or *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* ssp. *lactis* 420 does not reduce the risk of GDM, improve fasting glucose concentrations or reduce insulin resistance as compared to placebo in overweight and obese women (Study I). Additionally, this intervention does not affect GWG or maternal body composition as compared to placebo (Study II).
2. Women with GDM gain less FM than normoglycemic women. The body composition of overweight and obese women in early pregnancy does not predict the development of GDM at approximately 24–28 gestational weeks. (Study II).
3. Dietary protein intake and better diet quality were positively correlated with maternal FFM, while energy intake and physical activity were not associated with body composition. Compared to women with appropriate GWG, women with excess GWG gained more FM while women with an inadequate GWG lost more FM. Even appropriate GWG results in a mean reduction of FM in obese women. (Study III).
4. Measured TGV differs significantly from predicted TGV in pregnant women in early and late gestation. Measurement of TGV provides more accurate estimates of maternal adiposity than can be attained by prediction of TGV. (Study IV).

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