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INTERRELATIONS BETWEEN MICROBIOTA, METABOLISM AND LOW-GRADE INFLAMMATION WITH OBESITY AND GESTATIONAL DIABETES MELLITUS:

Means to intervene during pregnancy

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To my family

UNIVERSITY OF TURKU
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NOORA HOUTTU: Interrelations between microbiota, metabolism and low-grade inflammation with obesity and gestational diabetes mellitus: means to intervene during pregnancy
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ABSTRACT

Pregnant women with overweight and obesity may exhibit altered circulating low-grade inflammatory and metabolic profile and gut and vaginal microbiota which may contribute to the development of gestational diabetes mellitus (GDM). These are further exaggerated in obesity. In this thesis, the aim was to investigate the interaction of low-grade inflammation, metabolism and gut and vaginal microbiota in pregnant women with obesity and overweight and whether these are related to the onset of GDM. Further, the effect of dietary intervention with fish oil and/or probiotics on these factors were studied.

The study involved pregnant women with overweight and obesity (n = 99–434) in early and late pregnancy. Blood, faecal and vaginal samples were analysed for low-grade inflammatory and metabolic markers (metabolites, phosphorylated insulin-like growth factor binding-protein 1 (phIGFBP-1), IGFBP-1, active matrix metalloproteinase 8 (aMMP-8) and fatty acids) and gut and vaginal microbiota and vaginal aMMP-8, respectively. The women were randomised into four intervention groups (fish oil+placebo, probiotics+placebo, fish oil+probiotics, placebo+placebo) from early pregnancy onwards. The fish oil capsules contained 2.4 g of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs): 1.9 g docosahexaenoic acid, 0.22 g eicopentaenoic acid, and the remaining amount other n-3 fatty acids and probiotics *Lacticaseibacillus rhamnosus* HN001 and *Bifidobacterium animalis* ssp. *lactis* 420, each with 10^{10} colony-forming units per capsule.

In early pregnancy, the pregnant women with obesity had higher level of low-grade inflammatory markers and distinct metabolic profile and gut microbiota as compared to the women with overweight. Low serum phIGFBP-1, IGFBP-1 and high serum n-3 LC-PUFAs in early pregnancy were related to the onset of GDM. Some vaginal bacterial genera and species were related to GDM. The fish oil and/or probiotics intervention did not influence low-grade inflammation, serum phIGFBP-1, IGFBP-1 or serum/vaginal aMMP-8 but it reduced the relative abundance of some bacterial genera and species, namely fish oil reduced *Ureaplasma urealyticum*, probiotics *Ureaplasma*, *U. urealyticum* and *Prevotella disiens*, fish oil+probiotics *Dialister invisus* and *P. timonensis*, in vagina and increased serum n-3 LC-PUFAs.

In conclusion, obesity alters inflammatory and metabolic profile and gut microbiota in pregnant women. Serum phIGFBP-1, IGFBP-1 and serum n-3 LC-PUFAs as well as vaginal microbiota in early pregnancy were related to the onset of GDM. The fish oil and/or probiotics resulted lower relative abundance of potential pathobionts in vagina.

KEYWORDS: overweight, obesity, pregnancy, low-grade inflammation, metabolism, gut microbiota, vaginal microbiota, fatty acids

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TIIVISTELMÄ

Ylipaino ja lihavuus voivat nostaa veren matala-asteisen tulehduksen merkkiaineita ja johtaa aineenvaihdunnan epätasapainoon ja muuttaa mikrobistoa. Raskauden aikana nämä muutokset voivat aiheuttaa raskausdiabetesta. Tässä väitöskirjassa oli tavoitteena tutkia matala-asteisen tulehduksen, aineenvaihdunnan ja mikrobiston yhteyksiä keskenään sekä niiden yhteyttä raskausdiabeteksen puhkeamiseen ylipainoisilla sekä lihavilla raskaana olevilla naisilla. Lisäksi, tavoitteena oli tutkia kalaöljyn ja/tai probioottien vaikutusta näihin kolmeen eri tekijään.

Tutkimukseen osallistui ylipainoisia ja lihavia raskaana olevia naisia (n = 99–434) alku- ja loppuraskaudessa. Verinäytteistä analysoitiin matala-asteisen tulehduksen ja aineenvaihdunnan (aineenvaihdunnallinen profiili ja fosforyloitu insuliinin kaltaista kasvutekijää sitova proteiini 1 (phIGFBP-1), IGFBP-1 ja aktiivinen matriksin metalloproteinaasi 8 (aMMP-8) sekä rasvahappoja) merkkiaineita ja uloste- ja emätinnäytteistä mikrobiston koostumus. Tutkittavat satunnaistettiin neljään interventioryhmään (kalaöljy+lume, probiootit+lume, kalaöljy+probiootit, lume+lume) alkuraskaudessa. Kalaöjykapselit sisälsivät 2.4 g n-3 pitkäketjuisia monityydyttyneitä rasvahappoja (n-3 LC-PUFA) (1.9 g dokosaheksaeenihappoa ja 0.22 g eikosapentaeenihappoa) ja probioottikapselit *Lacticaseibacillus rhamnosus* HN001 ja *Bifidobacterium animalis* ssp. *lactis* 420 -kantojen bakteereja (10^{10} pesäkkeen muodostavaa yksikköä/kanta).

Matala-asteinen tulehduksen ja monien rasva-aineenvaihdunnan merkkiaineiden tasot veressä sekä suoliston Prevotellacaea-bakteerin suhteellinen osuus olivat korkeammat lihavilla verrattuna ylipainoisiin naisiin alkuraskaudessa. Loppuraskaudessa raskausdiabetekseen sairastuneiden naisten veren phIGFBP-1- ja IGFBP-1-tasot sekä emättimen eräiden bakteerien osuudet olivat matalammat ja veren n-3 LC-PUFA-tasot korkeammat alkuraskaudessa verrattuna naisiin, jotka eivät sairastuneet raskausdiabetekseen. Interventio vähensi emättimen *Ureaplasma*-, *Prevotella*- ja *Dialister*-suvun bakteereja ja nosti n-3 LC-PUFA-tasoja veressä.

Lihavuus voi muuttaa matala-asteista tulehdusta ja aineenvaihduntaa sekä mikrobistoa. Matalat phGFBP-1 ja IGFBP-1-tasot sekä muuttuneet n-3 LC-PUFA-tasot olivat yhteydessä raskausdiabetekseen. Kalaöljy ja/tai probiootit laskivat mahdollisesti haitallisten bakteerien määrää emättimessä.

AVAINSANAT: ylipaino, lihavuus, raskaus, matala-asteinen tulehdus, aineenvaihdunta, suoliston mikrobisto, emättimen mikrobisto, rasvahapot

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Main abbreviations

AA	Arachidonic acid
ALA	α-linolenic acid
aMMP-8	Active matrix metalloproteinase 8
BCAAs	Branched chain amino acids
BMI	Body mass index
CEs	Cholesteryl esters
CFU	Colony forming units
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
DGLA	Dihomo-γ-linolenic acid
GDM	Gestational diabetes mellitus
GlycA	Glycoprotein acetylation
hs-CRP	High sensitivity C-reactive protein
IGF-1	Insulin-like growth factor 1
IGFBP-1	Insulin-like growth factor binding-protein 1
IL-6	Interleukin 6
LA	Linoleic acid
LC-PUFAs	Long-chain polyunsaturated fatty acids
LPS	Lipopolysaccharide
NEFAs	Non-esterified fatty acids
NMR	Nuclear magnetic resonance
MUFAs	Monounsaturated fatty acids
PC	Phosphatidylcholine
phIGFBP-1	Phosphorylated insulin-like growth factor binding-protein 1
PUFAs	Polyunsaturated fatty acids
rRNA	Ridosomal ribonucleic acid
SFAs	Saturated fatty acids
TAGs	Triacylglycerols
TNF-α	Tumour necrosis factor α
VLDL	Very low density lipoprotein

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 Houttu N, Mokkala K, Laitinen K. Overweight and obesity status in pregnant women are related to intestinal microbiota and serum metabolic and inflammatory profiles. *Clinical Nutrition*, 2018; 6: 1955-1966.*

II Houttu N, Mokkala K, Koivuniemi E, Pellonperä O, Juhila J, Sorsa T, Laitinen K. The Impacts of fish oil and/or probiotic intervention on low-grade inflammation, IGFBP-1 and MMP-8 in pregnancy: a randomized, placebo-controlled, double-blind clinical trial. *Biomolecules*, 2020; 1: 5.

III Houttu N, Mokkala K, Saleem WT, Virtanen S, Juhila J, Koivuniemi E, Pellonperä O, Tertti K, Luokola P, Sorsa T, Salonen A, Lahti L, Laitinen K. Potential pathobionts in vaginal microbiota are affected by fish oil and/or probiotics intervention in overweight and obese pregnant women. *Biomedicine & Pharmacotherapy*, 2022; 149: 112841.

IV Houttu N, Vahlberg T, Miles EA, Calder PC. The impact of fish oil and/or probiotics on serum fatty acids and the interaction with low-grade inflammation in pregnant women with overweight and obesity: a randomized controlled trial. Manuscript.

* This article was published in Clinical Nutrition, Volume 37, Noora Houttu, Kati Mokkala and Kirsi Laitinen, Overweight and obesity status in pregnant women are related to intestinal microbiota and serum metabolic and inflammatory profiles, Pages 1955-1966, Copyright © Elsevier Ltd and European Society for Clinical Nutrition and Metabolism (2018).

1 Introduction

Currently, overweight and obesity affects 49.1% and 17.0% of Finnish pregnant women, respectively (N.B., 49.1% includes pregnant women with obesity, Official Statistics of Finland, 2020), and has increased during the last decade (Official Statistics of Finland, 2020). Overweight and obesity during pregnancy predisposes pregnant women to gestational diabetes mellitus (GDM) which is a serious condition affecting almost 16% of pregnancies (Official Statistics of Finland, 2020) and having an impact on maternal and child health. Indeed, obesity itself can be defined as a chronic relapsing progressive disease (Bray *et al*, 2017). GDM increases the risk for adverse pregnancy outcomes, such as macrosomia and shoulder-dystocia (Metzger *et al*, 2008), and the offspring have heightened risk for metabolic diseases in later life (Baird *et al*, 2017). GDM is diagnosed only during pregnancy and it is based on detection of glucose intolerance. Obesity during pregnancy and further diagnosed GDM may be considered as serious public health emergencies.

Low-grade inflammation has been detected in subjects with overweight and obesity and it has been suggested that low-grade inflammation may mediate the development of obesity related metabolic diseases, such as GDM in pregnancy (McElwain *et al*, 2021). It is of note that, the impact of the degree of overweight or obesity on low-grade inflammation is not clearly established and thus the studies are lacking. Other factors that contribute to the obesity mediated health effects are host's microbiota, and in particular gut microbiota may play a role (Cani *et al*, 2012). In addition, vaginal microbiota has vast importance on women's health. In pregnancy, it has been showed that altered i.e., dysbiotic vaginal microbiota may be associated even to preterm birth (Fettweis *et al*, 2019). However, the exact bacterial genera and species which contribute to the health, in particular in pregnancy with overweight and obesity and GDM, are unrevealed and more research are needed on this topic. Furthermore, the previous studies show that also metabolic profile is strongly linked to the subjects' increased adiposity (Payab *et al*, 2021) but less is known about the metabolic profile in pregnant women with overweight and obesity or GDM, and the impact of the degree of overweight or obesity on metabolic profile is not extensively studied. Thus, understanding the role of obesity, low-grade inflammation, gut and

vaginal microbiota in overweight/obesity and GDM would allow planning and setting up new interventions.

One opportunity to impact the risk for overweight and obesity associated metabolic conditions could be fish oil and probiotics. Fish oil is rich in n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), which are crucial during pregnancy since they support the visual and cognitive development of the foetus (Larqué *et al*, 2012) and have beneficial effects on glycaemic control in GDM (Gao *et al*, 2020). Similarly, probiotics may offer a mean to regulate glucose metabolism (Laitinen *et al*, 2009) and reduce the incidence of GDM (Luoto *et al*, 2010). Findings from diet intervention studies suggest the dietary interventions can decrease fasting glucose and lower the need for medication treatment in GDM (Yamamoto *et al*, 2018) as well as the incidence of GDM (Sparks *et al*, 2022), but the success is variable. Thus, new means for preventing GDM are called for. Due to low-grade inflammatory phenotype of overweight and obesity, n-3 LC-PUFAs and probiotics would benefit subjects with overweight and obesity as n-3 LC-PUFAs and probiotics show inflammation reducing capacity (Mokkala *et al*, 2017). However, the research in pregnant subject is lacking, and less is known about the synergistic effects of both fish oil and probiotics. Simultaneously, by identifying the pregnant women in increased risk for GDM, means for preventing the disease could be offered to those women specifically.

In this thesis the overall aim was to investigate the interaction of low-grade inflammation, metabolism and gut and vaginal microbiota in pregnant women with overweight and obesity and whether these are related to the onset of GDM. Further, the effect of dietary intervention with fish oil and/or probiotics on these factors were studied.

2 Review of the Literature

In this literature review, the current research on low-grade inflammation, gut and vaginal microbiota and metabolic profile and metabolic markers, these including IGFBP-1 and MMP-8 and fatty acids, in pregnant women with overweight and obesity will be covered, followed by a review of previous studies on the impact of fish oil and probiotics on these aforementioned factors in pregnant women.

2.1 Low-grade inflammation

Immunity can be divided into innate and adaptive immunity, which are activated, by acute infection or trauma leading to rapid responses as well as rapid resolution of the responses. In comparison to acute inflammatory responses, low-grade inflammation is persistent low level systemic inflammatory response to altered metabolism in overweight and obesity (Gregor & Hotamisligil, 2011). Low-grade inflammation arises from several tissues, including gut, adipose tissue, pancreas and liver (Cani *et al*, 2007; Ehses *et al*, 2007; Xu *et al*, 2003) as well as placenta (Hauguel-de Mouzon & Guerre-Millo, 2006). In these tissues, various cells exhibit boosted inflammatory signalling pathways leading in increased cytokine production, and simultaneously, these tissues can exhibit increased immune cell infiltration (Gregor & Hotamisligil, 2011). Innate immune cells infiltrating in the tissues include proinflammatory macrophages (Xu *et al*, 2003). Also, the immune cells of adaptive immunity, i.e. T and B cells, are increased (Pantham *et al*, 2015). Due to the boosted inflammatory signalling pathways in the cells, increased pro-inflammatory cytokines, such as interleukin 6 (IL-6), are detected in the tissues and circulation. Further, cytokines induce liver to produce C-reactive protein (CRP). CRP belongs to pentraxin protein family and it is ~25 kDA acute-phase protein which forms pentamere (~120 kDA) in circulation and is traditionally used as determinant of systemic low-grade inflammation, but also proinflammatory cytokines, such as tumour necrosis factor α (TNF-α) and IL-6, have been utilised. Glycoprotein acetylation (GlycA), which consist of acute-phase glycoproteins, is suggested to better reflect low-grade inflammation (Mokkala *et al*, 2020a; Chiesa *et al*, 2022). GlycA consists of several acute phase proteins while CRP is a single acute phase protein (Connelly *et al*, 2017). The main proteins that are detected in circulation to measure GlycA rise slightly after

than does hs-CRP (Connelly *et al*, 2017). Compared to high sensitivity (hs) CRP, GlycA exhibit less intra-individual variability (Connelly *et al*, 2017).

2.1.1 Low-grade inflammation in pregnant women with overweight and obesity

During pregnancy, the maternal immune system changes; the first and the third trimesters are characterised as pro-inflammatory (Th1) whereas the second trimester is characterised as anti-inflammatory (Th2), and involves both innate and adaptive immune system in tightly regulated manner to allow the implantation and placentation and not to reject the foetus as the pregnancy proceeds (Mor *et al*, 2011). This is part of normal pregnancy. Obesity challenges the immune regulation of pregnant women. In pregnant women with obesity, it has been shown that the switch from Th1 to Th2 response is impaired (Stewart *et al*, 2007), and circulating CRP increases slightly during early pregnancy but then decreases towards end of pregnancy in pregnant women with obesity, and the levels being higher in pregnant women with obesity as compared to women with normal weight (Pantham *et al*, 2015), and further this predisposes the pregnant women to adverse health effects. It is of note, that the obesity before pregnancy may elevate the levels of CRP which may influence the findings. Compared to non-pregnant women, normal pregnant women with uncomplicated pregnancy present higher concentrations of hs-CRP.

Specifically, low-grade inflammation in pregnant women with obesity was recognised 20 years ago when higher concentrations of plasma CRP and IL-6 were detected in pregnant women with obesity as compared to women with normal weight (Ramsay *et al*, 2002). Other studies since have found that increased circulating levels of CRP/hs-CRP is associated with increased adiposity, and it seems that this is observed in all three trimesters (**Table 1**). However, in one study serum CRP measured in the third trimester was not associated with obesity (Zacarías *et al*, 2018). Less is known about the relation of degree of obesity on CRP/hs-CRP. Few studies have investigated the impact of the degree of adiposity, evaluated by body mass index (BMI), and compared women with overweight and obesity (Friis *et al*, 2013; Fujimori *et al*, 2015; Jääskeläinen *et al*, 2018; Witteveen *et al*, 2022). These findings indicate pregnant women with obesity exhibited higher levels of CRP than their counterparts with overweight. This was also seen when the women were divided to two groups according to their pre-eclampsia status; levels of serum hs-CRP were increased in women with obesity compared to overweight or normal weight in women with and without pre-eclampsia (Jääskeläinen *et al*, 2018).

Table 1. Studies investigating the low-grade inflammation, measured as CRP/hs-CRP or cytokines/chemokines and other inflammatory markers, in pregnant women with normal weight, overweight and obesity.

Reference	Country	Study design	Study subjects*	Time of blood sampling	Low-grade inflammatory markers	Findings
(Ramsay *et al*, 2002)	United Kingdom	Cross-sectional	• Lean (early pregnancy BMI 22.1 kg/m^2): n = 24 • Obese (BMI 31.0 kg/m^2): n = 23	3rd trimester	Plasma CRP and IL-6	CRP and IL-6 ↑ in obese vs lean
(Bo *et al*, 2005)	Italy	Cross-sectional	• Normal weight: n = 154 • Overweight (BMI ≥ 25 kg/m^2, < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2): n = 36	32–36 gws	Serum CRP and TNF-α	CRP ↑ in overweight/obese vs normal weight, TNF-α -
(Bodnar *et al*, 2005)	USA	Cross-sectional	• Underweight (BMI < 18.5 kg/m^2): n = 14 • Normal weight (BMI 18.5–24.9 kg/m^2): n = 122 • Overweight (BMI 25.0–29.9 kg/m^2): n = 48 • Obese (BMI ≥ 30.0 kg/m^2): n = 36	≤ 20 gws	Serum CRP	CRP ↑ in women with overweight vs normal weight, CRP ↑ in obese vs under and normal weight
(Stewart *et al*, 2007)	United Kingdom	Longitudinal	• Lean (booking BMI < 30 kg/m^2): n = 30 • Obese (booking BMI ≥ 30 kg/m^2): n = 30	1st, 2nd, and 3rd trimesters (and at 4 months postpartum	Plasma CRP, TNF-α, IL-6, IL-10 and TNF-α/IL-6 ratio (index of Th1/Th2 response)	CRP ↑ in obese vs lean in all trimesters, TNF-α -, IL-6 ↑ in obese vs lean in 1st and 2nd trimester, IL-10 -, TNF-α/IL-6 ratio ↓ obese vs lean in all time points, CRP and TNF-α changed during pregnancy in both groups and IL-6 in obese

Reference	Country	Study design	Study subjects*	Time of blood sampling	Low-grade inflammatory markers	Findings
(Madan *et al*, 2009)	USA	Cross-sectional	• Normal weight (2nd trimester BMI < 26.5 kg/m^2): n= 20 • Overweight (2nd trimester BMI 26.5–31 kg/m^2): n = 19 • Obese (2nd trimester BMI > 31–41 kg/m^2): n = 21 • Morbidly obese (2nd trimester BMI > 41 kg/m^2): n = 20	1st trimester	Serum hs-CRP, IL-2, TNF-α, TGF-β1, HGF and MCP-1	Hs-CRP ↑ in obese and morbidly obese vs normal weight, hs-CRP ↑ in morbidly obese vs overweight, MCP-1 differed between the groups (↑ in morbidly obese), TNF-α -, HGF -, IL-2 -, TGF-β1 -
(Kac *et al*, 2011)	Brazil	Cross-sectional	• BMI quintile 1 (16.40–20.08 kg/m^2): n = 39 • Quintile 2 (20.09–22.12 kg/m^2): n = 29 • Quintile 3 (22.13–24.06 kg/m^2): n = 38 • Quintile 4 (24.07–27.83 kg/m^2): n = 38 • Quintile 5 (27.84–41.26 kg/m^2): n = 38	1st trimester	CRP and IL-6	CRP ↑ in quintile 4 vs 1 and in 5 vs 1 and in 2 vs 5, IL-6 -
(Friis *et al*, 2013)	Norway	Longitudinal	• Normal weight (BMI < 25 kg/m^2): n = 109 • Overweight (BMI 25–30 kg/m^2): n = 61 • Obese (BMI > 30 kg/m^2): n = 14	14–16, 22–24, 30–32 and 36–38 gws	Plasma CRP, IL-6, MCP-1, sTNF-RII, IL1-Ra and IL-10	CRP, MCP-1 differed between the groups (obese > overweight > normal weight) in visits 1-3, IL-6 differed between the groups (obese > overweight > normal weight) in visit 1, IL-Ra differed between the groups (obese > overweight > normal weight) in visits 1-4

Reference	Country	Study design	Study subjects*	Time of blood sampling	Low-grade inflammatory markers	Findings
(Christian & Porter, 2014)	USA	Longitudinal	• Normal weight (BMI 18.5–24.9 kg/m^2): n = 17 • Overweight (BMI 25-29.9 kg/m^2): n = 16 • Obese (BMI ≥ 30 kg/m^2): n = 24	1st, 2nd, and 3rd trimesters (and 4–6 wks postpartum)	Serum CRP, IL-6, IL-8, TNF-α and IL-1β	IL-6 ↑ in overweight or obesity vs normal weight in all time points, TNF-α ↑ in overweight vs normal weight in all time points, CRP ↑ in obese vs overweight or normal weight at postpartum, IL-8 -, IL-1β -
(de Oliveira *et al*, 2015)	Brazil	Prospective	• Normal weight (BMI 18.5–24.9 kg/m^2): n = 152 • Overweight (BMI 25–29.9 kg/m^2): n = 87 • Obese (BMI ≥ 30 kg/m^2): n = 40	5 to 13, 20 to 26, and 30 to 36 gws	Serum CRP	CRP correlated with BMI
(Fujimori *et al*, 2015)	Brazil	Cross-sectional	• Normal weight (BMI 18.5–24.9 kg/m^2): n = 25 • Overweight (BMI 25–29.9 kg/m^2): n = 24 • Obese (BMI ≥ 30 kg/m^2): n = 19	Prior to the beginning of labour	Serum IgA, IgM and IgG, C3 and C4, and CRP	IgA ↑ in obese vs normal weight, IgM -, IgG -, C3, C4 and CRP ↑ in obese vs normal weight or overweight
(Duran *et al*, 2016)	Turkey	Cross-sectional	Women with below and above VAT and SCFT cut-off points: • VAT < 4 cm n = 57 • VAT ≥ 4 cm n = 27 • SCFT < 2 cm n = 50 • SCFT ≥ 2 cm n = 34 • Women with BMI < 25 kg/m^2: n = 70	1st trimester	CRP	CRP ↑ in women with VAT ≥ 4 cm vs < 4 cm and in women with SCFT ≥ 2 cm vs vs < 2 cm

Reference	Country	Study design	Study subjects*	Time of blood sampling	Low-grade inflammatory markers	Findings
			• Women with BMI ≥ 25 kg/m^2: n = 14 (BMI ↑ in women with VAT ≥ 4 cm vs < 4 cm and in women with SCFT ≥ 2 cm vs < 2 cm & VAT ↑ and SCFT ↑ in women with BMI ≥ 25 kg/m^2 vs BMI < 25 kg/m^2)			
(Gillespie & Christian, 2016)	USA	Cross-sectional	• Normal weight (BMI 18.5–24.9 kg/m^2): n = 35 • Overweight (BMI 25–29.9 kg/m^2): n = 27 • Obese (BMI ≥ 30 kg/m^2): n = 43	2nd trimester	Serum CRP and IL-6	CRP and IL-6 ↑ in obese or overweight vs lean
(McDade *et al*, 2016)	Philippines	Prospective	• Women (BMI categories: < 18.5, < 23, < 25, < 27.5, > 27.5 kg/m^2): n = 309	3rd trimester	Dried blood spot converted to plasma equivalent values and plasma CRP	BMI during pregnancy was significantly and positively associated with CRP, and CRP ↑ along with increasing BMI (BMI from < 18.5 to > 27.5 kg/m^2)
(Fujimori *et al*, 2017)	Brazil	Cross-sectional	• Normal weight (BMI 18.5–24.9 kg/m^2): n = 15 • Overweight (BMI 25–29.9 kg/m^2): n = 15 • Obese (BMI ≥ 30 kg/m^2): n = 15	Prior to the beginning of labour	Serum CRP, IL-6, IL-10 and TNF-α	CRP and IL-6 differed between the groups (obese > overweight > normal weight), TNF-α -
(Shin *et al*, 2017)	USA	Cross-sectional	• Underweight (BMI < 18.5 kg/m^2): n = 31 • Normal weight (BMI 18.5–24.9 kg/m^2): n = 311	~2nd trimester	CRP	Pre-pregnancy BMIs of women with overweight and obesity had increased odds for being in the highest tertile of CRP

Reference	Country	Study design	Study subjects*	Time of blood sampling	Low-grade inflammatory markers	Findings
			• Overweight (BMI 25.0–29.9 kg/m^2): n = 153 • Obese (BMI ≥ 30 kg/m^2): n = 136			
(Jääskeläinen *et al*, 2018)	Finland	Cross-sectional case-control multicentre	Women with (n = 1447) and without (n = 1064) pre-eclampsia: • Normal weight (BMI < 25 kg/m^2): n = 852, 717 • Overweight (BMI 25–29.99 kg/m^2): n = 339, 242 • Obese (BMI ≥ 30 kg/m^2): n = 256, 104	1st and 3rd trimester	Serum hs-CRP	Hs-CRP ↑ in obese vs overweight or normal weight in both groups
(Zacarías *et al*, 2018)	Finland	Cross-sectional	• Normal weight (BMI 18.5–24.9 kg/m^2): n = 25 • Overweight (BMI 25–29.9 kg/m^2): n = 18 • Obese (BMI 30kg/m^2): n = 11	3rd trimester	Serum hs-CRP, haptoglobin and suPAR	CRP -, haptoglobin ↑ in overweight or obese vs normal weight, suPAR -
(Zambon *et al*, 2018)	Italy	Observational, longitudinal	• Normal weight (BMI ≥ 18.5, < 25 kg/m^2): n = 27 • Obese (BMI ≥ 30 kg/m^2): n = 35 (GDM, n = 17)	3rd trimester	Plasma CRP and PTX3	CRP ↑ in obese with and without GDM vs lean, PTX3 ↓ obese with and without GDM vs lean
(Bernhardt *et al*, 2022)	India	Prospective	• Non-obese (1st trimester or prepregnancy BMI < 30 kg/m^2): n = 30 • Obese (1st trimester or prepregnancy BMI ≥ 30 kg/m^2): n = 30	1st and 3rd trimester	Serum hs-CRP and ADA	Hs-CRP ↑ and ADA ↑ in obese vs non-obese, hs-CRP ↑ from 1st to 3rd trimester in obese, ADA ↑ from 1st to 3rd trimester in obese and non-obese,

Reference	Country	Study design	Study subjects*	Time of blood sampling	Low-grade inflammatory markers	Findings
						BMI correlated directly with hs-CRP in 1st and 3rd trimester, BMI correlated directly with ADA in 1st and 3rd trimester
(Li *et al*, 2022)	China	Cross-sectional	• Women with low BMI (BMI < 18.5 kg/m^2) • Normal weight (BMI 18.5–24.9 kg/m^2) • Overweight/obese (BMI ≥ 25.0 kg/m^2) • total n = 5476	The same day of delivery or on the day preceding delivery	Serum CRP LGI: CRP 3–10 mg/L CSI: CRP > 10 mg/L	CSI risk ↑ in overweight/obese or normal BMI vs low BMI weight, LGI risk ↑ in overweight/obese or normal BMI vs low BMI
(Witteveen *et al*, 2022)	Netherlands	Cross-sectional	• Underweight (BMI < 18.5 kg/m^2) • Normal weight (BMI 18.5–24.99 kg/m^2) • Overweight (BMI 25–29.99 kg/m^2) • Obese (BMI ≥ 30 kg/m^2) • Total n = 3547	Median of 13 gws (IQR 12–14)	Serum CRP	CRP ↑ in obese vs overweight or normal weight

*BMI is prepregnancy BMI unless stated otherwise

Statistically significant increase: ↑

Statistically significant decrease: ↓

No statistically significant difference: -

ADA, adenosinedeaminase; BMI, body mass index; C3 and 4, complement protein 3 and 4; hs-CRP, high sensitivity C-reactive protein; CSI, clinically significant inflammation; gws, gestational weeks; HGF, hepatocyte growth factor; IgA, M and G, immunoglobulins A, M and G; IL1-Ra, IL-1 receptor antagonist; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IQR, interquartile range; LGI, low-grade inflammation; MCP-1, monocyte chemotactic protein 1; PTX3, pentraxin 3; SCFT, subcutaneous fat tissue; suPAR, soluble urokinase plasminogen activating receptor; TGF-β1, transforming growth factor β1; TNF-α, tumour necrosis factor α; VAT, visceral adipose tissue; wks, weeks.

2.2 Microbiome

Human microbiome consist of multiple microbiomes in multiple sites in the body, e.g., gut and vagina. Human body serves as a host for microbes, including bacteria, viruses, fungi and archaea. Bacteria represents 1–3 % of human body weight (National Institutes of Health, 2012). However, the most recent estimates state that the percentage of bacteria of body weight is 0.3 % which accounts for $3.8x10^{13}$ bacterial cells, being the same as the number of human cells in the body (Sender *et al*, 2016).

In gastrointestinal track, in the colon, the bacterial mass is the highest (10^{11} cells/mL) followed by ileum (10^{8} cells/mL) and then duodenum and jejunum (10^{3}–10^{4} cells/mL) (Sender *et al*, 2016). The major taxa in the phylum level are Bacillota, Bacteroidota, Actinomycetota, Pseudomonadota and Verrucomicrobiota (formerly Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia, respectively (Oren & Garrity, 2021)) (Tremaroli & Bäckhed, 2012). *Ruminococcus*, *Clostridium*, *Lactobacillus*, *Eubacterium*, *Faecalibacterium* and *Roseburia* are genera that belongs to Bacillota (formerly Firmicutes), *Bacteroides*, *Prevotella* and *Xylanibacterdegrade* to Bacteroidota (Bacteroidetes formerly), *Collinsella* and *Bifidobacterium* in Actinomycetota (formerly Actinobacteria) and *Escherichia* and *Desulfovibrio* in Pesudomonadota (formerly Proteobacteria) (Tremaroli & Bäckhed, 2012). Gut microbiome has vast importance in human physiology and health, as it modulates host's metabolism and function of immune and nervous systems (Adak & Khan, 2019). Obesity can shift the gut microbial composition and diversity as showed in non-pregnant humans (Crovesy *et al*, 2020) and experimental animals (Ley *et al*, 2005). In obesity generally observed changes are increase in Bacillota (formerly Firmicutes) and decrease in Bacteroidota (formerly Bacteroides). Further, the obesity induced alterations in microbiota may lead to dysbiosis which is defined as an imbalanced gut microbiota with an increase in gram-negative bacteria. The gram-negative bacteria produce endotoxin lipopolysaccharide (LPS) (Cani *et al*, 2012), which is highly antigenic. The proposed mechanisms include increased intestinal permeability and LPS leakage. Obesity may increase intestinal permeability which means loss of intestinal epithelial cell tight junctions and increased passage of molecules from intestine to the body. Due to the increased intestinal permeability, LPS may leak from intestine to circulation (Vetrani *et al*, 2022). LPS is transferred to toll like receptor 4 and myeloid differentiation factor 2 complex in immune cells which activate proinflammatory signalling pathways and expression of transcription factors, such as nuclear factor κB (Page *et al*, 2022). Furthermore, LPS stimulates the proinflammatory macrophages. This all leads to increased levels of low-grade inflammatory markers.

Vaginal microbiota consist of ~10^{8} bacterial cells per millilitre of vaginal fluids (Ravel *et al*, 2011). It is of note that the amount of bacterial cells varies depending

on the vaginal microbial balance, e.g., in bacterial vaginosis the amount is higher, 10^{8-9} per gram (Danielsson *et al*, 2011). The most dominant phyla in the vagina are Bacillota, Pseudomonadota, Actinomycetota and Bacteroidota (formerly Firmicutes, Proteobacteria, Actinobaceria and Bacteroidetes, respectively) (Diop *et al*, 2019). Oestrogen induces glycogen formation which favours growth of lactobacilli, which makes the lactic acid producing *Lactobacillus* the major bacterial genus in vagina. Due to this high abundance of *Lactobacillus* species, the vaginal microbiota is less diverse than gut microbiota. The most dominant Lactobacillus species are *L. crispatus*, *L. iners*, *L. jensenii*, and *L. gasseri*, forming four community state types following the fifth one consisting of mainly strict anaerobic species from bacterial genera *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella* and other bacteria from order Clostridiales (Smith & Ravel, 2017). The acidic environment of the vagina, is a first-line defence against pathogens. In non-pregnant women obesity can shift the vaginal microbiota, as shown in previous study where women with obesity presented less *Lactobacillus* dominant microbiota and higher abundance of anaerobic bacterial species (*Dialister unclassified*, *P. timonensis*, *Anaerococcus vaginalis*) and α-diversity and richness as compared to non-obese women (Raglan et al., 2021). Also, increased expression of local pro-inflammatory markers were detected as compared to non-obese women (Raglan *et al*, 2021). This study was done on middle-aged pre- and post-menopausal women. However, similar finding has been found in reproductive aged women with overweight/obesity; decrease in *Lactobacillus* and increase in α-diversity were detected (Allen *et al*, 2022). Allen and co-workers (2022) suggested that even though the increased adiposity increases the oestrogenic levels, the increase in estrogenic levels have minor effects on vaginal microbiota and the changes due to obesity are independent of estrogenic levels.

2.2.1 Gut microbiota in pregnant women with overweight and obesity

During normal pregnancy, based on a few studies, gut microbiota appears to change during pregnancy. One study suggests that remodelling occurs in gut microbiota during pregnancy: from the first to the third trimester (Koren *et al*, 2012). However, another study did not detect such remodelling (DiGiulio *et al*, 2015). Those studies were done with pregnant women with varying BMI. In a study, which is part of the main trial of this thesis, the gut microbiota of pregnant women with overweight and obesity (n = 61; placebo group) changed from early to late pregnancy as analysed by metagenomics, however, the changes were not evident after correcting for multiple testing (Mokkala *et al*, 2021a).

As in non-pregnant individuals, overweight and obesity status can influence the gut microbiota in pregnancy. Previous studies show, that pregnant women with

overweight or obesity have decreased diversity as compared to their lean counterparts (Stanislawski *et al*, 2017; Collado *et al*, 2008). The differences in composition include lower abundance of bacteria belonging to the Bacillota (formerly Firmicutes) (genus *Lachnospira*, family Christensenellaceae and genus *Finegoldia* analysed by 16S ribosomal RNA (rRNA) gene sequencing (overweight/obese, n = 52; normal weight, n = 171, Stanislawski *et al*, 2017)) and higher abundance of those belonging to the Bacteroidota (formerly Bacteroidetes) (genus *Bacteroides*) analysed by fluorescent in situ hybridization coupled with flow cytometry and by quantitative real-time polymerase chain reaction (PCR) (overweight, n = 18; normal weight, 36, Collado *et al*, 2008) in pregnant women with overweight or/and obesity as compared to normal weight, faecal samples collected in the first and the third trimester. In contrast, higher number of bacteria belonging to the Bacillota (formerly Firmicutes, genus *Stafylococcus*) in the first (Collado *et al*, 2008) and the third trimesters (Zacarías *et al*, 2018; Collado *et al*, 2008) in women with overweight and obesity have been detected. In one study, *Bifidobacterium* and *Bacteroides* numbers were lower in overweight (n = 16) than in normal weight (n = 34) analysed by quantitative real-time PCR. However, the women with overweight had also lower fibre intake which may have affected the findings (Santacruz *et al*, 2010). Not all studies utilising 16S rRNA gene sequencing report association with BMI and gut microbiota (overweight/obese, n = 16; normal weight, n = 36, Aatsinki *et al*, 2018). However, in that study mothers were divided according to their microbiota, and Bacillota (formerly Firmicutes) dominant women had higher proportion of normal weight women than Bacteroidota (formerly Bacteroidetes) dominant women. Studies on the impact of the degree of adiposity i.e. overweight and obesity on gut microbiota during pregnancy are lacking. One study showed altered gut microbiota with increased BMI (Zacarías *et al*, 2018). In conclusion of these studies, the gut microbial signature is influenced by overweight and obesity during pregnancy, but whether the influence is similar to both BMI groups, is not known. Interestingly, one previous study showed that fat mass, another measure of adiposity which is derived from body composition measurement, correlated directly with *Bilophila* and indirectly with *Phascolarctobacterium* in pregnant women with normal weight and overweight/obesity (Ruebel *et al*, 2021). The studies on body composition and gut microbiota in pregnancy are scarce regardless.

2.2.2 Vaginal microbiota in pregnant women with overweight and obesity

During pregnancy vaginal microbiota changes as pregnancy proceeds; increase in species in *Lactobacillus* genus, and decrease in anaerobe or strict-anaerobe species

have been detected (Romero *et al*, 2014a) which supports the fact that oestrogene levels increases during pregnancy, as discussed by Allen et al. (2022). However, some studies do not agree and these studies report no changes in taxonomic composition or diversity (DiGiulio *et al*, 2015) and report rather stable microbiota during pregnancy as compared to in non-pregnant women (Romero *et al*, 2014b).

Studies on comparing vaginal microbiota in terms of normal weight, overweight and obesity during pregnancy are very scarce. One previous study, where vaginal samples were collected at 27–29 and 36–39 weeks of gestation from six normal weight and 15 women with obesity and analysed by 16S rRNA gene sequencing, showed trend of increased α-diversity and modulated taxonomic composition in genus level by obesity and gestational weight gain status, particularly in the first sampling time point (Faucher *et al*, 2020).

2.3 Metabolism

2.3.1 Metabolomics in pregnant women with overweight and obesity

Development of high-throughput techniques, including nuclear magnetic resonance (NMR) spectroscopy, has allowed investigation of human metabolism at a more detailed level as compared to standard clinical laboratory techniques. Studies using metabolomics techniques have shown that obesity modulate the metabolic profile of non-pregnant and pregnant human subjects analysed from serum, plasma, urine, serum of venous cord blood, adipose tissue, cord-blood, placenta tissue, and exhaled breath condensate samples according to a recent systematic review (Payab *et al*, 2021). According to that review particularly increased levels of branched-chain (BCAAs) and aromatic amino acids and some lipid derivates as well as some fatty acids are related to obesity.

During normal pregnancy the metabolic status changes as study using NMR spectroscopy showed; a decrease in plasma amino acids in early pregnancy and increase in lipids throughout the pregnancy were observed (Pinto *et al*, 2015). The study indicates also that higher BMI in the third trimester is associated with higher lipid and N-acetyl glycoprotein content. In other study, with a higher number of participants (pregnant women, n = 322; non-pregnant women n = 3938), serum lipoproteins and GlycA were increased during pregnancy (Wang *et al*, 2016).

The blood metabolic profile of pregnant women is altered due to overweight/obesity (Hellmuth *et al*, 2017; Shearer *et al*, 2021; Foratori-Junior *et al*, 2022; Shokry *et al*, 2019; Wahab *et al*, 2022; Sandler *et al*, 2017; Kivelä *et al*, 2021). In contrast, one small study did not find difference in lipids between pregnant women with obesity (n = 10) as compared to normal weight (n = 10), metabolites were

analysed by targeted liquid-chromatography tandem mass-spectrometry (Rauschert *et al*, 2019). Study using NMR metabolomics found higher levels of several lipoproteins all very low density lipoprotein (VLDL) subclasses and mean diameter of VLDL particles, small high density (HDL) particles, cholesterol and triglycerides in VLDL, total triglycerides, monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), and MUFAs to total fatty acids ratio, BCAAs and aromatic amino acids and GlycA while lower levels of very large and large HDL lipoprotein subclasses and mean diameter for HDL particles, and some fatty acid ratio measures (e.g., PUFAs to total fatty acids) in pregnant women with obesity (n = 347) as compared to normal weight (n = 267) (Kivelä *et al*, 2021). In another study, BCAAs and ketones (acetoacetate, β-hydroxybutyrate) were high while histidine was low as analysed by NMR spectroscopy in the second trimester in pregnant women with overweight and obesity (Shearer *et al*, 2021). In contrast, Hellmuth et al. (2017) or Wahab et al. (2022) did not find an association between BCAAs and prepregnancy BMI while Shokry et al. (2019) did. In particular, plasma non-esterified fatty acids (NEFAs) in the first and the second trimester, glutamic acid in the second trimester, and some phospholipids in the first trimester, were directly and asparagine and some phosphatidylcholines in the third trimester were indirectly associated with prepregnancy BMI, as detected by targeted liquid chromatography coupled with mass spectrometry (Hellmuth *et al*, 2017). Wahab et al. (2022) found that some maternal serum amino acids, NEFAs, phospholipid, sphingomyelins and carnitine concentrations analysed by targeted metabolomics in early pregnancy were related to overweight and obesity. In most of the studies pregnant women with overweight and obesity were combined into one group (Wahab *et al*, 2022) or handled as a continuous variable (Hellmuth *et al*, 2017; Shokry *et al*, 2019) or only individuals with obesity were included (Foratori-Junior *et al*, 2022), thus not allowing the evaluation of the degree of adiposity on metabolic profile. However, in a paper of Sherearer et al. (2021) distinct metabolic profile in all BMI groups (normal weight, overweight and obese) were visible in a heat map. Kivelä et al. (2021) found that the differences between normal weight versus obese and normal weight versus overweight were similar except the effects were less strong between normal weight and overweight than obese. All in all, metabolic profile is altered in pregnant women with overweight and obesity as compared to normal weight, however, the exact metabolites and their direction is not consistent between the studies and needs further investigation. Nevertheless, the strongest evidence comes from lipids and amino acids as those being higher in obesity. And less is known about the effect of the degree of adiposity on metabolic profile of pregnant women.

2.3.2 IGFBP-1 and MMP-8 in pregnant women with overweight and obesity

In non-pregnant humans insulin-like growth factor 1 (IGF-1) and its binding protein, insulin-like growth factor binding-protein 1 (IGFBP-1), are modulated by obesity; IGFBP-1 levels reduce and IGF-1 levels rise in circulation due to obesity (Nam *et al*, 1997). IGFBP-1 is regulated by insulin; insulin reduces the production of IGFBP-1 and increases the production of IGF-1 (Lewitt *et al*, 2014). Simultaneously, IGFBP-1 regulates the bioavailability of IGF-1. It is of note that, the phosphorylation of IGFBP-1 increases affinity of IGFBP-1 to IGF-1.

During normal pregnancy blood levels of IGFBP-1 have been mostly detected to increase during pregnancy from the first to the third trimester (Skjærbæk *et al*, 2004; Åsvold *et al*, 2011) and also slight decrease in the third trimester has been detected (Åsvold *et al*, 2011). Another study suggests increase in pregnancy in two peaks, one in gestational weeks 17–24 and other one just before delivery (Larsson *et al*, 2013). In a small study, which pregnant women (n = 23) with BMI ranking from 18 to 39 kg/m^2 were included, serum IGFBP-1 was negatively correlated with body weight and fat in gestational weeks 14 and 32 (Olausson *et al*, 2010). This was consistent with a bit larger study in which serum IGFBP-1 correlated negatively with BMI in the third trimester in pregnant women (Jansson *et al*, 2008). In another study serum IGFBP-1 correlated negatively with BMI but not with weight in normal weight pregnant women (n = 89) (Holmes *et al*, 2000). The studies focusing specifically on pregnant women with overweight and obesity are lacking, but one study including 12 pregnant women with obesity 12 normal weight pregnant women, did not find difference in serum IGFBP-1 levels at term between the groups (Ferraro *et al*, 2012). Regarding matrix metalloproteinase 8 (MMP-8), its levels in the blood have been detected to be increased in subjects with obesity (n = 34) but not in overweight (n = 76) as compared to normal weight subjects (n = 130) (Lauhio *et al*, 2016). The literature on pregnant women with overweight and obesity and their serum levels of MMP-8 are lacking.

2.3.3 Fatty acid metabolism in pregnant women with overweight and obesity

During pregnancy serum levels of total SFAs, MUFAs and polyunsaturated fatty acids (PUFAs) (both n-3 and n-6 LC- PUFAs) increases, however, eicosapentaenoic acid (EPA) and arachidonic acid (AA) decrease while docosahexaenoic acid (DHA) does not change according to a recent study with 479 pregnant women (fatty acid measured as absolute concentration, Aparicio *et al*, 2021b). However, one systematic review concluded an increase in DHA levels during pregnancy (measured as absolute concentration) (Wilson *et al*, 2019). Maternal fatty acid metabolism changes during

pregnancy to ensure optimal fatty acid transfer to foetus; in early pregnancy the lipid stores are built up and are later on broken down for the utilization of the growth and development of the foetus. For that, maternal diet is of importance to ensure optimal growth and development of the foetus. Diet allows supply of essential fatty acids (n-6 LC-PUFA, linoleic acid (LA) and n-3 LC-PUFA, α-linolenic acid (ALA)) which cannot be synthesised by the body. LA is converted into AA while ALA can be converted into EPA and DHA. In particular, DHA and AA are of importance since those are required for the neurological development of the foetus (Larqué *et al*, 2012). Due to low conversion rate of EPA and DHA as well as the high foetal needs, the intake of EPA and DHA from the diet is necessary.

Systemic review done in non-pregnant subjects show that overweight and obesity alter the fatty acid levels (Fekete *et al*, 2015). Similar to that overweight and obesity are associated with the blood fatty acid status in pregnant women (Tomedi *et al*, 2013; Al-Otaibi *et al*, 2020; Vidakovic *et al*, 2015; Aparicio *et al*, 2021a; Penfield-Cyr *et al*, 2018; Gázquez *et al*, 2021; Scifres *et al*, 2014; Cinelli *et al*, 2016; Chamorro *et al*, 2022). Specifically, studies show that the fatty acid status is influenced with increasing BMI; content of some fatty acids, e.g., proportional amount of red blood cell DHA and AA of total fatty acids at 20 gestational weeks reduce (Tomedi *et al*, 2013) and some, e.g., percentage by weight of total SFAs, total n-6 LC-PUFAs and LA of total fatty acids increase (measured soon after delivery) (Al-Otaibi *et al*, 2020) with increasing BMI (normal weight -> overweight -> obese). In line with the study conducted with 476 pregnant women showed that concentrations of total SFAs, n-6 LC-PUFAs, AA and ratio of n-6/n-3 LC-PUFAs were higher in the first trimester and SFAs in the third trimester in women with overweight and obesity than in normal weight (Aparicio *et al*, 2021a). One study did not find difference in SFAs or MUFAs but lower erythrocyte total n-3 LC-PUFAs, ALA, EPA, DHA and higher n-3/n-6 LC-PUFAs ratio (mol % of fatty acid) at 20–24 gestational weeks in pregnant women with obesity as compared to non-obese were detected (Chamorro *et al*, 2022). One study conducted with high number of study subjects (n = 5363) at 20.5 weeks showed higher total SFAs, palmitic acid, stearic acid and dihomo-γ-linolenic acid (DGLA) and AA concentrations while lower myristic acid and ALA concentrations in plasma glycerophospholipids in pregnant women with overweight and obese as compared to normal weight women, as well as higher palmitoleic while lower EPA and LA concentrations in pregnant women with obesity but not in overweight than in normal weight women, and higher total n-6 LC-PUFAs in overweight compared to normal weight (Vidakovic *et al*, 2015). This study shows that degree of adiposity is reflected in fatty acid levels in plasma, as in study of Gázquez et al. (2021), in which palmitic acid, stearic acid and n-6/n-3 LC-PUFAs ratio were higher while DHA was lower in serum phospholipids (g/100g of fatty acids) in pregnant women with obesity than in women with

overweight at 24 weeks of gestation. It seems that higher blood SFAs, n-6 LC-PUFAs and n-3/n-6 LC-PUFAs ratio while lower n-3 LC-PUFAs are related to overweight/obesity in pregnancy but not all studies are in line with each other.

2.4 Gestational diabetes mellitus

2.4.1 Pathogenesis

In early phase of normal pregnancy insulin sensitivity increases and simultaneously glucose stores increase by the glucose uptake by the tissue. Towards later stages of normal pregnancy insulin resistance takes over as stimulated by hormones that leads to increase in blood glucose and further its transport to the foetus (Plows *et al*, 2018). In pregnancy complicated by GDM this system fails. The two driving factors in pathophysiology of GDM are β-cell dysfunction (fail to sense blood glucose and release insulin) and insulin resistance (tissues fail to response to insulin due to the failure in insulin signalling) (Plows *et al*, 2018).

In the following chapter, the relation of low-grade inflammation, vaginal microbiota and circulating MMP-8, IGFBP-1 and fatty acids in the onset of GDM will be reviewed.

2.4.2 The relation of low-grade inflammation, vaginal microbiota, IGFBP-1, MMP-8 and fatty acids to the onset gestational diabetes mellitus

It has been postulated that low-grade inflammation may induce insulin resistance when cytokines activate inflammatory signalling pathways, and thus interfere with insulin signalling pathways (Chen *et al*, 2015). Previous studies indicate that the levels of CRP in early pregnancy are increased in women developing GDM in later pregnancy (n = 27–283 per group) (Bo *et al*, 2005; Qiu *et al*, 2004; Wolf *et al*, 2003; Smirnakis *et al*, 2007; Maged *et al*, 2014; Kansu-Celik *et al*, 2020), but it is of note that in some of those studies the findings were evident in both women with normal weight and those with overweight (Qiu *et al*, 2004) but in some studies the adjustment with BMI attenuated the results indicating that BMI rather than GDM influences the levels of CRP (Wolf *et al*, 2003). Syngelaki et al. (2016) did not report differences in the levels of hs-CRP between women who developed GDM (n = 200) and who did not (n = 800). In that study, the analyses were adjusted with many maternal characteristics, e.g., cigarette smoking, age, racial orgin and history of type 2 diabetes. Also, in cross-sectional setting in pregnant women with GDM, increased levels of CRP and hs-CRP have been detected as compared to women without GDM (Alyas *et al*, 2019; Sifnaios *et al*, 2019). According to a recent systematic review

(Amirian *et al*, 2020), out of 31 reviewed articles, in 20 of the articles, the association between hs-CRP or CRP and GDM was reported. Two of those articles were prospective. Interestingly, in one study the concentration of GlycA in early pregnancy was associated with the onset of GDM in later pregnancy (Mokkala *et al*, 2020b).

Disturbed vaginal microbiota may be linked to adverse pregnancy outcomes, such as preterm birth (Fettweis *et al*, 2019). GDM can increase the risk of preterm birth but whether GDM affects adversely on vaginal microbiota or vaginal microbiota causes GDM, is unclear. The literature on the relation of vaginal microbiota in the onset GDM is lacking. Nevertheless, previous studies in which the vaginal microbiota has been determined after the GDM diagnosis indicate that vaginal dysbiosis (Rafat *et al*, 2022) and specific bacterial phyla (Firmicutes, Actinobacteria, Bacteroidetes (formerly Bacillota, Actinomycetota, Bacteroidota)) and genera (*Prevotella*, *Aerococcus*, *Veillonella, Klebsiella, Escherichia-Shigella*, *Enterococcus*, *Enterobacter*, *Bacteroides*) (Wang *et al*, 2018; Cortez *et al*, 2019) and species (*L. listeri*, *L. amylovorus*, *L. fructivorans*) (Zhang *et al*, 2018) may be associated with GDM. Cortez et al. (2019) reported lower diversity in women with GDM than women without GDM.

As IGFBP-1 is regulated by insulin and may be linked to insulin resistance, systematic review on circulating IGF-axis markers and their relation to GDM has been conducted (Wang *et al*, 2019). Regarding IGFBP-1, its levels were reported in five studies out of 12 studies. One study reported that IGFBP-1 measured in early pregnancy was inversely associated with the development of GDM (GDM n = 74, control n = 757), and two studies found lower levels of IGFBP-1 in mid-pregnancy in women with GDM compared to controls. No studies on IGFBP-1 in late pregnancy and its relation to GDM existed but one study investigated the effect of GDM treatment on the serum levels of IGFBP-1 from early to later pregnancy when GDM develops (Huhtala *et al*, 2020). Metformin and insulin combined increased IGFBP-1 and metformin alone, as compared to insulin, increased IGFBP-1 more.

Lauhio et al. (2016) showed *in vitro* analyses that MMP-8 cleavages insulin receptor, and the authors suggested that MMP-8 has a role in insulin signalling and development of insulin resistance. The literature indicates that MMP-8 is related to GDM as measured in serum and gingival crevicular fluid at the time of diagnosis (Akcalı *et al*, 2017) and in gingival crevicular fluid measured in early pregnancy (Chaparro *et al*, 2021). Serum MMP-8 was not related to history GDM as measured postpartum (Vilmi-Kerälä *et al*, 2017). The literature on serum MMP-8 and its association with GDM is very scarce.

GDM seems to alter the fatty acids in red blood cells, plasma and serum as reviewed previously (Taschereau-Charron *et al*, 2017). Two of those studies measured serum/plasma absolute concentrations of fatty acids of which other study

showed higher SFAs and EPA in early pregnancy and higher MUFAs, AA, ALA, EPA and DHA in late pregnancy in women with GDM diagnosed in later pregnancy as compared to women without GDM. Two studies measured serum/plasma fatty acids as percentage of total fatty acids in late pregnancy and found higher in SFAs and MUFAs in total and SFA sand AA in phosphatidylcholine (PC) and cholesteryl esters (CEs) and DHA in triacylglycerols (TAGs) while lower MUFAs and ALA in PC and CEs in women with GDM diagnosed in late pregnancy. Tryggvadottir et al. (2021) found that concentration of SFAs, MUFAs, PUFAs, n-6 PUFAs, n-3 PUFas and total fatty acids in early pregnancy were higher in women who developed GDM. This results did not change after taking into account the women's BMI.

2.5 Dietary regulators of low-grade inflammation, microbiota and metabolism

In the two following paragraphs the randomised controlled trials on two dietary ingredients, namely n-3 LC-PUFAs and probiotics, on circulating low-grade inflammatory markers and vaginal microbiota and n-3 LC-PUFAs on blood fatty acids in pregnant women will be covered. It is of note that the literature on the impact of fish oil and probiotics on IGFBP-1 and MMP-8 and the impact of probiotics on fatty acids in pregnant women are lacking, and thus are not reviewed. However, the studies in non-pregnant subjects are described briefly in relation to IGFBP-1 and MMP-8.

2.5.1 N-3 LC-PUFAs

The effect of n-3 LC-PUFAs on low-grade inflammation in pregnant women has been evaluated in five studies (**Table 2**); only one study measured circulating CRP (Haghiac *et al*, 2015) while others mainly cytokines and chemokines (Krauss-Etschmann *et al*, 2008; Warstedt *et al*, 2009; García-Rodríguez *et al*, 2012; Valentine *et al*, 2021). Two studies did not find any effect (García-Rodríguez *et al*, 2012; Warstedt *et al*, 2009) as the others did (Krauss-Etschmann *et al*, 2008; García-Rodríguez *et al*, 2012; Haghiac *et al*, 2015; Valentine *et al*, 2021). The study in which CRP was measured included women with overweight or obesity. The consumption of 800 mg DHA and 1200 mg of EPA, a total of 2 g of n-3 LC-PUFAs daily (n = 25), from before 16 gestational weeks until delivery resulted in decrease in plasma CRP as compared to placebo (n = 24). However, no effect was observed in two proinflammatory cytokines, IL-6 or interleukin 8. They also measured adipose and placental tissue messenger RNA levels of cytokines in a subset of women (n = 16) and found reduced levels of IL-6, interleukin 8, TNF-α and toll-like receptor 4. The studies in which cytokines and chemokines were measured, n-3 LC-PUFAs

were related to a decrease in tumour necrosis factor β and increase in interferon γ (INF-γ) and interleukin 1 and no effect was seen in pro-inflammatory cytokines, interleukin 4 or interleukin 13 (Krauss-Etschmann *et al*, 2008). Surprising was that in one study the increase in IL-6 was associated with higher dosage of DHA (Valentine *et al*, 2021).

Only one previous study has investigated the effect of n-3 LC-PUFAs on vaginal microbiota in pregnant women, however, leading to no statistically significant results (Hjelmsø *et al*, 2020). The previous evidence from non-pregnant human subjects is lacking as well.

No previous studies on the impact of n-3 LC-PUFAs on blood IGFBP-1 or MMP-8 in pregnant women exist. Yet one study demonstrated that fatty fish (150g/week) does not affect the levels of plasma matrix metalloproteinase 9 in pregnant women (García-Rodríguez *et al*, 2012). The evidence from studies including non-pregnant subjects shows fish oil supplementation (5 mL/d fish oil, n-3 cod liver oil) increases the levels of IGF-1 in boys (n = 26) and reduces the serum levels of insulin-like growth factor binding-protein 3 in girls (n =30) as compared to sunflower oil (n = 33, n = 26, respectively) in infants aged 9–18 months (Damsgaard *et al*, 2016). Similarly, an increase in serum IGF-1 and decrease in IGFBP-1 were detected in men with cardiovascular disease after 8-week 720 mg EPA and 480 mg DHA daily supplementation (n = 31) as compared to placebo (paraffin, n =31) (Gholamhosseini *et al*, 2015). Oppositely, 8-week low fat diet with n-3 LC-PUFAs (3% of energy from n-3 LC-PUFAs) increased the levels of insulin-like growth factor binding-protein 3 in postmenopausal women (n = 16) (Young *et al*, 2013). Increase in IGF-1 was also detected. One study had similar findings as the previous studies but did not find effect on IGFBP-1 after n-3 LC-PUFAs (powder 3 grams daily, n = 17) or n-6 LC-PUFAs supplementation (powder 9 grams daily, n = 13) in patients with Crohn's disease (Eivindson *et al*, 2005).

The effect of n-3 LC-PUFAs on circulating fatty acids levels, has been evaluated in supplement (Dunstan *et al*, 2004; Bergmann *et al*, 2008; Krauss-Etschmann *et al*, 2007; Farshbaf-Khalili *et al*, 2017) and whole fish (Miles *et al*, 2011) as well as dietary counselling studies (Hautero *et al*, 2013). The studies demonstrate that all approaches are able to increase the n-3 LC-PUFA levels in pregnancy as measured from serum, plasma and blood cells. Some of the studies show effect on other fatty acid classes e.g., n-6 LC-PUFAs, MUFAs and SFAs (Dunstan *et al*, 2004; Hautero *et al*, 2013) but not all (Bergmann *et al*, 2008; Farshbaf-Khalili *et al*, 2017). The studies evaluating the effect of n-3 LC-PUFAs on blood fatty acids included women with varying BMI, and some even excluded women with obesity.

Table 2. Intervention studies investigating the effect of n-3 LC-PUFAs on circulating low-grade inflammatory markers and fatty acids as well as vaginal microbiota in pregnant women.

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
Low-grade inflammation:						
(Krauss-Etschmann *et al*, 2008)	2008	Germany, Hungary and Spain	Pregnant women • Fish oil: n = 49 • 5-MTHF: n = 49 • Fish oil+5-MTHF: n = 49 • Placebo: n = 50 No information on BMI, but weight and height at study entry were: Fish oil: 69.4 (66.6–72.2) kg, 164.3 (160.1–168.6) cm 5-MTHF: 67.5 (64.4–70.6) kg, 164.4 (159.9–168.8) cm fish oil+5-MTHF: 66.7 (63.9–69.4) kg, 165.7 (162.2–169.2) cm Placebo: 67.3 (64.3–70.4) kg, 167.6 (163.2–171.9)	• Fish oil (0.5 g DHA and 0.15 g EPA) • 5-MTH (400 µg) • Fish oil+5-MTHF • Placebo (90% cow's milk fatty acids)	22 gws until delivery, sampling at study entry and 30 gws, mRNA levels in blood	TGF-β ↑ in fish oil vs placebo, IL-1 and IFN-γ ↓ in fish oil vs placebo, CXCR3 -, CRTH2 -, IL-4 -, IL-13 -, CCR4 - in fish oil vs placebo, TGF-β ↑ in fish oil+5-MTHF vs placebo, CRTH2 ↓ and CCR4 ↓ in fish oil+5-MTHF vs placebo, IL-1 -, IFN-γ -, CXCR3 -, IL-4 -, IL-13 - in fish oil+5-MTHF vs placebo

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
(Warstedt *et al*, 2009)	2009	Sweden	Pregnant women with allergic disease in the immediate family, whole blood cultures with LPS • n-3 LC-PUFAs: n = 28 • Placebo: n = 31 No information about BMI	• 2.7 g n-3 LC-PUFAs, 1.6 g EPA and 1.1 g DHA, 9 capsules • Placebo (soybean oil) 9 capsules	25 gws until 1 week postpartum, average 30.5 wks, sampling at entry and within 1 wk postpartum	Whole blood cell culture with or without LPS: IL-1β -, IL-6 -, CXCL8 (IL-8) -, IL-10 -, IL-12p70 -, TNF -, CCL2 (MCP-1) -, and CCL3 (MIP-1α) -, IL-5 - and IFN-γ -
(García-Rodríguez *et al*, 2012)	2012	United Kingdom	Pregnant women • Intervention: n = 54 • Control: n = 54 No information on BMI, but in the main publication of the study the height and weight at study entry are as follows: Salmon: 67.5 ± 1.6 kg, 165.4 ± 0.8 cm (n = 62) Placebo: 71.3 ± 2.0 kg, 165.6 ± 0.9 cm (n = 61) (Miles *et al*, 2011)	• Habitual diet + 2 portions/wk of farmed salmon (150 g/portion; 0.57 g EPA, 0.35 g 1.16 g DHA, total 3.56 g n-3 LC-PUFAs) • Control (habitual diet)	From 20 gws until delivery, sampling 20, 34 and 38 gws	IL-6 -, IL-8 -, TNF-α -, MCP-1 -, HGF -
(Haghiac *et al*, 2015)	2015	USA	Pregnant women with BMI ≥ 25 kg/m^2 • Intervention: n = 25 • Placebo: n = 24 Inclusion criteria BMI ≥ 25 kg/m^2 at the first antenatal visit	• Oral 800 mg DHA, and 1200 mg of EPA, total of 2 g of n-3 LC-PUFAs, 4 capsules • Matching placebo (wheat germ oil)	Prior to 16 gws until delivery, sampling 8–16 and 34–36 gws	Plasma CRP change ↓ n-3 LC-PUFAs vs placebo, Plasma IL-6 change -, plasma IL-8 change -, adipose and placental tissue IL-6, IL-8, TNF-α and TLR4 mRNA expression ↓ n-3 LC-PUFAs vs placebo

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
(Valentine *et al*, 2021)	2021	USA	Pregnant women • DHA 1000 mg/day: n = 465 • Placebo DHA 200 mg/day: n= 437 BMI at study entry: 200 mg/day: Obese n = 138 (31.9%) Other (BMI < 30 kg/m^2) n = 294 (68.1%) 1000 mg/day: Obese n = 159 (34.7%) Other (BMI < 30 kg/m^2) n = 299 (65.3%)	• Algal oil daily, 800 mg of DHA, 2 capsules + 200mg DHA supplement • Placebo (soybean and corn oil, 0 mg of DHA) + 200mg DHA supplement	Sampling 12-20 gws and at delivery	sRAGE ↓ and IL-6 ↑ were associated with a probability (Bayesian model) that 1000 mg/day is better than 200 mg/day, IL-1β -, TNF-α -, IFNγ -
Vaginal microbiota:						
(Hjelmsø *et al*, 2020)	2020	Denmark	Pregnant women • n-3 LC-PUFAs: n = 344 • Placebo for n-3 LC-PUFAs: n = 349 • Vitamin D: n = 294 • Placebo fot vitamin D: n = 286 No information on BMI	• 2.4 g of n-3 LCPUFA (55% EPA and 37% DHA) • Placebo for n-3 LC-PUFAs (olive oil) • Vitamin D_3 2400 IU • Placebo for vitamin D_3 (Camette A/S)	24 gws until 1 wk postpartum, sampling 24 and 36 gws	α-diversity -, β-diversity -

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
Fatty acids:						
(Dunstan *et al*, 2004)	2004	Australia	Pregnant women • Intervention: n = 36 • Placebo: n = 37 BMI at entry: Intervention group: 25.6 ± 0.7 kg/m^2 Placebo group: 25.9 ± 0.6 kg/m^2	• 4 g of fish oil (56% DHA) and 28% EPA) • Placebo (olive oil)	20 gws until delivery (sampling 20, 30, 37 gws and 6 weeks postpartum)	Erythrocyte phospholipids, % of total fatty acids: EPA and DHA ↑ in fish oil vs placebo in 30 and 37 gws, AA ↓ in fish oil vs placebo in 30 and 37 gws, DGLA ↓ in fish oil vs placebo in 30, adrenic acid ↓ in fish oil vs placebo in 37 gws, total n-6 PUFAs ↓ in fish oil vs placebo in 30 and 37 gws, total n-3 PUFAs ↑ in fish oil vs placebo in 30 and 37 gws, n-3 to n-6 ↑ in fish oil vs placebo in 30 and 37, myristic acid, palmitic acid, palmitoleic acid, stearic, oleic acid or DPA -
(Krauss-Etschmann *et al*, 2007)	2007	Germany, Hungary and Spain	Pregnant women • Fish oil: n = 69 • 5-MTHF n = 65 • Fish oil+5-MTHF: n = 64 • Placebo: n = 72 Inclusion criteria: body weight from > 50 kg to 92 kg BMI at study entry: Fish oil: 25.2 (18.5–35.2) kg/m^2 5-MTHF: 24.6 (18.9–39.1) kg/m^2 Fish oil+5-MTHF: 24.9 (19.5–32.4) kg/m^2	• Fish oil (0.5 g DHA and 0.15 g EPA) • 5-MTH (400 µg) • Fish oil+5-MTHF • Placebo (90% cow's milk fatty acids)	20 gws until birth (sampling 20 and 30 gws)	Plasma phospholipids, % by wt: DHA ↑ in fish oil, DHA ↑ in 5-MTHF, DHA fish oil supplementation×MTHF supplementation×time interaction -, EPA ↑ in fish oil, EPA - in 5-MTHF, EPA fish oil supplementation×MTHF supplementation×time interaction -

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
			Placebo: 24.1 (18.5–35.5) kg/m^2			
(Hautero *et al*, 2013)	2013	Finland	Pregnant women • Intervention: n = 45 • Control: n = 45 Prepregnancy BMI: Intervention group: 24.2 (4.2) kg/m^2 Control group: 23.1 (3.4) kg/m^2	Individual dietary counselling: support the food choices that will increase the intake of unsaturated fats and lower the intake of SFAs	The first trimester until one month after delivery (sampling the first and the third trimester of pregnancy and 1 month after delivery)	Serum phospholipids, % of total fatty acids: MUFAs and n-3/n-6 PUFAs ↓ in intervention vs control, PUFAs, DHA and sum n-3 PUFAs ↑ in intervention vs control in the third trimester, Serum cholesteryl esters, % of total fatty acids: EPA and sum n-3 PUFAs ↑ in intervention vs control, MUFAs ↓ in intervention vs control in the third trimester, Serum triacylglycerols, % of total fatty acids: PUFAs, ALA, LA, GLA, AA, sum n-3 and n-6 PUFAs ↑ in intervention vs control, SFAs ↓ in intervention vs control in the third trimester
(Bergmann *et al*, 2008)	2008	Germany	Pregnant women • Vit/Min: n = 48 • FOS: n = 47 • DHA-FOS: n = 41 Pre-pregnancy BMI: Vit/Min: 23.0 ± 5.16 kg/m^2 FOS: 22.2 ± 2.82 kg/m^2 DHA-FOS: 22.2 ± 4.29 kg/m^2	• Vitamin-mineral supplement (Vit/Min group) • Vit/Min plus 4.5 g fructo-oligosaccharide (FOS group) • Vit/Min plus 4.5 g FOS plus 200 mg fish oil-derived DHA (DHA-FOS group)	21 to 37 gws (sampling 21 and 38 gws, and 3 months postpartum)	Red blood cell phospholipids, % of total fatty acids: DHA and EPA in DHA-FOS ↑ vs other groups in 37 gws, AA -

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
(Miles *et al*, 2011)	2011	UK	Pregnant women • Intervention: n = 62 (20 gws), 55 (34 gws), 53 (38 gws) • Placebo n = 62 (20 gws), 56 (34 gws), 54 (38 gws) Weight and height at study entry: Salmon: 67.5 ± 1.6 kg, 165.4 ± 0.8 cm (n = 62) Placebo: 71.3 ± 2.0 kg, 165.6 ± 0.9 cm (n = 61)	• Habitual diet + 2 portions/wk of farmed salmon (150 g/portion; 0.57 g EPA, 0.35 g 1.16 g DHA, 3.56 g total n-3 LC-PUFA) • Control (habitual diet)	20 gws until delivery (sampling 20 and 32-24 and 38 gws)	Plasma phosphatidylcholine, % of total fatty acids: DHA and EPA ↑ in fish oil, DHA and EPA ↓ in placebo, DHA and EPA ↑ in fish oil vs placebo in 34 and 38 gws, changes in DHA and EPA between 20 and 34 and 20 and 38 gws were significantly different between groups
(Farshbaf-Khalili *et al*, 2017)	2017	Iran	Pregnant women • Intervention: n = 67 • Placebo: n = 68 Exclusion criteria included BMI > 20 kg/m^2 Prepregnancy BMI: Intervention group: 23.7 (3.5) kg/m^2 (n = 72) Placebo group: 23.9 (3.7) kg/m^2 (n = 72) (there were women from BMI classes underweight, normal weight and overweight)	• 1000 mg fish oil supplements: 120 mg DHA and 180 mg EPA • Placebo (liquid paraffin)	21 gws until delivery (sampling baseline and 35–37 gws)	Serum phospholipids, % of total fatty acids: DHA, n-3 PUFAs and stearic acid ↑ in fish oil vs placebo, n-6 PUFAs/n-3 PUFAs ↓ in fish oil vs placebo, EPA, LA, ALA, AA, n-6 PUFAs, myristic acid, palmitic acid, palmitoleic acid, oleic acid, SFAs, MUFAs, SFA/MUFA -

Significant increase: ↑
Significant decrease: ↓
No significant difference: -
5-MTHF, 5-methyl-tetra-hydrofolic acid; AA, arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; CCL2, CC chemokine ligand 2; CCL3, CC chemokine ligand 3; CCR4, CC chemokine receptor 4; CRP, C-reactive protein; CRTH2, chemoattractant receptor-homologous molecule expressed on

Th2 lymphocytes; CXCL8, CXC chemokine ligand 8; CXCR3, CXC chemokine receptor 3; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GDLA, dihomo-γ-linolenic acid; gws, gestational weeks; HGF, hepatocyte growth factor; IL-1, interleukin 1; IL-1β, interleukin 1β; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IL-12p70, interleukin 12p70; IL-13, interleukin 13; IFN-γ, interferon γ; LA, linoleic acid; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein 1; MIP-1α, macrophage inflammatory protein 1α; MUFAs, monosaturated fatty acids; mRNA, messenger ribonucleic acid; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; TLR4, toll-like receptor 4; TGF-β, tumour necrosis factor β; TNF-α, tumour necrosis α; sRAGE, soluble receptor for advanced glycation end products, wks, weeks; wt, weight.

2.5.2 Probiotics

Only two previous studies have investigated the effect of probiotics on low-grade inflammation in pregnant women (Jafarnejad *et al*, 2016; Hajifaraji *et al*, 2018). The studies included women with GDM and the intervention lasted eight weeks but the women were supplemented with different probiotic mixtures (**Table 3**). Both probiotic mixtures resulted decrease in CRP and TNF-α. The other study found decrease in IL-6 after probiotic consumption (Jafarnejad *et al*, 2016) while the other did not (Hajifaraji *et al*, 2018) when compared to the placebo.

Eight studies have evaluated the effect of probiotics on vaginal microbiota (**Table 3**). Out of those, five studies found no effect (Vitali *et al*, 2012; McMillan *et al*, 2018; Bisanz *et al*, 2015; Gille *et al*, 2016; Husain *et al*, 2020). Only one study showed decrease in α-diversity and β-diversity after consumption of probiotics including *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, respectively (Zheng *et al*, 2020)) for 30 days (Vasundhara *et al*, 2021). Information of women's adiposity was not given. In phylum level Liang et al. (2021) found no effect while Vasundhara et al. (2021) did, as they found that probiotics increased relative abundance of Bacillota (formerly Firmicutes) and decrease in Bacteroidota (formerly Bacteroidetes) and Actinomycetota (formerly Actinobacteria) and no change in these bacterial in placebo group. Those two studies used two different kind of probiotic mixtures. Liang et al. (2021) found also changes in genus level as *Ureaplasma* and *Gardnerella* increased in probiotic group as compared to control group while *Prevotella* and *Sneathia* increased in control group as compared to probiotic group. One study did not find effect in genus or species level (McMillan *et al*, 2018). Two studies found effect in species level (Vasundhara *et al*, 2021; Yang *et al*, 2020). In both studies bacterial species e.g., *G. vaginalis*, *Fannyhessea vaginae* (formerly *Atopobium vaginae* (Plummer *et al*, 2021)), *L. acidophilus* as well as *Lactobacillus* sp. were affected.

No previous studies on the impact of probiotics on blood IGFBP-1 or MMP-8 in pregnant women exist. A recent study showed that 3-month supplementation with probiotics (*L. rhamnosus* GG) was not able to affect serum MMP-8 in non-pregnant patients with myocardial infarction as compared to placebo (inulin) (Moludi *et al*, 2021). The effect of probiotics on IGFBP-1 or other IGFBPs in blood in non-pregnant humans is not documented.

The effect of probiotics on circulating fatty acids in pregnant women has not been evaluated previously but studies utilising other biological specimens have shown that probiotics combined with dietary counselling modifies placental (Kaplas *et al*, 2007) and breast milk (Hoppu *et al*, 2012) fatty acids.

Table 3. Intervention studies investigating the effect of probiotics on circulating low-grade inflammatory markers and vaginal microbiota in pregnant women. The studies on IGFBP-1, MMP-8 and circulating fatty acids are lacking.

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
Low-grade inflammation:						
(Jafarnejad *et al*, 2016)	2016	Iran	Pregnant women with GDM in mid-pregnancy • Intervention: n = 37 • Placebo: n = 35 BMI: Intervention group: 26.8 ± 2.7 kg/m^2 Placebo group: 27.4 ± 3.1 kg/m^2	• VSL#3, eight strains of lactic acid bacteria (*Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*), *Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*), and *Lactobacillus delbrueckii* subsp. *bulgaricus*), 2 capsules, 112.5 × 10^9 CFU/capsule • Placebo capsules containing 40 mg microcrystalline cellulose	8 weeks	Serum hs-CRP change ↓ probiotics vs placebo, serum TNF-α change ↓ probiotics vs placebo, IL-6 change ↓ probiotics vs placebo, IFN-γ -, IL-10 -
(Hajifaraji *et al*, 2018)	2018	Iran	Pregnant women with GDM at 24–28 gws • Intervention: n = 27 • Placebo: n = 29	• Probiotic capsule contained *Lactobacillus acidophilus* LA-5, *Bifidobacterium* BB-12, *Streptococcus thermophilus* STY-31 and *Lactobacillus delbrueckii* subsp. *bulgaricus* (formerly *Lactobacillus delbrueckii bulgaricus*) LBY-27, 4 capsules, 10^9 CFU/capsule	8 consecutive weeks	Serum hs-CRP change ↓ probiotics vs placebo, serum TNF-α change ↓ probiotics vs placebo, IL-6 -

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
			Inclusion criteria: prepregnancy BMI ≥ 18.5 kg/m^2 Intervention group: 31.4 ± 3.9 kg/m^2 Placebo group: 29.9 ± 3.4 kg/m^2	• Placebo capsules without bacteria had the same specifications as probiotic capsules		
Vaginal microbiota:						
(Vitali *et al*, 2012)	2012	Italy	Pregnant women • Intervention: n = 15 • Control: n = 12 No information on BMI	• VSL#3 sachet contains 900 billion viable lyophilized bacteria consisting of 4 strains of *Lactobacillus* (*Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*), *Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*), *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*), 3 strains of *Bifidobacterium* (*Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*) and 1 strain of *Streptococcus thermophilus*, 1 sachet • Control (no placebo)	4 wks, from 33 to 37 gws, sampling 33 and 37 gws	Change in α-diversity (richness) - in probiotics or control, principal bacterial populations -
(Bisanz *et al*, 2015)	2015	Tanzania	Pregnant women • Intervention: n = 23	• Probiotic yogurt *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 ~10^{10} CFU	Study entry to 1 wk to 1 month postpartum, 88 ± 31 days	Diversity -, composition -

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
			• Placebo: n = 24 Women with obesity excluded	per 250 g unit and 4.3 g of dried ground Moringa oleifera leaves • Control group		
(Gille *et al*, 2016)	2016	Germany	Pregnant women • Intervention: n = 135 • Placebo: n = 136 No information on BMI	• *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 and *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) RC-14, 10^9 CFU each/capsule, 1 capsule • Placebo that contained lactose	8 wks, from < 12 gws, sampling before and after intervention	Frequency of normal microbiota (Nugent score <4) -, frequency of bacterial vaginosis (Nugent score 4-7) -
(McMillan *et al*, 2018)	2018	Africa	Pregnant women • Intervention: n = 17 (one month), n = 8 (birth) • Placebo: n = 13 (one month), 5 (birth) No information on BMI	• *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 and *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) RC-14, one billon each, 1 capsule • Placebo (calcium carbonate)	One month, sampling at recruitment, after 1 month of treatment and at birth	α-diversity (Shannon index) -, composition genus and species level -
(Husain *et al*, 2020)	2019	United Kingdom	Pregnant women • Intervention: n = 115 • Placebo: n = 123	• *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 and *Limosilactobacillus reuteri* (formerly *Lactobacillus*	9-14 gws to end of pregnancy, sampling 18-20 and 34-36 gws	Rate of bacterial vaginosis (Nugent score ≥ 7) -, diversity -, rates of colonisation with *Lacticaseibacillus*

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
			BMI: Intervention group at study entry: 25.4 (5.0) kg/m^2 Placebo group: 25.1 (4.2) kg/m^2	*reuteri*) RC-14 2.5 billion CFUs each • Placebo excipients alone		*rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1, *Escherichia coli* or group B streptococci -
(Liang *et al*, 2021)	2020	China	Pregnant women • Intervention: n = 13 • Control: n = 15 Prepregnancy BMI: Intervention group: 21.01 ± 2.20 kg/m^2 Control group: 19.92 ± 1.79 kg/m^2	• *Bifidobacterium longum* 0.5 x 10^7 CFU, *Lactobacillus delbrueckii subsp. bulgaricus* (formerly *Lactobacillus delbrueckii bulgaricus*) 0.5 x 10^6 CFU and *Streptococcus thermophilus* 0.5 x 10^6 CFU, 2 tablets • No placebo tablets	< 32 gws until birth, sampling at study entry and before birth	α-diversity (Shannon and Simpson index) -, β-diversity -, phylum level -, genus level: *Ureaplasma* and *Gardnerella* ↑ in probiotics vs control, *Prevotella* and *Sneathia* ↑ in control vs probiotics
(Yang *et al*, 2020)	2020	Canada	Pregnant women who had an intermediate or bacterial vaginosis Nugent score (≥ 4) at 13 gws • Intervention: n = 32 • Placebo: n = 34 Prepregnancy BMI: Intervention group: 22.5 ± 3.2 kg/m^2	• *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 2.5 × 10^9 CFU and *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) RC-14 2.5 × 10^9 CFU, 2 capsules, • Placebo capsules (powder without the organisms)	12 wks, sampling 13, 28 and 35 gws	α-diversity (Shannon) -, statistically significant difference between groups (time point cannot be specifies): *Lactobacillus iners*, *Gardnerella vaginalis*, *Fannyhessea vaginae* (formerly *Atopobium vaginae*), *Lactobacillus acidophilus*, *Atopobium rimae*, *Bacillus cereus*, *Lactobacillaceae bacterium*, *Escherichia coli*, *Desulfotomaculum*

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
			Placebo group: 22.4 ± 3.1 kg/m^2			*halophilum*, *Streptococcus thermophiles*, *Erythrobacter flavus*, *Prevotella denticola*, *Coynebacterium pseudogenitalium*, *Facklamia hominis*, *Corynebacterium amycolatum*, *Clostridiales coagulans*, *Varibaculum cambriense*, *Campylobacter ureolyticus*, *Corynebacterium coyleae*, *Prevotella disiens* and *Cryptobacterium curtum*
(Vasundhara *et al*, 2021)	2021	India	Pregnant women with bacterial vaginosis (Nugent score and Amsel criteria) • Intervention: n = 8 • Placebo n = 8 (and control with normal vaginal microbiota n = 16) No information on BMI	• *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 and *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) RC-14 10^9 CFU each • Matching placebo (dextrose)	30 days from the third trimester, sampling at baseline and after 30 days of intervention	Change in α-diversity and β-diversity ↓ in probiotics group, phylum level: change in Bacillota (formerly Firmicutes) ↑ in probiotics, change in Bacteroidota (formerly Bacteroidetes) and Actinomycetota (formerly Actinobacteria) ↓ in probiotics, change in Firmicutes - in placebo, change in Actinomycetota (formerly Actinobacteria) or Pseudomonadota (formerly Proteobacteria) - in placebo, species level: change in *Gardenerella vaginalis*, *Acidaminococcus* sp. D21, *Fannyhessea vaginae*

Author	Year	Country	Study subjects	Intervention, daily dosage	Intervention duration, sampling timing	Intervention effect
						(formerly *Atopobium vaginae*), *Lactobacillus acidophilus* and *Lactobacillus gasseri* ↓ in probiotics, change in *Gardnerella vaginalis* - in placebo, change in *Lactobacillus iners*, *Prevotella copri*, *Corynebacterium tuberculostearicum*, *Cricetulus griseus*, *Lactobacillus iners*, *Lactobacillus* sp., *Pediococcus adidilactici* and *Vibrio* sp. SI9 ↑ in probiotics, change in *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus)* GR-1 or *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) RC-14 - in probiotics or placebo, change in *Lactobacillus helveticus*, *Lactobacillus gasseri* and *Megasphaera genosmosp* type_1 ↓ in placebo vs probiotics

Significant increase: ↑
Significant decrease: ↓
No significant difference: -
BMI, body mass index; CFU, colony forming units; GDM, gestational diabetes mellitus; gws, gestational weeks; hs-CRP, high sensitivity C-reactive protein; IFN-γ, interferon γ; IL-6, interleukin 6; IL-10, interleukin 10; sp, species; TNF-α, tumour necrosis α; wks, weeks.
The new names of the bacteria in genus *Lactobacillus* were obtained from the emended description of the genus *Lactobacillus* (Zheng *et al*, 2020).

2.6 Summary of the literature

Pregnancy itself induces major changes in women's physiology, however, in a tightly regulated manner to ensure growth and development of the foetus. Overweight and obesity can adversely affect these regulated changes which indeed may contribute to the development of adverse pregnancy outcomes both in short and long term, including GDM. These changes are detected in inflammatory and metabolic profile as well as microbiota. However, it is not known well whether the degree of adiposity is reflected in the changes, or which inflammatory and metabolic markers or bacteria are involved and whether those are interrelated in pregnancy. Similarly, the relation of these changes in development of GDM needs further investigation to fill in the gap in knowledge. Further, to modulate these complex interrelations dietary interventions should be explored as potential benefits are expected. The approach studied in the current thesis was intervention by fish oil and/or probiotics in an at-risk target group of pregnant women with overweight or obesity.

2.7 Hypothesis

It is hypothesised that there is an interaction between microbiota, metabolism and low-grade inflammation, and this further impacts maternal health in pregnant women with overweight and obesity (**Figure 1**). Subsequently it may be possible to intervene these factors with fish oil and probiotics. Specifically, it is hypothesised that the levels of circulating low-grade inflammatory and metabolic markers as well as gut microbiota are altered in obesity as compared to overweight, and further the altered levels of circulating low-grade inflammatory and metabolic markers as well as the unbalanced vaginal microbiota can increase the incidence of GDM. Eventually, the altered levels of circulating low-grade inflammatory and metabolic markers as well as vaginal microbiota can be modulated by fish oil and/or probiotics in pregnant women with overweight and obesity.

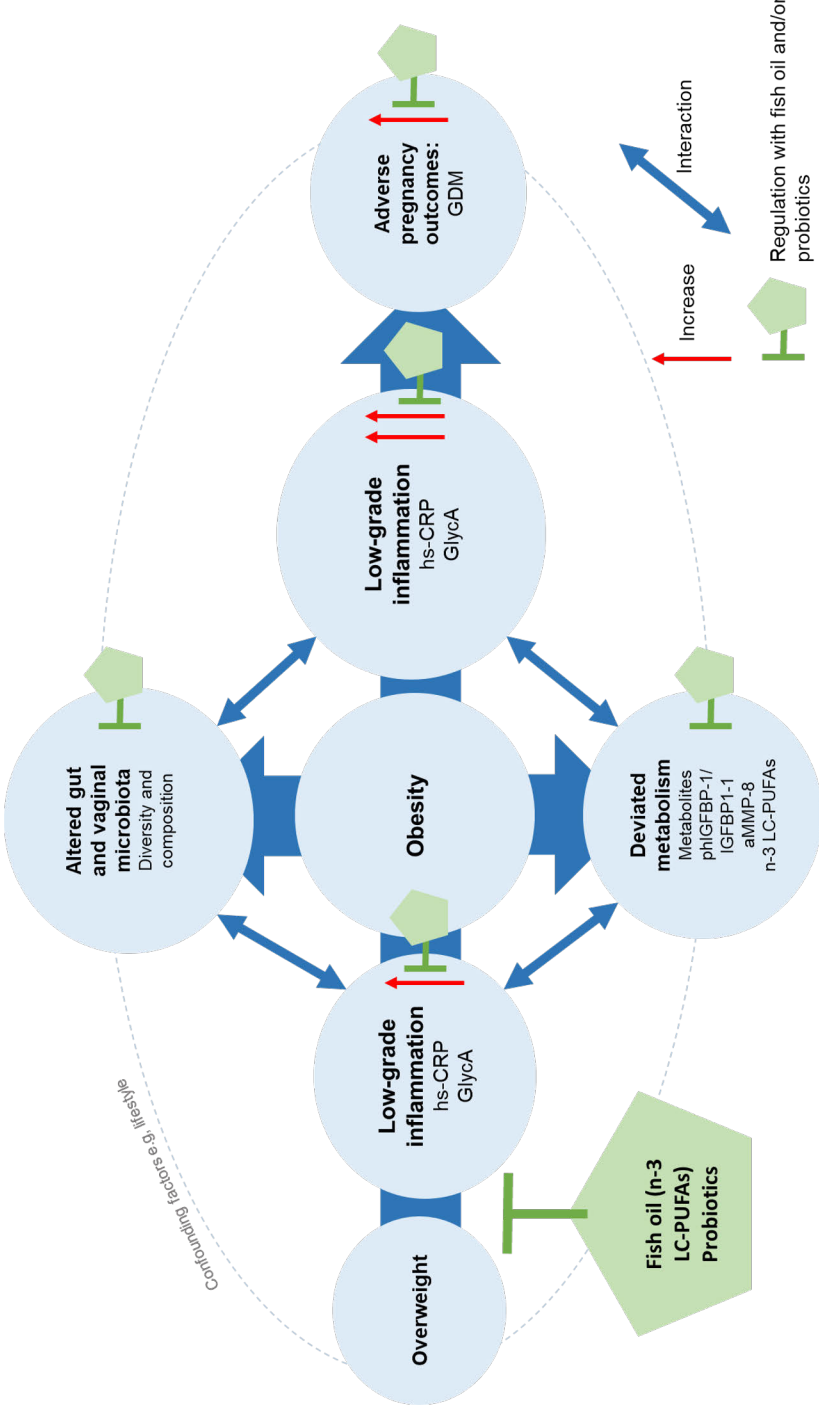


Figure 1. Modification of microbiota, metabolism and low-grade inflammation by n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) and probiotics offers novel opportunity to regulate interaction between these three factors with likely beneficial health effects on pregnant women with overweight and obesity in increased risk for gestational diabetes mellitus (GDM). aMMP-8, active matrix metalloproteinase 8; GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; hs-CRP, high sensitivity C-reactive protein; n-3 LC-PUFAs, n-3 long-chain polyunsaturated fatty acids; phIGFBP-1, phosphorylated insulin-like growth factor binding-protein 1.

3 Aims

In this study, the overall aim was to investigate the interrelations of circulating markers of low-grade inflammation and metabolism as well as microbiota, and the means to intervene this complex relationship in pregnant women with overweight and obesity.

The specific aims were to study in pregnant women with overweight and obesity:

1. The difference in circulating low-grade inflammatory markers and metabolites as well as gut microbiota between pregnant women with overweight and obesity (study I)
2. The interaction between circulating low-grade inflammatory and metabolics markers (study I, II and IV) and vaginal microbiota (study III)
3. The relationships of circulating and vaginal low-grade inflammatory and metabolic markers with the onset of GDM (studies II–IV)
4. The impact of fish oil and/or probiotics on circulating low-grade inflammatory markers and circulating and vaginal metabolic markers (study II and IV) and on vaginal microbiota (study III)

4 Materials and Methods

4.1 Study design, participants and conduct

The data is derived from mother-infant clinical trial with double-blind randomised placebo-controlled study design (ClinicalTrials.gov, NCT01922791). For the study I, the baseline data are utilised and for studies II, III and IV the baseline in early pregnancy and the second time point data in later pregnancy are used. The trial was conducted in the Turku University Hospital and University of Turku in Finland and the participants were recruited from Turku and neighbouring cities between October 2013 and July 2017. The recruitment protocol included recruitment advertisements distributed to maternal welfare clinics and ultrasound measurement units as well as advertisements shared in newspapers and social media. The inclusion criteria were a signed consent form, age 18–45 years, BMI ≥ 25 kg/m^2, early pregnancy (< 18 weeks of gestation) and absence of chronic diseases impacting metabolic and gastrointestinal health, including type 1 and 2 diabetes and coeliac disease (asthma and allergies were allowed). Exclusion criteria were BMI ≤ 25 kg/m^2, mid- or late pregnancy, chronic diseases, continuous intake of other probiotic or fish/vegetable oil supplements and increased bleeding tendency. In total 439 pregnant women were recruited. One woman was later excluded since it was revealed that she had familiar hypercholesterolemia, and thus did not fulfil the inclusion criteria, and therefore in total 438 women were included in the study. The women were allocated into four intervention groups according to their parity and history of GDM, and stratified randomization (random permuted blocks of 4) and randomisation list of three blocks was done by a statistician (T. Poussa, STAT-Consulting, Nokia, Finland). The four dietary intervention groups were: 1) fish oil and placebo for probiotics, 2) probiotics and placebo for fish oil, 3) fish oil and probiotics and 4) placebo and placebo.

The women visited the study centre in early and late pregnancy; the allocation to the intervention groups was done at the first visit i.e. early pregnancy, and the second visit took place at late pregnancy. Biological samples, i.e., serum, faecal and vaginal samples were collected in early and late pregnancy, and those were used for determining the predefined secondary outcomes of the main trial (hs-CRP, microbiota, metabolites, fatty acids, IGFB-1, phIGFBP-1 and aMMP-8) (**Figure 2**).

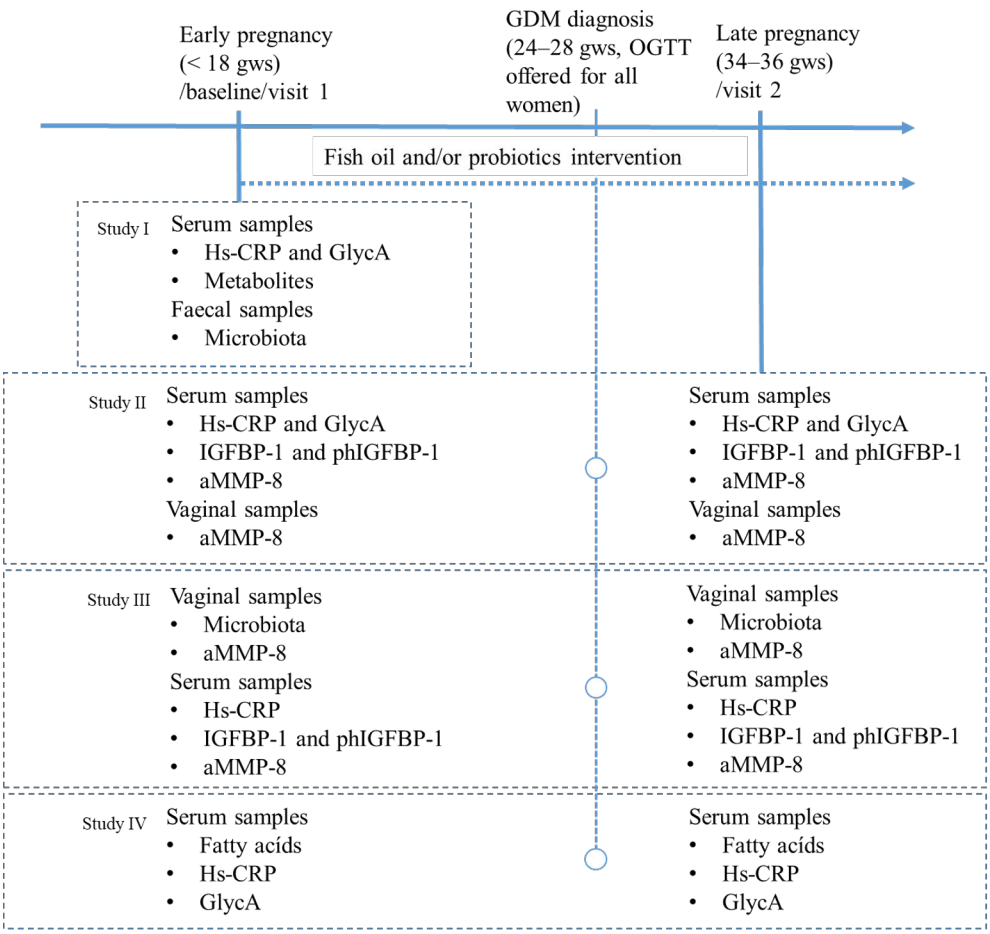


Figure 2. Timeline of the secondary analyses of the main trial included in this thesis. aMMP-8, active matrix metalloproteinase 8; GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; gws, gestational weeks; hs-CRP, high-sensitivity C-reactive protein; phIGFBP-1, phosphorylated insulin-like growth factor binding-protein 1; OGTT, oral glucose tolerance test.

4.2 Ethics

The study conduct followed the guidelines laid down in the Declaration of Helsinki. The Ethics Committee of the Hospital district of Southwest Finland approved the study protocol, which describes all the procedures done for study participants. Also, the University hospital of Turku gave permission to the study conduct. All participants who were included in the study gave written informed consent.

4.3 Dietary intervention supplements

The women were provided two fish oil capsules and one probiotics capsule daily. The fish oil capsules (Croda Europe Ltd., Leek, UK, Incromega E1070) contained 2.4 g of n-3 fatty acids in two capsules; 1.9 g docosahexaenoic acid (22:6 n-3, DHA), 0.22 g eicosapentaenoic acid (20:5 n-3, EPA) and the remaining amount other n-3 fatty acids such as DPA. The probiotics capsule contained *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus* HN001) (ATCC SD5675; DuPont, Niebüll, Germany) and *Bifidobacterium animalis* ssp. *lactis* 420 (DSM 22089; DuPont), each with 10^{10} colony-forming units in one capsule.

The placebo capsules for fish oil consisted of 2.4 g medium-chain fatty acids (capric acid C8 54.6% and caprylic acid C10 40.3%) while the placebo for the probiotics consisted of microcrystalline cellulose. The flavour, size, shape and colour was same for the respective active intervention capsule.

Of the women 88.4% reported good compliance (study capsules consumed ≥ 5 days/week at both time points) and 91.8 ± 15.9% of the capsules had been consumed as calculated from the returned fish oil capsules (Pellonperä *et al*, 2019). Further, principal component analysis showed a clear separation of the intervention groups according to the lipids that reflect the intake of fish oil indicating a good compliance (Mokkala *et al*, 2021b).

4.4 Clinical parameters

4.4.1 Body mass index

Prepregnancy BMI (kg/m^2) was calculated by dividing the weight in kilograms with height in meters. The weight was self-reported and obtained from maternal welfare clinic records. Height was measured at the first study visit by using a wall stadiometer in 0.1 cm accuracy. Overweight is BMI ≥ 25 kg/m^2 and < 30 kg/m^2 while obesity ≥ 30 kg/m^2.

4.4.2 Body composition

The body composition of the pregnant women was measured by air-displacement plethysmography (the Bod Pod system, software version 5.4.0, COSMED Inc.) and the subsequent fat percentage was estimated using formula developed specifically for pregnant women by Raaij (1988). The measurement of body composition is described in more detail previously (Pellonperä *et al*, 2021).

4.4.3 Diagnosis of gestational diabetes mellitus

The diagnosis of GDM was based on a 75 g 2-hour oral glucose tolerance test. The test was offered to the pregnant women late pregnancy 24–28 gestation weeks (**Figure 2**) in the maternal welfare clinics. GDM was diagnosed when one or more values were at or above the threshold level: 0 h ≥ 5.3, 1 h ≥ 10.0, and 2 h ≥ 8.6 mmol/L, according to the Finnish Current Care Guidelines (The Finnish Medical Society Duodecim, 2022).

4.4.4 Dietary intake

The women were instructed to fill in 3-day food diary prior to the study visits including one weekend day and two weekdays. The research personnel checked the food diaries to ensure the reporting was accurate and it was done with the help of portion picture booklet. The daily intake of energy, energy yielding nutrients (energy percent (E%) and grams (g)) and fibre (g) was calculated with a software (AivoDiet 2.0.2.3, Aivo, Turku, Finland). The food composition database was provided by the the Finnish National Institute for Health and Welfare (www.fineli.fi).

4.4.5 Questionnaires and interview

The women fill in questionnaires about their clinical background information and diaries about their health status and medication usage (e.g., antibiotics usage: the women who had used vaginal antibiotics were excluded from the analyses in study III) during pregnancy. The women were interviewed for usage of additional fish oil and probiotics supplements after and during the study.

4.5 Blood sampling and analyses

Fasting blood samples (at least 9 hours overnight fasting) were drawn from antecubital vein of the women in a certified laboratory (TYKSLAB, the Hospital District of Southwest Finland) at both visits. The whole blood was separated to plasma and serum. The serum aliquots were stored in -80˚C for further analyses of metabolomics, IGFBP-1, aMMP-8 and fatty acids.

4.5.1 Low-grade inflammation

The concentration of fasting serum hs-CRP was determined by an automated colorimetric immunoassay on the Dade Behring Dimension RXL autoanalyzer (Siemens Healthcare, Camberly, Surrey, UK) in a certified laboratory (TYKSLAB).

The concentration of fasting serum GlycA was quantified by a high-throughput proton NMR spectroscopy (Nightingale, Helsinki, Finland). GlycA consist of multiple acute phase glycoproteins (α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin) of which signals are detected as NMR-signals from N-acetyl sugar groups of glycoproteins (Otvos *et al*, 2015).

4.5.2 Metabolites

The metabolic profile of fasting serum samples was analysed by NMR spectroscopy (Nightingale, Helsinki, Finland). The details of the analyses are described previously (Soininen *et al*, 2015). The metabolomics platform quantified metabolites and their ratios, these including 213 lipids and nine amino acids in the pregnant women.

4.5.3 phIGFBP-1 and IGFBP-1

The concentrations of fasting serum and vaginal IGFBP-1 and phIGFBP-1 were analysed by immunoenzymometric assays (Actim, Espoo, Finland) as described previously (Nuutila *et al*, 1999; Kruit *et al*, 2018).

4.5.4 aMMP-8

The concentration of fasting serum and vaginal aMMP-8 was analysed by a solid-phase immunoenzymometric assay (MMP-8 IEMA, Actim, Espoo, Finland); the analyses are described previously in Myntti et al. (2016), Myntti et al. (2017) and Tuomainen et al. (2007) and in Publication II.

4.5.5 Fatty acids

The fatty acid composition of four serum lipid fractions (PC, CEs, TAGs, NEFAs) was determined by gas chromatography (Agilent Technologies). The methodology of these analyses are described in more detail in Fisk et al (2018). Briefly, internal standards, dipentadecanoyl-PC, heneicosanoic acid, cholesteryl heptadecanoate and tripentadecanoin, were added to each serum sample. Lipid was extracted into chloroform–methanol (2:1 vol/vol). PC, NEFAs, CEs and TAGs were separated by solid-phase extraction on aminopropyl silica cartridges. Fatty acids were removed and simultaneously methylated to produce fatty acid methyl esters (FAMEs) by heating in methanolic sulphuric acid. FAMEs were separated by gas chromatography and were identified by comparison with retention times of thirty-seven FAMEs standards run alongside the samples. Finally, the FAMEs were quantified using ChemStation software (Agilent Technologies) and Microsoft Excel (Microsoft

Corporation). The data are expressed as concentration (μg/mL) and percentage of total fatty acids (%).

4.5.6 Glucose metabolism

The concentration of fasting plasma glucose was determined by an enzymatic method utilizing hexokinase (Cobas 8000 automatic c702-analyzer, Roche Diagnostics GmbH, Mannheim, Germany), fasting plasma insulin by immunoelectrochemiluminometric assay (a modular E170 automatic analyser, Roche Diagnostics GMbH, Mannheim, Germany) (TYKSLAB). Homeostatic model assessment (HOMA2-IR) was calculated from fasting plasma glucose and insulin (Wallace *et al*, 2004).

4.5.7 Endotoxin and intestinal permeability

Serum fasting LPS activity was determined by Limulus amebocyte lysate assay coupled with a chromogenic substrate (HyCult Biochemistry, Uden, the Netherlands). Serum fasting zonulin was measured by using a zonulin ELISA kit (Immundiagnostik AG) as previously described (Mokkala *et al*, 2016).

4.6 Faecal and vaginal sampling and analyses

The pregnant women were asked to collect faecal sample in a sterile plastic pot at home before the both study visit or previous evening. The samples were collected by the study personnel in the visit and stored at 4˚C and then transferred for DNA extraction, which was done during the same day as the study visits. Extracted DNA was frozen in -70˚C for further analysis.

The vaginal samples for IGFBP-1 and aMMP-8 and microbiota analysis were obtained by research personnel by sterile swabs in both visits as described previously in Publication II and III. The samples for IGFBP-1 and aMMP-8 analyses were stored in -20 °C, and samples for vaginal microbiota in -20 °C and then transferred to -80 °C within one week and stored there until analysis.

4.6.1 Analysis of gut microbiota

Gut microbiota composition was analysed using 16S RNA gene sequencing as described in detail previously (Mokkala *et al*, 2016). The relative abundance was determined using operating taxonomic units which were a total of 731. The bacteria with relative abundance >1% were included in the analyses. Chao1, observed

species, phylogenetic diversity and Shannon index were analysed to study the diversity and richness.

4.6.2 Analysis of vaginal microbiota

Bacterial DNA was extracted from the vaginal swab samples using a bead beating method as previously described (Virtanen *et al*, 2019). Sample preparation and Illumina MiSeq sequencing of the V3–V4 16S rRNA gene amplicons were performed as previously described (Virtanen *et al*, 2019) using 2 × 300 bp reads and a MiSeq v3 reagent kit at the Biomedicum Functional Genomics Unit (FuGU), Helsinki, Finland. In total 14,195,964 paired end reads for 234 samples were obtained and the median read count was 56,205/sample (180–244,519 reads per sample) (Publication III). The pre-processing trimming and annotation of sequencing data are described in more detail in Publication III.

4.7 Statistics

The description of data analysed and statistical tests used in the studies I–IV are summarised in **Table 4A–B**. The analyses of impact of the intervention on the concentration of GlycA was only included in this thesis, for this Kruskal-Wallis and One-way ANOVA were used. The statistical analyses in studies I–IV were conducted with SPSS Statistics 24.0 (IBM, Chicago, IL, USA) for Windows. For study III also R 3.6.3 was utilised.

Table 4A. Description of data analysed in studies I–IV with n of subjects, exposures, outcomes and covariates.

Study	Number of study subjects	Exposures	Outcomes	Covariates
I	Early pregnancy: 99	• Overweight and obesity status • Serum hs-CRP and GlycA	• Serum hs-CRP, GlycA, metabolites and gut microbiota • Serum metabolites	-
II	Early pregnancy: 434 Late pregnancy: 369 (367 early-late pairs)	• Fish oil and/or probiotics • Serum hs-CRP, phIGFBP-1, IGFBP-1 and serum/vaginal aMMP-8	• Serum hs-CRP, phIGFBP-1, IGFBP-1 and serum/vaginal aMMP-8 • Onset of GDM	Intervention and prepregnancy BMI (in GDM analysis)
III	Early pregnancy: 112 Late pregnancy: 116 (82 early-late pairs)	• Fish oil and/or probiotics • Serum hs-CRP, phIGBFP-1, IGFBP-1 and serum/vaginal aMMP-8 • Vaginal microbiota	• Vaginal microbiota • Onset of GDM	-
IV	Early pregnancy: 431 Late pregnancy: 361 (344 early-late pairs)	• Fish oil and/or probiotics • Serum hs-CRP and GlycA • Serum fatty acids	• Serum fatty acids • Onset of GDM	Intervention (in GDM analysis)

aMMP-8, active matrix metalloproteinase 8; BMI, body mass index; GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; hs-CRP, high sensitivity C-reactive protein; n-3 LC-PUFAs, n-3 long-chain polyunsaturated fatty acids; phIGFBP-1, phosphorylated insulin-like growth factor binding-protein 1.

Table 4B. Summary of study designs and statistical analyses used in each study.

Study	Study design	Statistical tests	Adjusted for multiple comparisons
I	Cross-sectional	Mann-Whitney *U* test, Independent Samples T-test, Spearman rank order test	Yes (the analyses of gut microbiota and metabolites, Benjamini-Hochberg method)
II	RCT, prospective, longitudinal	One-way ANOVA, Kruskal-Wallis, ANOVA, Pearson correlation analysis, Logistic regression analysis	No
III	RCT, prospective, longitudinal	Kruskal-Wallis, Wilcoxon test, DESeq2, Spearman rank order test, Chi-square test	Yes (the analyses of vaginal microbiota, Benjamini-Hochberg method)
IV	RCT, prospective, longitudinal	One-way ANOVA + Tukey's post-hoc test, Welch ANOVA + Tamhane's T2 post-hoc test, Spearman rank order test, Logistic regression analysis, Kruskal-Wallis, Chi-square test	No

ANOVA, analysis of variance; RCT, randomised controlled trial.

Post-hoc power was calculated for study I (GlycA and Prevotellaceae) and II (aMMP-8) (**Table 5**). Calculations were done using two tailed test and 5 % significance level with G*Power (version 3.1.9.7).

Table 5. Post-hoc power calculations for serum GlycA and Prevotellaceae (study I) and serum aMMP-8 (study II) between groups of interest.

Outcome	Mean ± SD n	Mean ± SD n	Effect size	Achieved power
Serum GlycA (mmol/L)	1.45 ± 0.11 52	1.57 ± 0.19 48	0.77	97 %
Prevotellaceae[a] (relative abundance, %)	-8.24 ± 3.35 48	-9.17 ± 3.54 43	0.27	25 %
Serum aMMP-8* (ng/mL)[b]	2.75 ± 0.71 90	2.83 ± 0.70 87	0.11	12 %

[a]Natural log-transformed variable

[b]For serum aMMP-8 the groups are fish oil+probiotics and placebo+placebo.

5 Results

5.1 Clinical characteristics of the pregnant women

The clinical characteristics of the pregnant women are presented in the **Table 6** and **Table 7**. In the study I there were 99 women in early pregnancy who were divided according to the prepregnancy BMI; overweight BMI 25–29.99 kg/m^2 and obese BMI ≥ 30 kg/m^2 included. The pregnant women with obesity had higher body fat percentage ($p<0.001$). The daily dietary intake of energy (overweight vs obese; 8090 ± 1503 vs 8194 ± 2203 kJ), carbohydrates (45 ± 7 vs 47 ± 6 E%) , protein (18 ± 5 vs 17 ± 3 E%), fat (35 ± 7 vs 34 ± 6 E%) or fibre (19 ± 6 vs 20 ± 7 g) did not differ between the women with overweight or obesity. The pregnant women with obesity had higher plasma fasting insulin (14.0 (9.0–17.0) vs 9.0 (7.0–11.0) mU/L, $p<0.001$) and glucose (4.9 ± 0.3 vs 4.7 ± 0.3 mmol/L, $p=0.015$) than their overweight counterparts.

The baseline characteristics of the women who completed the intervention are presented in **Table 7**. The characteristics of the women in the groups did not differ except for number of women who smoked before pregnancy in study III and IV. The daily dietary intake of carbohydrates, protein, fat, and fibre, PUFAs, MUFAs, SFAs, n-3 PUFAs or n-6 PUFAs did not differ between intervention groups in study IV (see Table 1 in publication IV).

Table 6. Clinical characteristics of the pregnant women with overweight and obesity. Modified from Original publication I.

Characteristics	Pregnant women with overweight	Pregnant women with obesity
n	52	48
Age (years)	30 ± 5	30 ± 5
Gestational weeks	13 ± 3	13 ± 3
Prepregnancy BMI (kg/m^2)	27 ± 2	34 ± 4
Fat percentage (%)	39.8 ± 4.6	48.3 ± 3.8

Data are presented as mean ± standard deviation.

Table 7. Baseline clinical characteristics of the pregnant women in the intervention. Modified from Original publication III and IV. The number of subjects (n) is presented as fish oil+placebo/probiotics+placebo/fish oil+probiotics/placebo+placebo/all women.

Characteristics	Pregnant women in study III		Pregnant women in study IV	
Age (years)	30.4 (28.2–33.9)	n = 35/39/36/36/146	30.6 ± 4.5	n = 90/92/91/88/361
Prepregnancy BMI (kg/m^2)	28.4 (26.5–30.9)	n = 35/39/36/36/146	28.7 (26.5–32.0)	n = 90/92/91/88/361
Obese (%)	34.2	n = 35/39/36/36/146	39.3	n = 90/92/91/88/ 361
Women developing GDM in later pregnancy (%)	27.8	n = 32/36/31/27/126	23.0	n = 79/86/83/78/326
Smoking before pregnancy (%)	21.5	n = 34/37/34/30/135	20.5	n = 90/90/90/86/356

Data are presented as mean ± standard deviation or median (interquartile range) or percentage (%). The number of subjects in study II were somewhat similar to study IV, and thus not included in the table.

5.2 The difference in circulating low-grade inflammatory markers and metabolites and gut microbiota between pregnant women with overweight and obesity (study I)

The pregnant women with obesity exhibited statistically significantly higher level of serum low-grade inflammatory markers, hs-CRP and GlycA, as compared to pregnant women with overweight in early pregnancy (**Table 8**). Additionally, the differences in serum zonulin, a marker of intestinal permeability, and a marker of endotoxemia, serum LPS, were investigated, but the values of those markers did not differ between the groups.

Table 8. Low-grade inflammatory, intestinal permeability and endotoxemia markers in pregnant women with overweight and obesity. Modified from Original publication I.

	Pregnant women with overweight	Pregnant women with obesity	P value
n	52	48	
hs-CRP (mg/L)	4.0 (1.8–6.9)	6.1 (4.0–10.0)	0.002
GlycA (mmol/L)	1.45 ± 0.11	1.57 ± 0.19	<0.001
Zonulin (ng/mL)	44.6 ± 8.7	48.9 ± 13.1	0.05
LPS (EU/mL)	0.37 ± 0.06	0.37 ± 0.08	0.80

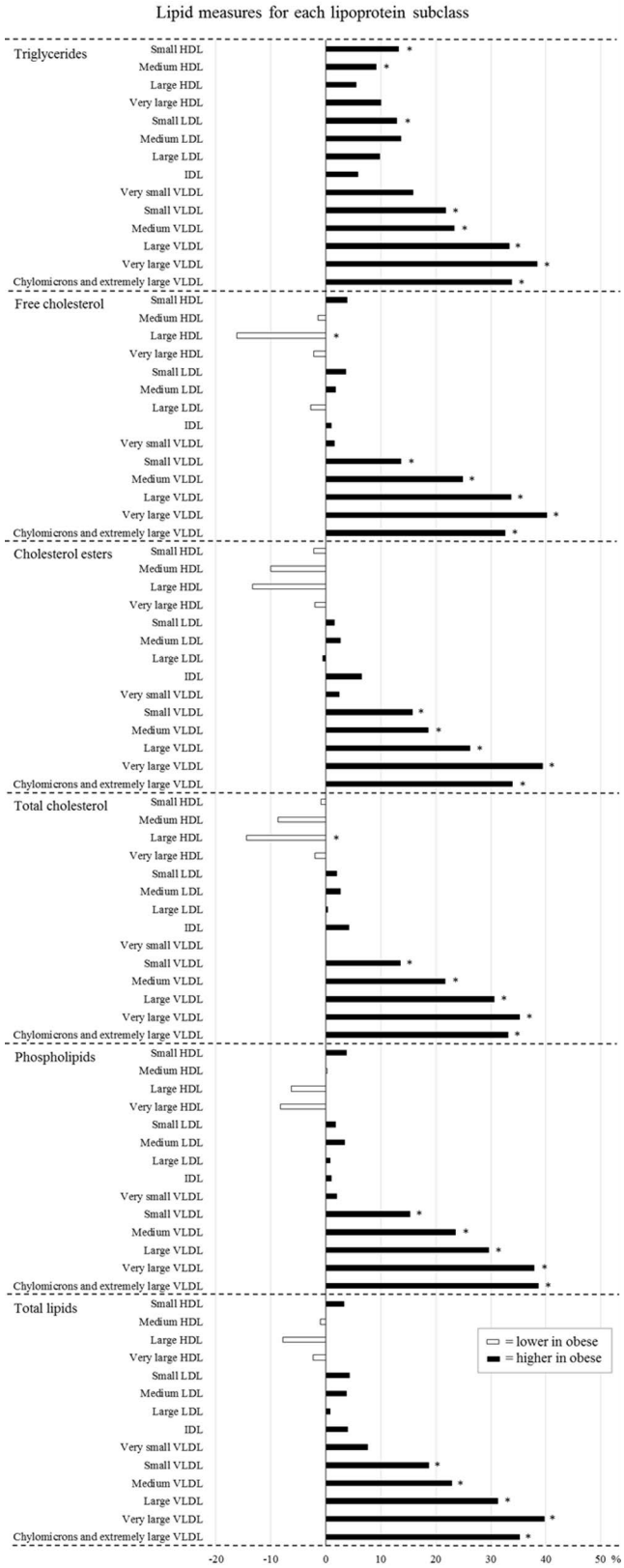
Data are presented as mean ± standard deviation or median (interquartile range). P value <0.05 is considered statistically significant.

The evaluation of nine amino acids and 219 lipids from NMR spectroscopy analysis revealed that the concentrations of three BCAAs and one aromatic amino acid (**Table 9**) and 54 lipids (**Figure 3**) were higher in pregnant women with obesity than women with overweight. The lipids that were higher in obese consisted mainly of concentrations of several VLDL particles and lipid measures in several VLDL particles.

Table 9. The concentrations of branched chain and aromatic amino acids in pregnant women with overweight and obesity. Modified from Original publication I.

Characteristics	Pregnant women with overweight	Pregnant women with obesity	Adj. P value
n	52	48	
Isoleucine (mmol/L)	0.04 (0.04–0.05)	0.05 (0.04–0.06)	0.024
Leucine (mmol/L)	0.06 (0.06–0.07)	0.07 (0.06–0.08)	0.026
Valine (mmol/L)	0.1 (0.1–0.2)	0.2 (0.1–0.2)	0.10
Phenylalanine (mmol/L)	0.08 (0.07–0.08)	0.08 (0.08–0.09)	0.050

Data are presented as median (interquartile range). The adjusted P value <0.12 is considered statistically significant.


Lipid measures for each lipoprotein subclass
Triglycerides
Free cholesterol
Cholesterol esters
Total cholesterol
Phospholipids
Total lipids
Small HDL
Medium HDL
Large HDL
Very large HDL
Small LDL
Medium LDL
Large LDL
IDL
Very small VLDL
Small VLDL
Medium VLDL
Large VLDL
Very large VLDL
Chylomicrons and extremely large VLDL
= lower in obese
= higher in obese
-20
-10
0
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20
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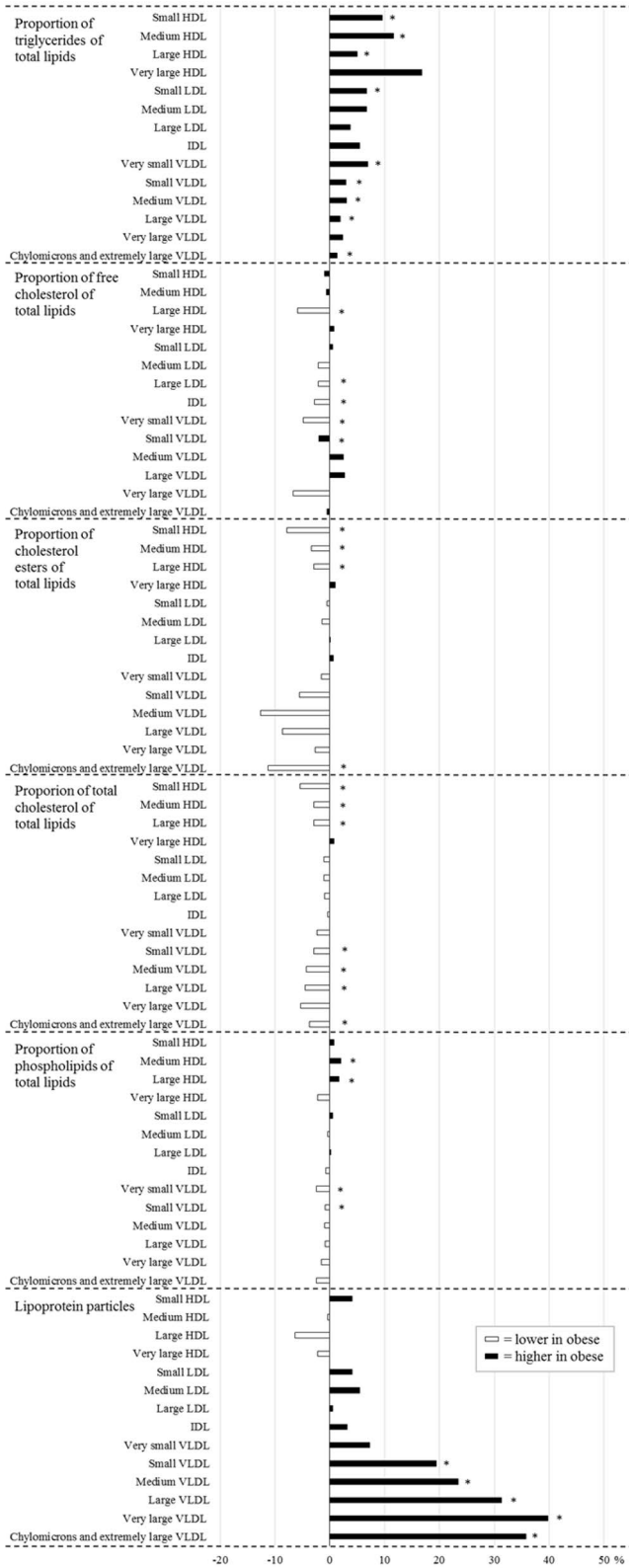
40
50 %


Proportion of triglycerides of total lipids
Proportion of free cholesterol of total lipids
Proportion of cholesterol esters of total lipids
Proporion of total cholesterol of total lipids
Proportion of phospholipids of total lipids
Lipoprotein particles
Small HDL
Medium HDL
Large HDL
Very large HDL
Small LDL
Medium LDL
Large LDL
IDL
Very small VLDL
Small VLDL
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Very large VLDL
Chylomicrons and extremely large VLDL
= lower in obese
= higher in obese
-20
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50 %

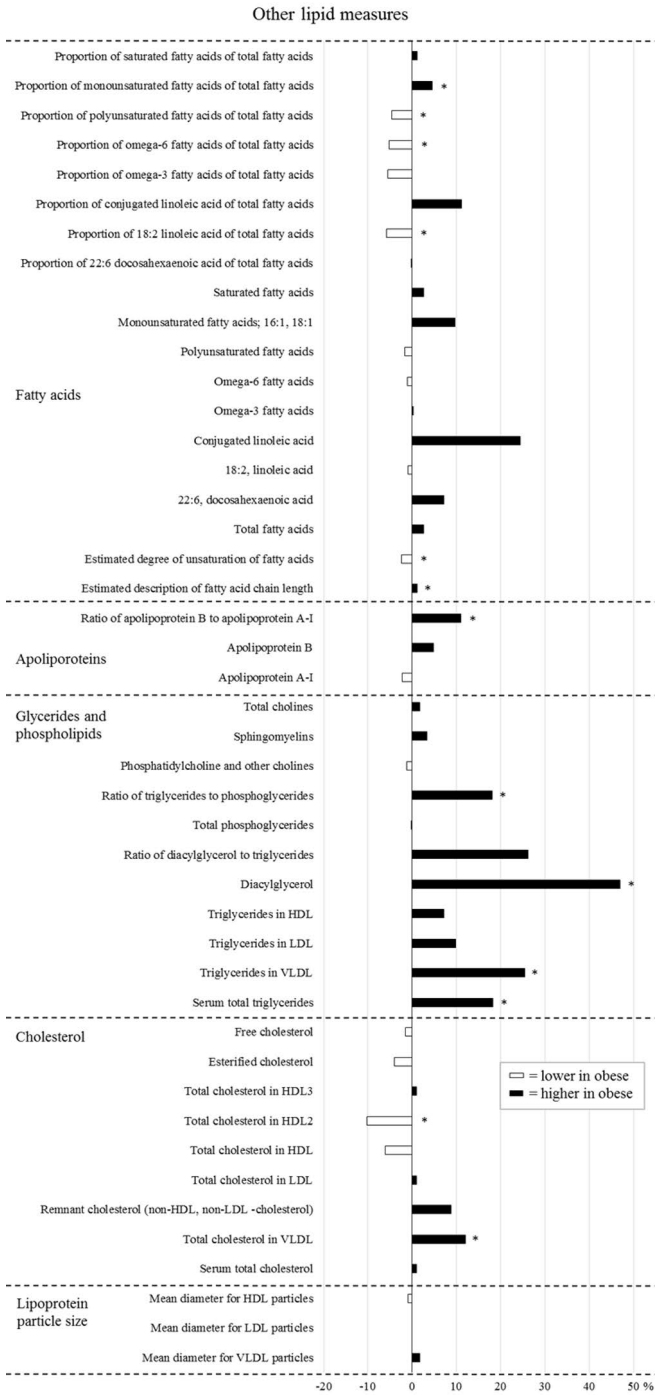


Figure 3. Median percentage differences in measures of lipids of pregnant women with obesity as compared to pregnant women with overweight. Adjusted P values < 0.12 are considered statistically significant. From Original publication I.

The gut microbiota differed between the groups as the relative abundance of bacterial family Prevotellaceae was higher in pregnant women with obesity as compared to the pregnant women with overweight after adjusting for multiple variable comparisons (3.69 ± 9.03 vs 2.50 ± 6.82, adj. p=0.19). α-Diversity indices (richness index Chao1 (376.4 ± 58.7 vs 387.5 ± 56.6, p=0.36), observed species (336.2 (296.2–372.1) vs 348.6 (296.2–372.1), p=0.38), phylogenetic diversity (36.5 (31.7–39.8) vs 36.93 (31.5–41.8), p=0.53), Shannon index (5.4 (5.0–5.9) vs 5.48 (5.3–5.8), p=0.64) or Firmicutes to Bacteroidetes ratio (0.89 (0.61–1.22) vs 0.92 (0.62–1.23), p=0.59) did not differ between the groups.

5.3 The interaction between low-grade inflammatory and metabolic markers and vaginal microbiota

5.3.1 The interaction between circulating low-grade inflammatory markers and metabolites, phIGFBP-1, IGFBP-1 and aMMP-8 (study I & II)

In early pregnancy hs-CRP correlated with 59 lipids and GlycA with 171 lipids in all pregnant women with overweight and obesity (n = 99) (adj. p<0.05, see exact values in original publication I, Supplementary table 1c). GlycA and hsCRP correlated directly with isoleucine and phenylalanine, and GlycA correlated with leucine and alanine (**Table 10**).

Table 10. The Spearman correlation coefficients (rho) between GlycA and hs-CRP and amino acids in pregnant women with overweight and obesity (n = 99). Modified from Original publication I.

	The correlation between GlycA with amino acids		**The correlation between hs-CRP with amino acids**	
Amino acids	**Rho**	**Adj. P value**	**Rho**	**Adj. P value**
Isoleucine (mmol/L)	0.64	0.01	0.27	0.03
Leucine (mmol/L)	0.44	0.04	0.23	0.07
Phenylalanine (mmol/L)	0.50	0.01	0.40	<0.001
Valine (mmol/L)	0.08	0.39	0.11	0.34
Glycine (mmol/L)	-0.02	0.39	0.04	0.67
Histidine (mmol/L)	-0.09	0.07	-0.19	0.14
Tyrosine (mmol/L)	-1.6E-04	0.67	0.12	0.34
Alanine (mmol/L)	0.36	<0.001	0.15	0.24
Glutamine (mmol/L)	-0.09	0.15	-0.06	0.60

Adjusted P value <0.05 is considered statistically significant.

Serum hsCRP and phIGFBP-1, IGFBP-1 and aMMP-8 correlated weakly between each other's in the women (n = 346–428). In particular, direct correlations were seen between serum hs-CRP and serum IGFBP-1 (r=0.121, p=0.02) in early pregnancy. In late pregnancy hs-CRP correlated directly with serum aMMP-8 (r=0.137, p=0.01) and change in serum aMMP-8 (r=0.118, p=0.03). Further, the change in hs-CRP correlated directly with late pregnancy serum IGFBP-1 level (r=0.142, p=0.008). Indirect correlations were detected between hs-CRP in early pregnancy and the serum phIGFBP-1 in early and late pregnancy (r=-0.131, p=0.007, r=-0.206, p<0.001, respectively) and serum MMP-8 (r=-0.189, p<0.001) in late pregnancy.

5.3.2 The interaction between circulating low-grade inflammatory marker, phIGFBP-1, IGFBP-1, aMMP-8 and vaginal aMMP-8 and vaginal microbiota (study III)

Serum hs-CRP, phIGBFP-1, IGFBP-1 or aMMP-8 did not correlate with vaginal α-diversity either at early or late pregnancy but vaginal aMMP-8 correlated directly with α-diversity in late pregnancy (rho=0.38, p<0.001). Any of the markers did not correlate with bacteria at genus level whilst vaginal aMMP-8 correlated negatively with species level bacteria, *L. fornicalis* (rho=-0.38, p=0.02) in early pregnancy and *L. crispatus* (rho=-0.40, p=0.005) in late pregnancy.

5.3.3 The interaction between circulating low-grade inflammatory markers and fatty acids (study IV)

The correlations between hs-CRP, GlycA and n-3 LC-PUFAs are visualised in **Figure 4** and **5**. The concentration of hs-CRP correlated inversely with % of DHA and % of total n-3 LC-PUFAs in serum PC and % of total n-3 LC-PUFAs in serum TAGs whereas GlycA directly with the concentration of DHA and the concentration of total n-3 LC-PUFAs in serum PC, CEs and TAGs and concentration of EPA in serum PC and TAGs in early pregnancy. EPA was not associated with hs-CRP. In late pregnancy hs-CRP did not correlate with DHA or total n-3 LC-PUFAs but GlycA correlated directly with the concentrations of DHA in serum NEFAs and the concentration of DHA and total n-3 LC-PUFAs in serum TAGs but also indirectly with % of DHA and % of total n-3 LC-PUFAs in serum TAGs.

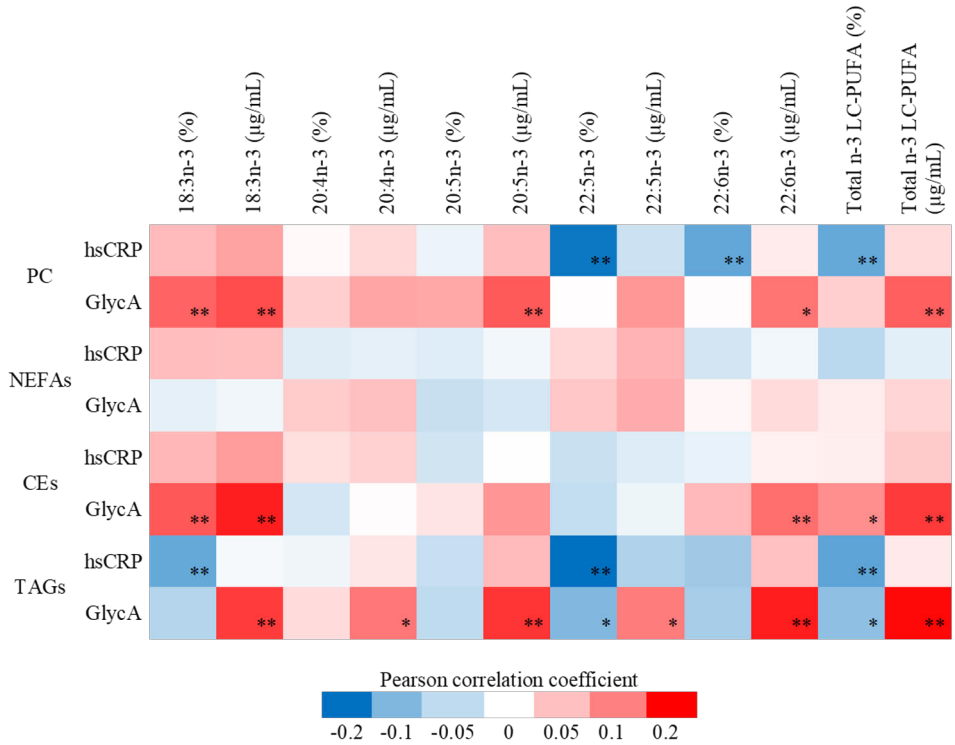


Figure 4. The heatmap describing the Pearson correlation coefficients between early pregnancy serum hs-CRP and GlycA and n-3 LC-PUFAs in PCs, NEFAs, CEs and TAGs. Red colour indicates positive correlations while blue negatives, **p<0.01, *p<0.05. n varies between 359 and 361. The following variables were natural log-transformed: hsCRP, PC 18:3n-3 %, PC 18:3n-3 µg/mL, PC 20:4n-3 %, PC 20:4n-3 µg/mL, PC 20:5n-3 %, PC 20:5n-3 µg/mL, PC 22:5n-3 µg/mL, NEFA 18:3n-3 %, NEFA 18:3n-3 µg/mL, NEFA 20:4n-3 %, NEFA 20:4n-3 µg/mL, NEFA 20:5n-3 %, NEFA 20:5n-3 µg/mL, NEFA 22:5n-3 µg/mL, NEFA 22:6n-3 %, NEFA 22:6n-3 µg/mL, CE 18:3n-3 %, CE 18:3n-3 µg/mL, CE 20:4n-3 %, CE 20:4n-3 µg/mL, CE 20:5n-3 %, CE 20:5n-3 µg/mL, CE 22:5n-3 %, CE 22-5n:3 µg/mL, CE 22:6n-3 %, CE 22:6n-3 µg/mL, TAG 18:3n-3 %, TAG 18:3n-3 µg/mL, TAG 20:4n-3 %, TAG 20:4n-3 µg/mL, TAG 20:5n-3 %, TAG 20:5n-3 µg/mL, TAG 22:5n-3 µg/mL, TAG 22:6n-3 %, TAG 22:6n-3 µg/mL, PC n-3 total µg/mL, NEFA n-3 total %, NEFA n-3 total µg/mL, CE n-3 total %, CE n-3 total µg/mL, TAG n-3 total %. From Original publication IV.

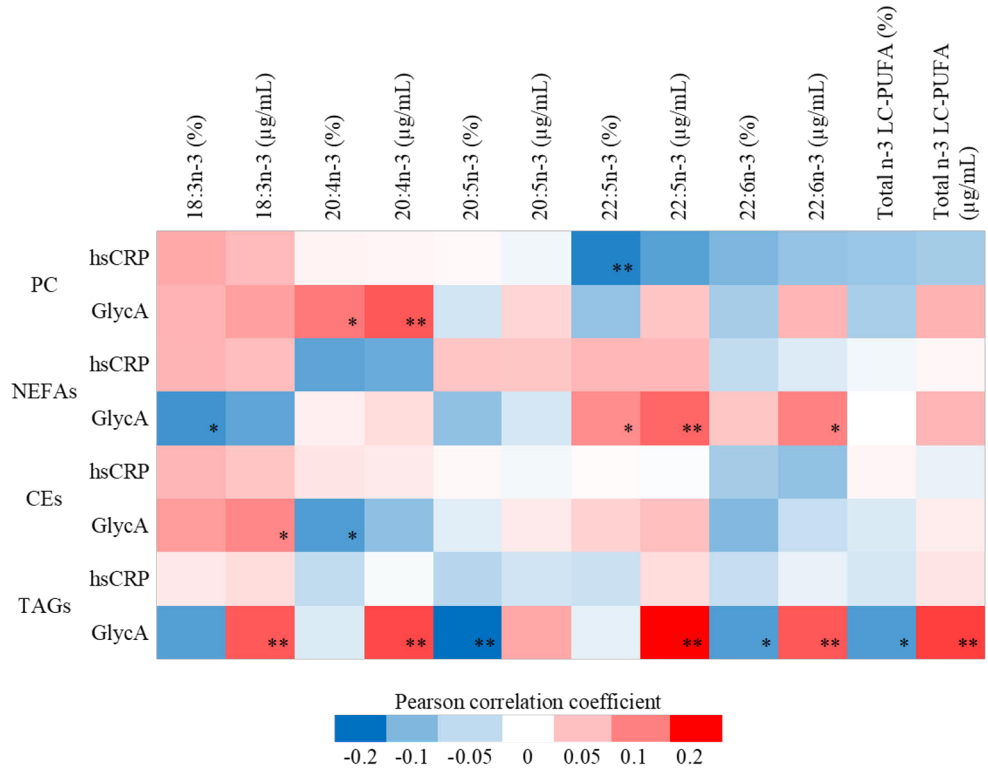


Figure 5. The heatmap describing the Pearson correlation coefficients between late pregnancy serum hs-CRP and GlycA and n-3 LC-PUFAs in PCs, NEFAs, CEs and TAGs. Red colour indicates positive correlations while blue negatives, **p<0.01, *p<0.05. n varies between 307 and 311. The following variables were natural log-transformed: hsCRP, PC 18:3n-3 %, PC 18:3n-3 ug/mL, PC 20:4n-3 %, PC 20:4n-3 ug/mL, PC 20:5n-3 %, PC 20:5n-3 ug/mL, PC 22:5n-3 ug/mL, PC 22:6n-3 ug/mL, NEFA 18:3n-3 %, NEFA 18:3n-3 ug/mL, NEFA 20:4n-3 %, NEFA 20:4n-3, ug/mL, NEFA 20:5n-3 %, NEFA 20:5n-3 ug/mL, NEFA 22:5n-3 %, NEFA 22:5n-3 ug/mL, NEFA 22:6n-3 %, NEFA 22:6n-3 ug/mL, CE 18:3n-3 %, CE 18:3n-3 ug/mL, CE 20:4n-3 %, CE 20:4n-3 ug/mL, CE 20:5n-3 %, CE 20:5n-3 ug/mL, CE 22:5n-3 %, CE fatty acid 22:5n-3 ug/mL, CE 22:6n-3 %, CE 22:6n-3 ug/mL, TAG 18:3n-3 %, TAG 18:3n-3 ug/mL, TAG 20:4n-3 %, TAG 20:4n-3 ug/mL, TAG 20:5n-3 %, TAG 20:5n-3, ug/mL, TAG 22:5n-3 ug/mL, TAG 22:6n-3 %, TAG 22:6n-3 ug/mL, PC n-3 ug/mL, NEFA n-3 total %, CE n-3 total %, TAG n-3 total %, NEFA n-3 total ug/mL. From Original publication IV.

5.4 The relation of low-grade inflammatory and metabolic markers and vaginal microbiota to the onset of gestational diabetes mellitus

5.4.1 The relation of circulationg low-grade inflammatory marker, phIGFBP-1, IGFBP-1, aMMP-8 and vaginal aMMP-8 to the onset of gestational diabetes mellitus (study II)

The concentration of marker of low-grade inflammation, hs-CRP, or aMMP-8 in early pregnancy were not associated with the onset GDM. Interestingly, phIGFBP-1 and IGFBP-1 were related to the onset of GDM as their concentrations in early pregnancy were lower in women who developed GDM in later pregnancy as compared to those women who did not develop GDM (**Table 11**).

Table 11. The concentrations of serum aMMP-8, phIGFBP-1 and IGFBP-1 and vaginal aMMP-8 measured in early pregnancy in those women developed GDM and in those who did developed GDM later in pregnancy. Modified from Original publication II.

	Women who developed GDM		**Women who did not develop GDM**		**P value[a,c]**
	mean ± SD	n	mean ± SD	n	
Serum hs-CRP (mg/L)[b]	1.7 ± 0.6	83	1.6 ± 0.8	276	0.22
Serum phIGFBP-1 (ng/mL)	635 ± 315	82	753 ± 335	271	0.005
Serum IGFBP-1 (ng/mL)[b]	3.8 ± 0.7	82	4.0 ± 0.7	269	0.042
Serum aMMP-8 (ng/mL)[b]	2.9 ± 0.6	82	2.9 ± 0.6	270	0.77
Vaginal aMMP-8 (ng/mL)[b]	3.0 ± 1.5	29	3.4 ± 1.5	69	0.19

[a]Test between women who developed GDM and who did not: ANOVA
[b]Natural log-transformed variables
[c]Adjusted for intervention
P value <0.05 is considered statistically significant.

The results of phIGFBP-1 and IGFBP-1 were confirmed with logistic regression analysis. Higher values of early pregnancy phIGFBP-1 (OR for 100 unit increase in phIGFBP-1 values 0.89, 95%CI 0.82–0.97, p=0.005, adjusted for intervention) and IGFBP-1 (OR for one unit increase in log scale in IGFBP-1 values 0.70, 95%CI 0.50–0.99, p=0.044, adjusted for intervention) were associaciated with decreased risk for GDM. When the analyses were adjusted for prepregnancy BMI and intervention, the result remained essentially the same for phIGFBP-1 (OR for 100 unit increase in phIGFBP-1 values 0.90, 95%CI 0.82–0.98, p=0.019), but IGFBP-1 was not related to the risk of GDM anymore (OR for one unit increase in log scale in IGFBP-1 values 0.75, 95%CI 0.52–1.09, p=0.13).

5.4.2 The relation of vaginal microbiota to the onset of gestational diabetes mellitus (study III)

Vaginal microbiota in early pregnancy was altered in women who developed GDM in later pregnancy. There were differences in bacterial genera and species between women who developed and who did not develop GDM, in particular the relative abundance of *Megasphaera* and *Corynebacterium*, *Ureaplasma*, *M. elsdenii*, *Veillonella montpellierensis* and *Bifidobacterium dentium* were lower in women who developed GDM as compared to those women who did not develop GDM in later pregnancy (**Table 12**).

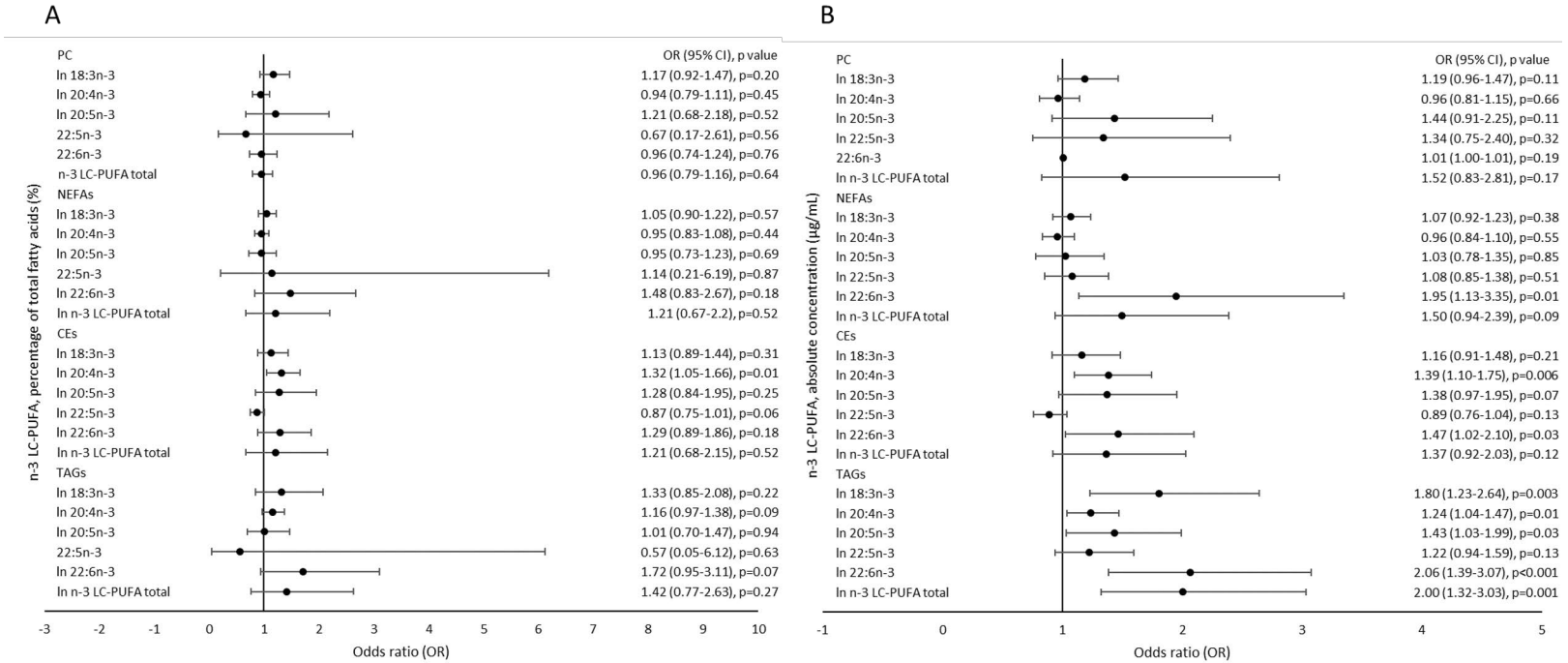
Table 12. The genera and species with FDR < 0.25 in early pregnancy in those women developing GDM in later pregnancy and those not developing GDM. Modified from Original publication III.

	Mean relative abundance (5/95% quantile) (%)			
Taxon	**Women developing GDM**	**Women not developing GDM**	**Effect size (Log2FC)**	**Adj. P value**
Megasphaera	0.54 (<0.01/0.02)	1.23 (<0.01/10.31)	-23.3	<0.001
Corynebacterium	<0.01 (<0.01/0.02)	0.01 (<0.01/0.06)	5.1	0.01
Ureaplasma	0.01 (<0.01/0.04)	0.01 (<0.01/0.07)	5.0	0.01
Gardnerella	7.80 (<0.01/48.83)	7.32 (<0.01/47.26)	3.2	0.22
Megashaera elsdenii	0.54 (0/0.02)	1.23 (0/10.31)	-24.3	<0.001
Veillonella montpellierensis	0.01 (0/0)	0.16 (0/0.38)	-24.0	<0.001
Bifidobacterium dentium	0 (0/0)	0.68 (0/0)	-23.0	<0.001

Adjusted P value <0.05 is considered statistically significant.

5.4.3 The relation of circulating fatty acids to the onset of gestational diabetes mellitus (study IV)

When the relation of serum fatty acids to the risk of developing GDM was investigated (**Figure 6 a–d**), it was shown that the concentration of DHA (22:6n-3) in serum CEs was associated with 47% increased risk of GDM (p=0.03), in serum TAGs, the concentrations of EPA (20:5n-3), DHA (22:6n-3) and total n-3 LC-PUFAs were associated with 43% (p=0.03), 106% (p<0.001), 100% (p=0.001), increased risk for GDM as well as in serum NEFAs the concentration of DHA was associated with 95% increased risk for GDM (p=0.01). Also, some n-6 LC-PUFAs were associated with increased risk for GDM, e.g., the concentration of AA and concentration of total n-6 LC-PUFAs were associated with 70% (p=0.01) and 108% (p=0.008) increased risk for GDM. However, the % of total n-6 LC-PUFAs was associated with 75% decreased risk for GDM (p=0.03).


A
PC
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
22:5n-3
22:6n-3
n-3 LC-PUFA total
NEFAs
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
22:5n-3
ln 22:6n-3
ln n-3 LC-PUFA total
CEs
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
ln 22:5n-3
ln 22:6n-3
ln n-3 LC-PUFA total
TAGs
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
22:5n-3
ln 22:6n-3
ln n-3 LC-PUFA total
OR (95% CI), p value
1.17 (0.92-1.47), p=0.20
0.94 (0.79-1.11), p=0.45
1.21 (0.68-2.18), p=0.52
0.67 (0.17-2.61), p=0.56
0.96 (0.74-1.24), p=0.76
0.96 (0.79-1.16), p=0.64
1.05 (0.90-1.22), p=0.57
0.95 (0.83-1.08), p=0.44
0.95 (0.73-1.23), p=0.69
1.14 (0.21-6.19), p=0.87
1.48 (0.83-2.67), p=0.18
1.21 (0.67-2.2), p=0.52
1.13 (0.89-1.44), p=0.31
1.32 (1.05-1.66), p=0.01
1.28 (0.84-1.95), p=0.25
0.87 (0.75-1.01), p=0.06
1.29 (0.89-1.86), p=0.18
1.21 (0.68-2.15), p=0.52
1.33 (0.85-2.08), p=0.22
1.16 (0.97-1.38), p=0.09
1.01 (0.70-1.47), p=0.94
0.57 (0.05-6.12), p=0.63
1.72 (0.95-3.11), p=0.07
1.42 (0.77-2.63), p=0.27
n-3 LC-PUFA, percentage of total fatty acids (%)
-3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Odds ratio (OR)
B
PC
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
ln 22:5n-3
22:6n-3
ln n-3 LC-PUFA total
NEFAs
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
ln 22:5n-3
ln 22:6n-3
ln n-3 LC-PUFA total
CEs
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
ln 22:5n-3
ln 22:6n-3
ln n-3 LC-PUFA total
TAGs
ln 18:3n-3
ln 20:4n-3
ln 20:5n-3
ln 22:5n-3
ln 22:6n-3
ln n-3 LC-PUFA total
OR (95% CI), p value
1.19 (0.96-1.47), p=0.11
0.96 (0.81-1.15), p=0.66
1.44 (0.91-2.25), p=0.11
1.34 (0.75-2.40), p=0.32
1.01 (1.00-1.01), p=0.19
1.52 (0.83-2.81), p=0.17
1.07 (0.92-1.23), p=0.38
0.96 (0.84-1.10), p=0.55
1.03 (0.78-1.35), p=0.85
1.08 (0.85-1.38), p=0.51
1.95 (1.13-3.35), p=0.01
1.50 (0.94-2.39), p=0.09
1.16 (0.91-1.48), p=0.21
1.39 (1.10-1.75), p=0.006
1.38 (0.97-1.95), p=0.07
0.89 (0.76-1.04), p=0.13
1.47 (1.02-2.10), p=0.03
1.37 (0.92-2.03), p=0.12
1.80 (1.23-2.64), p=0.003
1.24 (1.04-1.47), p=0.01
1.43 (1.03-1.99), p=0.03
1.22 (0.94-1.59), p=0.13
2.06 (1.39-3.07), p<0.001
2.00 (1.32-3.03), p=0.001
n-3 LC-PUFA, absolute concentration (µg/mL)
-1 0 1 2 3 4 5
Odds ratio (OR)

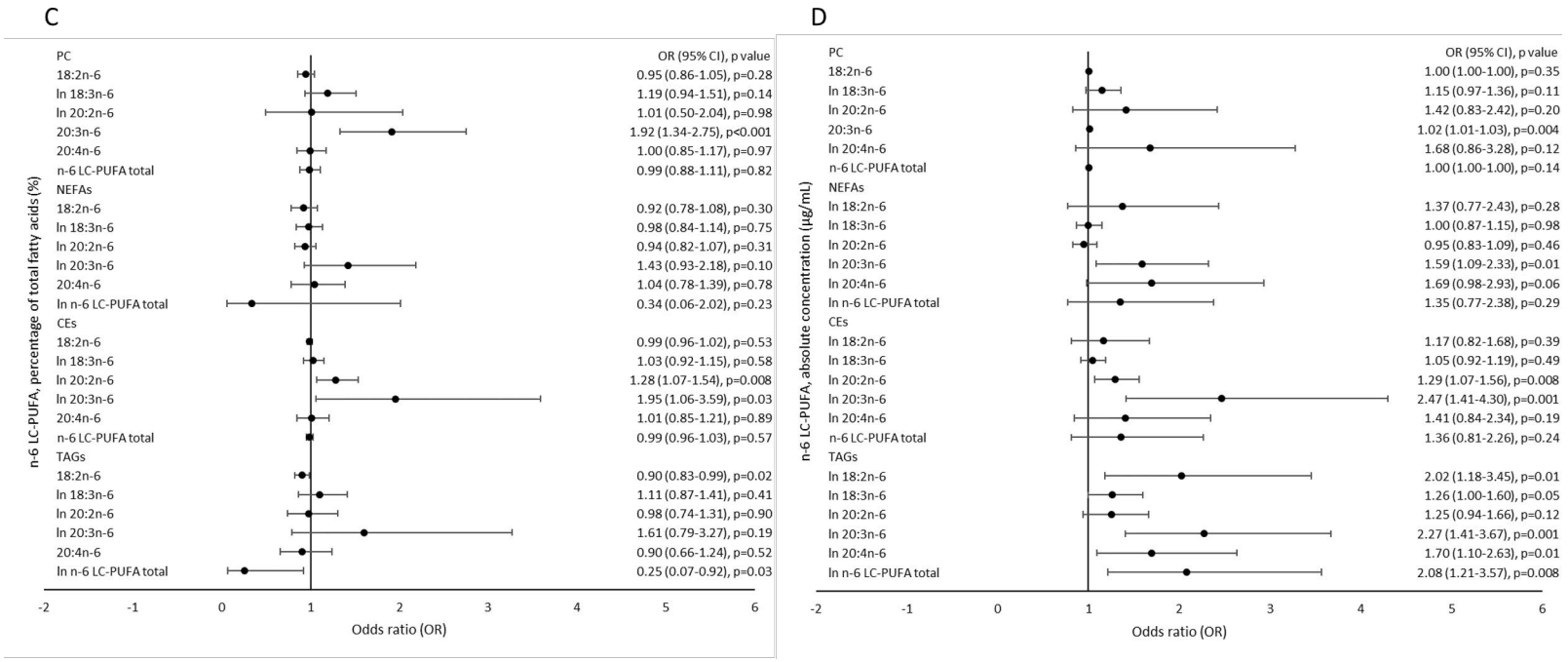


Figure 6 **a–d.** The association of n-3 and n-6 LC-PUFAs in early pregnancy evaluated as proportion of total fatty acids (%) and absolute concentration (µg/mL) in serum PC, NEFAs, CEs and TAGs and the risk of developing GDM (GDM positive, n=81; GDM negative PC n=274, NEFAs, CEs and TAGs n=275). Logistic regression, adjusted for intervention group, natural log-transformed variables are labelled with "ln". From Original publication IV. P value <0.05 is considered statistically significant.

5.5 The impact of fish oil and/or probiotics on low-grade inflammatory and metabolic markers and vaginal microbiota

5.5.1 Circulating low-grade inflammatory marker, phIGFBP-1, IGFBP-1, aMMP-8 and vaginal aMMP-8 (study II)

The intervention had no effect on the concentrations of serum hs-CRP (**Table 13**), GlycA (**Table 14**) or serum phIGFBP-1, IGFBP-1, aMMP-8 or vaginal aMMP-8 (**Table 15**).

Table 13. The concentrations of early and late pregnancy as well as change from early to late pregnancy of serum hs-CRP in the intervention groups. Data are expressed as mean ± SD and 95% Confidence Intervals for mean change (95%CI). Modified from Original publication II.

Hs-CRP (mg/l)[b]	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	n	P value[a]
Early pregnancy	1.68 ± 0.76	1.63 ± 0.79	1.58 ± 0.74	1.57 ± 0.80	108/109/108/109	
Late pregnancy	1.39 ± 0.85	1.32 ± 0.80	1.29 ± 0.78	1.26 ± 0.74	93/94/90/92	0.72
Mean change	-0.29	-0.33	-0.32	-0.30	92/94/90/91	0.97
95%CI	-0.43–(-0.15)	-0.46–(-0.20)	-0.45–(-0.18)	-0.44–(-0.16)		

[a]Test between the intervention groups: One-way ANOVA

[b]Natural log-transformed variables

P value <0.05 is considered statistically significant.

Table 14. The concentrations of early and late pregnancy as well as change from early to late pregnancy of serum GlycA in the intervention groups. Data are expressed as mean ± SD or median (interquartile range).

GlycA (mmol/L)	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	n	P value
Early pregnancy	1.23 ± 0.12	1.21 ± 0.10	1.21 ± 0.11	1.20 ± 0.10	108/109/107/109	
Late pregnancy	1.42 ± 0.12	1.42 ± 0.15	1.41 ± 0.14	1.41 ± 0.13	90/92/90/90	0.88[a]
Change	0.18 (0.13–0.23)	0.20 (0.14–0.29)	0.19 (0.13–0.25)	0.19 (0.12–0.28)	89/92/90/89	0.59[b]

[a]Test between intervention groups: One-way ANOVA

[b]Test between intervention groups: Kruskal-Wallis

P value <0.05 is considered statistically significant.

Table 15. The concentrations of early and late pregnancy as well as the change from early to late pregnancy of serum aMMP-8, phIGFBP-1, IGFBP-1 and vaginal aMMP-8 in the intervention groups. Data are expressed as mean ± SD and 95% Confidence Intervals for mean change (95%CI). Modified from Original publication II.

	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	n	P value[a]
Serum aMMP-8 (ng/mL)[b]						
Early pregnancy	3.04 ± 0.66	2.90 ± 0.64	2.89 ± 0.60	2.87 ± 0.58	107/106/106/108	
Late pregnancy	2.87 ± 0.73	2.79 ± 0.64	2.75 ± 0.71	2.83 ± 0.70	89/89/90/87	0.68
Mean change	-0.15	-0.11	-0.13	-0.06	87/86/89/86	0.89
95%CI	-0.30–0.10	-0.28–0.06	-0.27–0.02	-0.22–0.09		
Serum phIGFBP-1 (ng/mL)						
Early pregnancy	708.53 ± 343.32	701.59 ± 340.79	702.04 ± 309.79	696.04 ± 355.21	107/107/106/108	
Late pregnancy	1160.02 ± 509.38	1236.36 ± 489.00	1179.84 ± 422.35	1153.10 ± 380.46	89/88/90/87	0.61
Mean change	452.97	490.88	466.85	467.59	87/86/89/86	0.93
95%CI	377.67–528.26	404.56–577.21	379.56–554.15	404.64–530.54		
Serum IGFBP-1 (ng/mL)[b]						
Early pregnancy	3.85 ± 0.74	3.84 ± 0.78	3.90 ± 0.73	3.90 ± 0.70	107/106/105/108	
Late pregnancy	4.16 ± 0.63	4.26 ± 0.56	4.23 ± 0.56	4.21 ± 0.60	89/88/90/86	0.72
Mean change	0.28	0.35	0.33	0.34	87/86/89/85	0.83
95%CI	0.17–0.39	0.24–0.46	0.21–0.44	0.22–0.46		
Vaginal aMMP-8 (ng/mL)[b]						
Early pregnancy	3.45 ± 1.62	3.22 ± 1.46	3.06 ± 1.95	3.46 ± 1.59	28/29/29/28	
Late pregnancy	3.44 ± 1.34	3.96 ± 1.48	3.33 ± 1.67	3.64 ± 1.21	35/35/27/27	0.31
Mean change	0.04	0.55	0.26	-0.04	25/24/19/17	0.48
95%CI	-0.55–0.63	0.16–0.93	-0.41–0.98	-0.88–0.79		

[a]Test between the intervention groups: One-way ANOVA

[b]Natural log-transformed variables

P value <0.05 is considered statistically significant.

5.5.2 Vaginal microbiota (study III)

The intervention influenced the vaginal microbiota in genus and species level. Specifically, the following changes were detected from early to late pregnancy: the relative abundance of *Corynebacterium* increased in fish oil+placebo group, the relative abundance of *Sneathia*, *Prevotella, Fenollaria, Peptinophilus*, *Dialister* and *Campylobacter* decreased in the probiotics+placebo group, the relative abundance of *L. acidophilus* decreased in the fish oil+probiotics group and the relative abundance of *Parvimonas* and *P. micra* increased in the placebo+placebo group (all adj. $P<0.05$).

Additionally, the following differences were seen in late pregnancy when the active groups were compared to the placebo+placebo group: the relative abundance of *G. vaginalis* and *U. urealyticum* were lower in the fish oil+placebo group, the relative abundance of *Ureaplasma*, *U. urealyticum* and *P. disiens* were lower in the probiotics+placebo group and the abundance of *Corynebacterium*, *Anaerococcus* and *D. invisus* and *P. timonensis* were lower in fish oil+probiotics group when compared to the placebo+placebo group (all adj. $P<0.05$).

It is of note that the difference in relative abundances of *Corynebacterium* and *Anaerococcus* between the fish oil+probiotics and the placebo+placebo group and the difference in the abundance *G. vaginalis* between fish oil+placebo and the placebo+placebo group comparison was evident already at baseline ($p<0.001$).

α-Diversity increased in the fish oil+placebo group ($p=0.03$). No differences were seen in α- or β-diversity at late pregnancy between the active groups and placebo+placebo group. See details in the Publication III.

5.5.3 Circulating fatty acids (study IV)

The impact of the intervention on the serum n-3 and n-6 LC-PUFAs in PC, NEFAs, CEs and TAGs are presented in the Tables 1-4 in the publication IV. Here, the results on fatty acids on PC fraction are described. Regarding n-3 LC-PUFAs, the % and concentration of DHA, EPA and total n-3 LC-PUFAs differed between fish oil and fish oil+probiotics groups compared to probiotics and placebo groups, all of those being statistically significantly higher in the fish oil and fish oil+probiotics groups compared to probiotics and placebo groups ($p<0.05$ in all comparisons).

Regarding the n-6 LC-PUFAs, there were lower % or concentrations of total n-6 LC-PUFAs, LA, eicosadienoic acid (EDA) and DGLA in groups receiving fish oil than groups receiving probiotics or placebo. Specifically, the % of total n-6 LC-PUFAs was statistically significantly lower in the fish oil and fish oil+probiotics groups compared to the probiotics and placebo groups ($p<0.001$ for all comparisons). The % of LA was lower in the fish oil+probiotics group compared to the probiotics group ($p=0.02$) and the % of DGLA was lower in fish and fish oil+probiotics group

as compared to probiotics group ($p<0.001$) and placebo groups ($p=0.002$) and also the concentration of DGLA was lower in fish oil group as compared to the probiotics group ($p=0.01$). There were also differences between the groups in % and the concentration of EDA between fish oil and probiotics ($p=0.001$ & $p=0.005$) or placebo group ($p=0.01$) (see details on EDA in study IV).

The n-6/n-3 PUFA ratio were lower in the fish oil and fish oil+probiotics group compared to probiotics and placebo group ($p<0.001$).

6 Discussion

To summarise, it was shown that the obesity status was related to increase in circulating low-grade inflammatory markers and lipid and amino acid metabolites as well as changes in gut microbiota composition. In particular, hs-CRP, GlycA, lipids (mainly concentrations of several VLDL particles and lipid measures in several VLDL particles) and Prevotellaceae tended to be higher in obesity. The circulating low-grade inflammatory markers were associated with circulating metabolites, phIGFBP-1, IGFBP-1, aMMP-8 and n-3 LC-PUFAs. Importantly, vaginal aMMP-8 was directly associated with α-diversity while indirectly with two *Lactobacillus* species. The circulating levels of phIGFBP-1 and IGFBP-1 were lower and circulating levels of n-3 LC-PUFAs were higher in women who developed GDM in later pregnancy than compared to women who did not. The relative abundance of some vaginal microbial genera and species in early pregnancy were affected by the GDM in late pregnancy, namely the relative abundance of *Megasphaera* and *Corynebacterium*, *Ureaplasma*, *M. elsdenii*, *V. montpellierensis* and *B. dentium* being lower in women developing GDM in women who developed GDM in later pregnancy as compared to those who did not. Lastly, the intervention with fish oil and/or probiotics had an effect on serum fatty acids as fish oil and fish oil+probiotics increased the levels of several n-3 LC-PUFAs and moreover fish oil, probiotics and fish oil+probiotics decreased the abundance of potential pathobionts, namely fish oil *Ureaplasma urealyticum*, probiotics *Ureaplasma*, *U. urealyticum* and *P. disiens* and fish oil+probiotics *D. invisus* and *P. timonensis*. However, the intervention did not influence the circulating levels of hs-CRP, GlycA, phIGFBP-1, IGFBP, aMMP-8 or vaginal aMMP-8. The summary of the findings is presented in **Figure 7**.

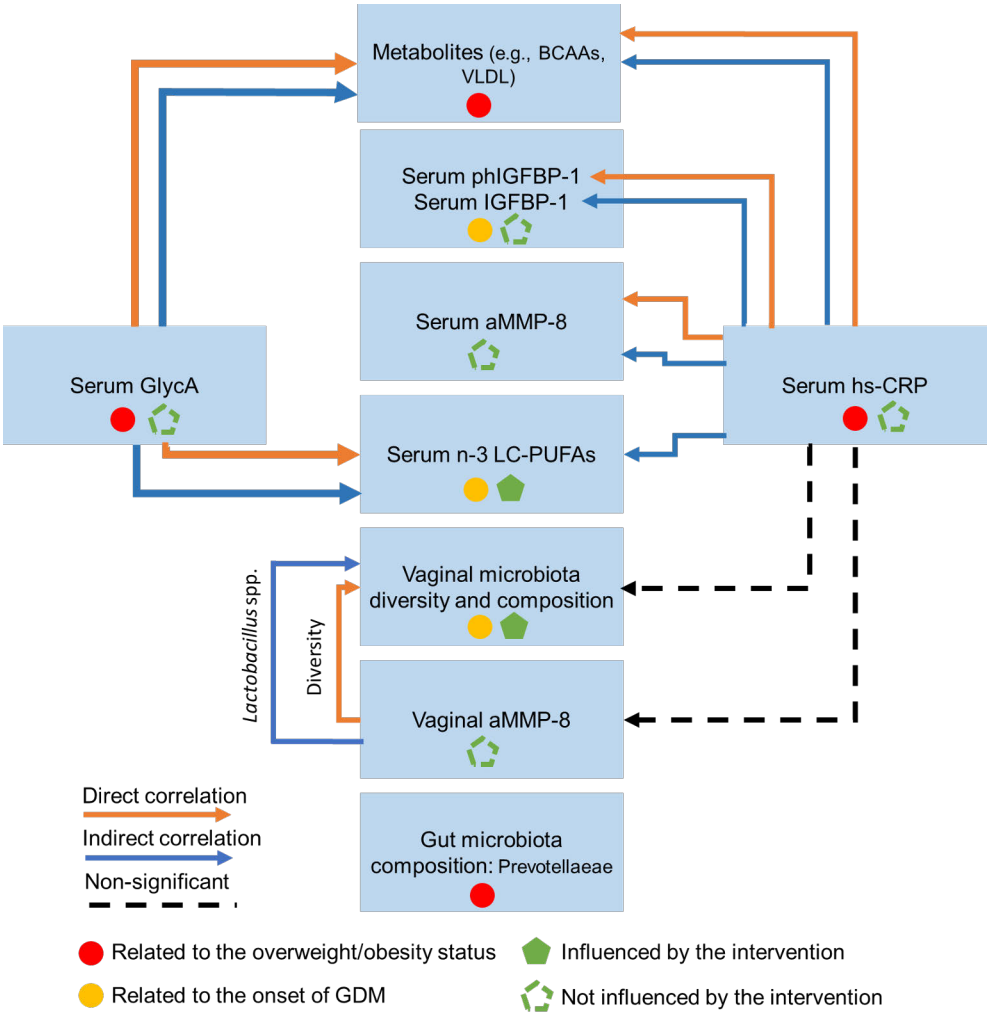


Figure 7. Summary of the results on the interrelations of low-grade inflammatory and metabolic markers as well as gut and vaginal microbiota and their role in obesity and GDM and the effect of the fish oil and/or probiotics intervention in this thesis. aMMP-8, active matrix metalloproteinase 8; BCAAs, branched-chain amino acids; GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; hs-CRP, high sensitivity C-reactive protein; n-3 LC-PUFAs, n-3 long-chain polyunsaturated fatty acids; phIGFBP-1, phosphorylated insulin-like growth factor binding-protein 1, VLDL, very low density lipoprotein.

6.1 The obesity status: the relation to health effects and mechanisms

The obesity associated low-grade inflammation as found in this thesis may be linked to changes in lipid and amino acid metabolism (increased VLDL lipoproteins and BCAAs) which may contribute to adverse pregnancy outcomes as GDM (Mokkala

et al, 2020b). Interestingly, increased VLDL lipoproteins and BCAAs have been previously linked to metabolic aberrations such as type 2 diabetes (Wang *et al*, 2011). The previous studies in pregnant women are few as shown in the literature review of this thesis but the literature indicates that as the adiposity increases there are changes in low-grade inflammation (Friis *et al*, 2013), metabolism (Kivelä *et al*, 2021) and gut microbiota (Zacarías *et al*, 2018), however, the previous literature focuses mainly on comparing normal weight to overweight or obese. As the adiposity increases in pregnancy, the risk for a congenital malformation and premature death, miscarriages, GDM, macrosomia and shoulder dystocia, both preterm and post-term birth, caesarean section, infections after caesarean section, thromboembolism and even maternal mortality increase (The Finnish Medical Society Duodecim, 2021). Indeed, development of new risk markers for identification of those women who are at increased risk for adverse health effects would benefit the patient themselves but also the public health, further decreasing the burden and costs in health care. Based on this study, circulating low-grade inflammatory and metabolic markers, i.e. metabolites, and gut microbiota, could be potential markers to be used in identification of those women who are at increased risk for adverse pregnancy outcomes. Yet, more studies on their potential role of markers in overweight/obesity status in pregnancy and subsequent adverse pregnancy outcomes are needed.

The mechanisms that contribute to the increasing inflammatory status with increasing adiposity may lie in the altered immunoregulation of enlarged adipose tissue. In obesity, increased CD8+ and CD4+ T cells can express INF-γ which promote proinflammatory macrophage accumulation and stimulate pro-inflammatory cytokine expression in adipose tissue (Choe *et al*, 2016) whereas regulatory T cells and invariant natural killer T cells which secrete anti-inflammatory cytokines are decreased (Zatterale *et al*, 2020). Thus, the infiltration of proinflammatory macrophages are increased which leads to increased expression of proinflammatory cytokines e.g., IL-6 and TNF-α (Zatterale *et al*, 2020). Also, the increased circulating LPS, which passage may be increased due to gut microbiota dysbiosis and increased intestinal permeability, can activate proinflammatory macrophages (Wu *et al*, 2016), linking gut microbiota to immune regulation of adipose tissue in obesity.

In this thesis the pregnant women with obesity exhibited tendency to have higher the relative abundance of bacterial family Prevotellaceae as compared to women with overweight. Family Prevotellaceae consists of gram-negative bacteria, e.g., Prevotella, and belongs to the phylum Bacteroidota (formerly Bacteroidetes) (Rosenberg, 2014). Previously, Prevotellaceae has been detected in individuals with obesity (Duan *et al*, 2021) but also metabolically healthy individuals with obesity (Olivares *et al*, 2021). It might be that the increased LPS derived from Prevotellaceae have a role in obesity induced low-grade inflammation. However, the levels of LPS

did not differ between the women with overweight and obesity in this thesis, suggesting that Prevotellaceae alone is not able to induce detectable levels of LPS.

Concentrations of triglycerides, free cholesterol, cholesterol esters, phospholipids and total lipids in several sizes of VLDL particles, total cholesterol in VLDL and different sizes of VLDL particles were higher in pregnant women with obesity than in overweight. As reviewed by Franssen et al. (2011), obesity can cause dyslipidemia, which includes increased secretion and clearance of VLDL which may cause increased levels of VLDL and simultaneously decreased levels of HDL. The imbalance between VLDL and HDL particles may be due to the enzyme cholesteryl ester transfer protein which mass and activity may increase in obesity, and thus increase the transfer of cholesterol esters from HDL to VLDL (Arai *et al*, 1994; Franssen *et al*, 2011). Also, in obesity the free fatty acids levels increase due to increased release and decreased clearance (Boden, 2008), which contributes to increased triglyceride content of VLDL (Nielsen & Karpe, 2012).

Regarding the increased levels of BCAAs in women with obesity, it may be due to the altered catabolism of BCAAs, e.g., the previous studies suggest that the enzymes that regulate BCAAs catabolism loss their function, or it may be that the increased insulin levels stimulate protein breakdown from muscle, and thus the levels of BCAAs increase in circulation (Vanweert *et al*, 2022).

6.2 Interaction between low-grade inflammation, metabolism and microbiota

There was an interaction between circulating low-grade inflammatory and metabolic markers, in particular the concentrations of hs-CRP and GlycA were associated with several lipid metabolites and amino acids. Previous studies in pregnant women are lacking but the studies done in non-pregnant women show that higher circulating hs-CRP are linked with increased circulating BCAAs (Hamaya *et al*, 2021). In that same study reduced HDL-cholesterol and increased triglycerides were related to higher levels of BCAAs. It seems that obesity related low-grade inflammation is related to alterations in lipid and amino acid metabolism. It is of note that GlycA correlated with higher amount of metabolites than hs-CRP suggesting that GlycA may be better marker than hs-CRP. This was seen also in correlation analyses between hs-CRP, GlycA and n-3 LC-PUFAs. However, the associations between hs-CRP and metabolites were more consistent than the associations between GlycA and fatty acids, e.g., the correlations between hs-CRP and fatty acids are only negative while correlations between GlycA and fatty acids are both positive and negative which may be due to the multiple acute phase proteins of GlycA (Otvos *et al*, 2015). It is of note that GlycA correlated higher number of n-3 LC-PUFAs than did hs-CRP.

The level of hs-CRP was associated with phIGFBP-1 and IGFBP-1, negatively with phIGFBP-1 and positively with IGFBP-1. A negative association of IGFBP-1 and hs-CRP have been detected previously in non-pregnant subjects (Heald *et al*, 2003; Rahman *et al*, 2021). The associations between hs-CRP and aMMP-8 were both positive and negative. In one previous study done in women with history of GDM, hs-CRP was directly associated with circulating MMP-8 (Vilmi-Kerälä *et al*, 2017).

Interestingly, vaginal aMMP-8 was directly associated with vaginal microbial α-diversity while indirectly with two *Lactobacillus* species. When vaginal microbiota goes towards dysbiosis e.g., in bacterial vaginosis (Danielsson *et al*, 2011) the diversity increases and *Lactobacillus* species decrease which indicates that aMMP-8 may be linked with altered vaginal microbiota.

6.3 Gestational diabetes mellitus: mechanisms

There are current consensus about the pathophysiology of GDM as presented in the literature of this thesis. However, the the exact molecular mechanisms of the pathophysiology of GDM are not known. The mechanism may be related to the outcomes that were studied in this thesis. In contrast to the hypothesis, hs-CRP in early pregnancy did not differ between women who developed GDM and who did not in this thesis, but previous studies suggest that low-grade inflammation has role in GDM pathophysiology (Amirian *et al*, 2020). Mechanistically, proinflammatory cytokines, e.g., TNF-α, and IL-6, can activate protein kinases (protein kinase C via IκB kinase) which can inhibit insulin receptor substrate 1 (IRS-1) in β-cells. By inhibiting IRS-1, insulin and its binding to the insulin receptor do not result in activation of further downstream insulin signalling cascades, and thus glucose uptake is not activated by glucose transporter 4 (Plows *et al*, 2018), which can result in insulin resistance and further hyperglycaemia.

GDM may induce changes in metabolism, the known changes include increased triglyceride and decreased HDL (Lain & Catalano, 2007). In this thesis that was not investigated but previously it has been shown that metabolomics profile in early pregnancy is associated to the onset GDM (Mokkala *et al*, 2020b).

When comparing the groups, concentrations of phIGFBP-1 and IGFBP-1 were lower in women who developed GDM later pregnancy as compared to those who did not. This was confirmed with logistic regression analyses in which both markers were associated with decreased risk of GDM. When the logistic regression analysis was adjusted for prepregnancy BMI, lower IGFBP-1 was not related to the risk of GDM anymore. This means that the relation of IGFBP-1 on GDM is dependent on BMI, and thus phIGFBP-1 but not probably IGFBP-1 could be a marker of GDM regardless of the BMI. The production of IGFBP-1 is regulated by insulin; insulin

suppresses the gene expression of IGFBP-1 and also regulates the acute endogenous levels of IGFBP-1 (Rajpathak *et al*, 2009), as detected in insulin resistance as insulin levels increases. Moreover, IGFBP-1 can block the binding of IGF-1 to IGF receptor and further inhibit the action of IGF-1 (Aguirre *et al*, 2016). Thus, in diabetic state insulin levels rises, which decreases the the levels of IGFBP-1 which increases the IGF-1 binding and further the tissue specific effects. E.g., IGF-1 have been linked to excess foetal growth (Liu *et al*, 1996), possibly linking the reduced IGFBP-1 levels and GDM to macrosomia. As stated in the literature review of this thesis, the phosphorylation of IGFBP-1 increases affinity of IGFBP-1 to IGF-1. This relates to findings of one study in which the highly phosphorylated isoform of IGFBP-1 in cord blood was decreased in women with GDM as compared to women without GDM, suggesting that GDM affects the levels of the highly phosphorylated phosphoisoform which may lead to increased bioavailability of IGF-1 and increase the foetal growth (Loukovaara *et al*, 2005).

As described before in this thesis, MMPs may cleavage insulin receptor (Delano & Schmid-Schönbein, 2008) and Lauhio et al. (2016) suggest that MMP-8 has a role in insulin signalling and development of insulin resistance. They found that serum levels of MMP-8 were higher in subjects with obesity as compared to normal weight subjects in clinical study setting. Furthermore, the insulin receptor was degraded by MMP-8 *in vitro*. Therefore, as the insulin receptor is degraded the insulin signalling fails, further inducing insulin resistance.

It is not known whether altered vaginal microbiota are the cause or consequence of GDM. However, there might be a link between GDM, bacterial vaginosis and further preterm birth, e.g., one study found association between vaginal dysbiosis, GDM and e.g., increased rate of bacterial vaginosis, premature rupture of membranes and preterm birth (Rafat *et al*, 2022). The link between vaginal microbiota and preterm birth might lie in increased microbe-induced inflammation possibly due to e.g., bacterial vaginosis or urinary tract infection, via premature rupture of membranes (Fettweis *et al*, 2019). Thus, it would be important to find novel biomarkers e.g., possibly MMP-8 in identifying those cases in early phases of GDM development to intervene the drastic and reversible outcomes, such as preterm birth. The alterations in vaginal microbiota leads to reduced abundance of beneficial *Lactobacillus* species. The importance of *Lactobacillus* dominant vaginal microbiota lies in following facts (Amabebe & Anumba, 2018): Oestrogen stimulates glycogen formation in vaginal epithelium which is further metabolised α-amylase to maltose, maltotriose and α-dextrines by human which are further metabolised to lactic acid by *Lactobacillus* species. Lactic acid lowers the pH and protects the vaginal epithelium from pathogens that cannot live in acidic environment. Other factors that have a role in the first-line defence in vagina, are bacteriocins produced by

lactobacilli and mucin that serves an antimicrobial, physical and immunological defence against invading pathogens.

N-3 LC-PUFAs can resolve inflammation caused by pro-inflammatory cytokines and AA derived eicosanoids, and thus the protective role of serum n-3 LC-PUFAs against GDM could be their inflammation resolving properties. N-3 LC-PUFAs can bound to peroxisome proliferator-activated receptors which inhibits the production of pro-inflammatory cytokines via inhibiting the translocation of transcription factor to the nucleus in adipose tissue or even pancreatic tissue (Wang & Chan, 2015). Moreover, n-3 LC-PUFAs may modulate the function and structure of lipid rafts in cell membranes of β-cells resulting changes in β-cells' function (Wang & Chan, 2015). Another protective mechanism could that n-3 LC-PUFAs may stimulate the adipokine secretion from adipose tissues which in turn promotes insulin secretion from β-cells (Wang & Chan, 2015).

It is of note that studies have been able to find different physiological subtypes of GDM which exhibit distinct risks for adverse perinatal outcomes (Powe *et al*, 2020). This aspect would be important to take into account in future studies.

6.4 N-3 LC-PUFAs and probiotics

The intervention with n-3 LC-PUFAs and/or probiotics did not influence circulating low-grade inflammatory marker, hs-CRP, or phIGFBP-1, IGFBP-1, aMMP-8 or vaginal aMMP-8, however, it had an effect on vaginal microbiota and circulating fatty acids.

N-3 LC-PUFAs and probiotics have inflammation resolving capacity (Mokkala *et al*, 2017). It was hypothesised that the consumption of fish oil rich in n-3 LC-PUFAs (Mokkala *et al*, 2017) would have an impact on low-grade inflammation, however, it was not detected. The reason that the intervention did not have an impact on the low-grade inflammation, remains to be unsolved. It might be that the overweight and obesity of the women is so high burden to women that the intervention is no able to impact on the circulating levels of hs-CRP. It might be also that the GDM status influences the findings as in this same trial it was previously shown that the response to the fish oil and/or probiotics intervention was related to the GDM status; the women with GDM were resilient to the intervention effects in terms of metabolites (Mokkala *et al*, 2021b). It remains to be solved if the response to the intervention in terms of low-grade inflammation is related to GDM status. It is of note that in the same trial, the incidence of GDM, was not influenced by the intervention (Pellonperä *et al*, 2019). These two results are in line since one would expect to find an effect on both, as insulin resistance may be mediated through low-grade inflammation. One could also speculate that as the effects of probiotics are strain-specific, that in this trial the probiotic strains were not optimal in modulation

of inflammation, or the timing or duration was not right. However, the probiotic strains were chosen based on scientific basis. *L. rhamnosus* HN001 is a well-characterised probiotic strain (Dekker *et al*, 2007) and *B. lactis 420* has shown to decrease tissue inflammation, insulin resistance and hyperglycaemia in experimental animals (Amar *et al*, 2011) and inflammation in humans (Roessler *et al*, 2008; Klein *et al*, 2008) as discussed in Publication II. In this trial the intervention was initiated in early pregnancy (mean 13.8 ± 2.1 gestational weeks) and lasted to late pregnancy (mean of 35.2 ± 1.0 gestational weeks) i.e., the duration of the intervention being approximately 22 weeks, while in previous studies done in pregnant women which have found statistically significant decrease in hs-CRP after probiotic intervention the duration was eight weeks (Jafarnejad *et al*, 2016; Hajifaraji *et al*, 2018) indicating that the duration of the intervention in this trial was not optimal. However, in those two studies, the timing of the intervention and the population were different as those were initiated in mid-pregnancy at the time of GDM diagnosis, and further done in women with GDM. Additionally, the studies were done in Iranian pregnant women while those of this thesis were European.

The previous research on the effects of fish oil and/or probiotics on the levels of phIGFBP-1, IGBFP-1 or aMMP-8 is very scarce and conducted mainly in non-pregnant women. More studies are needed to fill in the gap in knowledge on this topic.

In contrast, the fish oil and fish oil+probiotics were able to induce changes in the serum fatty acids and vaginal microbiota. Indeed, by dietary means i.e., increase in n-3 LC-PUFAs intake, one can beneficially affect the circulating fatty acid levels which is supported by other studies (see **Table 2**). Novel is the investigation of the effect of probiotics on serum fatty acids, which did not result in statistically significant results. This might be due to the probiotic-strain or duration as a study investigating the effects of 4-week standardised diet plus the probiotic *L. plantarum* 299v on cardiovascular health resulted in decrease in percentage of MUFAs and an increase in PUFAs in plasma phospholipids in patients with rheumatoid arthritis (Hulander *et al*, 2021). In contrast, one other study found no effects on serum fatty acids in PC, CEs, TGs after 3-week *L. rhanmosus* GG probiotic intervention in healthy non-pregnant subjects (Lahti *et al*, 2013), suggesting that the effects of probiotics might be related to the study population. The previous studies investigating the effects of probiotics on circulating fatty acids in pregnant women are lacking, and thus consistent conclusions cannot be made.

As probiotics target microbiota, the results that probiotics modulate vaginal microbiota are not surprising, however, the finding is important since the probiotics were able to reduce the relative abundance of potential low-abundant pathobionts. Pathobionts have symbiotic relationship with its host, however, in case of perturbations in the environment potential pathobionts can act as a pathogens and

cause harm to the host. Thus, when there are reductions in beneficial lactic acid producing lactobacilli e.g., in disease state such as GDM, the pathobionts might take over, in worst case scenario, and lead to adverse pregnancy outcomes. Probiotics and/or fish oil could be beneficial in preventing adverse pregnancy outcomes by inhibiting the growth of potential harmful bacteria. The mechanisms how oral probiotics may act in vagina may include modulation of vaginal immunity (Vitali *et al*, 2012) or production of possible metabolites by probiotics or by direct modulation of the microbial composition. Similarly, the fish oil and the combination of fish oil and probiotics reduced the potential pathobionts, which can be considered novel finding. The mechanisms behind the ability of fish oil to modulate vaginal microbiota are not known, it might be related to oestrogen metabolising action of the probiotics (Baker *et al*, 2017) or the n-3 LC-PUFAs incorporation of the probiotic strain cell wall (Kankaanpää *et al*, 2004) and its effect on the function of the probiotic.

6.5 Strengths and limitations

The strength of this thesis is well-characterised study population with relatively high number of study subjects. Another strength is the study design of randomised controlled trial. Novelty of this study lies in the intervention with combination of fish oil and probiotics of which effect on circulating low-grade inflammatory markers, phIGFBP-1, IGFBP-1, aMMP-8, fatty acids or vaginal aMMP-8 or vaginal microbiota in pregnant women that has not been investigated before. However, although predefined, the power calculations of the trial was originally calculated on the bases of incidence of GDM and fasting glucose (Pellonperä *et al*, 2019) and the child allergic diseases but not on the basis of the outcomes of this thesis. The post-hoc power calculations were conducted to estimate retrospectivly the achieved power of the study I regarding outcomes e.g., GlycA and Prevotellaceae and study II on aMMP-8. However, the post-hoc power calculations are not recommended by the statistical experts (Heckman *et al*, 2022) but these calculations may guide in designing the further studies and to evaluate if used sample size is efficient to detect the difference repeatedly. It is of note that the statistical and clinical significance are two different aspects (Ranganathan *et al*, 2015). For example, in study I Prevotellaceae was statistically significantly higher in women with obesity compared to overweight, but the achieved power was low. This was probably due to the high individual variability. It may be assumed that BMI affects gut microbial composition but on the other hand it is likely that other factors, such as dietary intake of fibre (Desai *et al*, 2016), can modulate gut microbiota.

Advantage is that the reported compliance of the intervention was good as of the women 88.4% reported good compliance and 91.8 ± 15.9% of the capsules had been

consumed as calculated from the returned fish oil capsules (Pellonperä *et al*, 2019). This was confirmed with principal component analysis which showed a clear separation of the intervention groups according to the lipids reflecting the intake of fish oil (Mokkala *et al*, 2021b). Moreover, the intervention with fish oil and/or probiotics was well-tolerated with no major adverse effects (Pellonperä *et al*, 2019). Advantages related to the well-characterised study population include the information on usage of antibiotics which may affect vaginal microbiota, and thus the antibiotic users within eight weeks before the sampling were excluded in study III. However, we did not exclude the antibiotic users in the study I in which the gut microbiota was assessed, yet, there were no antibiotic users in the time of the sampling. However, a recent antibiotic usage before the sampling may influence the gut microbiota (Elvers *et al*, 2020). Another aspect that might influence the findings of the vaginal microbiota is the contamination of the samples during the sampling process. The well-characterised data allowed also excluding subjects with current acute infections that could confound the findings from analysis of low-grade inflammatory markers in the study IV. We did not assess the association of GlycA with phIGFBP-1, IGFBP-1, aMMP-8 or vaginal aMMP-8 or vaginal microbiota, which needs to be done in future studies.

The utilisation of metabolomics platform which detects over 200 metabolites increases the knowledge of metabolism in pregnancy and its relation to the overweight/obesity status. However, targeted NMR based method was utilised which compromises known metabolites in the body. Non-targeted metabolomics would offer screening of novel metabolites related to the thesis outcomes. The development of "omics" techniques allows deeper understanding and also the field of gut microbiota research is developing fast. In the trial 16S rRNA sequencing was chosen for gut and vaginal microbiota composition analysis as the purpose was to obtain a comprehensive view of the gut and vaginal microbial composition. Since methods are developing, the metagenomics approach would provide deeper and finer understanding of the taxonomical features and even functional capacity gut microbiota of the overweight/obesity status.

7 Conclusions

The main findings of this thesis are:

1. The circulating low-grade inflammatory markers, metabolites and gut microbiota differ in pregnant women with overweight and obesity; the low-grade inflammatory markers, several lipid measures in VLDL particles and BCAAs and Prevotellaceae being higher in pregnant women with obesity.
2. The interaction between the circulating low-grade inflammatory markers, metabolites, phIGFBP-1, IGFBP-1, aMMP-8 and fatty acids as well as vaginal aMMP-8 and vaginal microbiota was evident, in particular the higher level of hs-CRP was associated with the lower level of phIGFBP-1 and n-3 LC-PUFAs and the higher level of IGFBP-1. However, hs-CRP was not associated with vaginal microbial diversity, genera or species. Interestingly, the higher vaginal aMMP-8 was associated with the higher α-diversity and lower *L. fornicalis* and *L. crispatus*. The higher level of GlycA was associated with the higher and lower levels of n-3 LC-PUFAs. Both hs-CRP and GlycA were associated with several lipids and BCAAs.
3. The lower circulating levels of phGFBP-1 and IGFBP-1 and the higher levels of n-3 LC-PUFAs as well as the lower abundance of few vaginal microbial genera and species were related to the onset of GDM.
4. The intervention with fish oil and/or probiotics did not have an effect on the circulating low-grade inflammatory markers or phIGFBP-1, IGFBP-1, aMMP-8 or vaginal aMMP-8 but it influenced the vaginal microbiota by decreasing the relative abundance of potential pathobionts, and the circulating fatty acids as fish oil and the combination of fish oil and probiotics increased the levels of n-3 LC-PUFAs. However, the probiotics alone did not have an effect on the circulating fatty acids.

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References

Aatsinki AK, Uusitupa HM, Munukka E, Pesonen H, Rintala A, Pietilä S, Lahti L, Eerola E, Karlsson L & Karlsson H (2018) Gut Microbiota Composition in Mid-Pregnancy Is Associated with Gestational Weight Gain but Not Prepregnancy Body Mass Index. *J Womens Health (Larchmt)* 27: 1293–1301.

Adak A & Khan MR (2019) An insight into gut microbiota and its functionalities. *Cell Mol Life Sci* 76: 473–493.

Aguirre GA, Ita JR, Garza RG & Castilla-Cortazar I (2016) Insulin-like growth factor-1 deficiency and metabolic syndrome. *Journal of Translational Medicine 2016 14:1* 14: 1–23.

Akcalı A, Gümüş P, Özçaka Ö, Öztürk-Ceyhan B, Tervahartiala T, Husu H, Heikkinen AM, Sorsa T & Buduneli N (2017) Proteolytic Mediators in Gestational Diabetes Mellitus and Gingivitis. *J Periodontol* 88: 289–297.

Allen NG, Edupuganti L, Edwards DJ, Jimenez NR, Buck GA, Jefferson KK, Strauss JF, Wickham EP & Fettweis JM (2022) The vaginal microbiome in women of reproductive age with healthy weight versus overweight/obesity. *Obesity (Silver Spring)* 30: 142–152.

Al-Otaibi, Hussein, Mustafa & Al-Mudaires (2020) Obesity, gestational weight gain, and polyunsaturated fatty acids profile in pregnant Saudi women. *Bioinformation* 16: 493–500.

Alyas S, Roohi N, Ashraf S, Ilyas S & Ilyas A (2019) Early pregnancy biochemical markers of placentation for screening of gestational diabetes mellitus (GDM). *Diabetes Metab Syndr* 13: 2353–2356.

Amabebe E & Anumba DOC (2018) The vaginal microenvironment: The physiologic role of Lactobacilli. *Front Med (Lausanne)* 5: 181.

Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermúdez-Humarán LG, Smirnova N, Bergé M, Sulpice T, Lahtinen S, *et al* (2011) Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO Mol Med* 3: 559–572.

Amirian A, Rahnemaei FA & Abdi F (2020) Role of C-reactive Protein(CRP) or high-sensitivity CRP in predicting gestational diabetes Mellitus:Systematic review. *Diabetes Metab Syndr* 14: 229–236.

Aparicio E, Martín-Grau C, Bedmar C, Orus NS, Basora J & Arija V (2021a) Maternal Factors Associated with Levels of Fatty Acids, Specifically n-3 PUFA during Pregnancy: ECLIPSES Study. *Nutrients* 13: 1–19.

Aparicio E, Martín-Grau C, Hernández-Martinez C, Voltas N, Canals J & Arija V (2021b) Changes in fatty acid levels (saturated, monounsaturated and polyunsaturated) during pregnancy. *BMC Pregnancy Childbirth* 21.

Arai T, Yamashita S, Hirano KI, Sakai N, Kotani K, Fujioka S, Nozaki S, Keno Y, Yamane M, Shinohara E, *et al* (1994) Increased plasma cholesteryl ester transfer protein in obese subjects. A possible mechanism for the reduction of serum HDL cholesterol levels in obesity. *Arterioscler Thromb* 14: 1129–1136.

Åsvold BO, Eskild A, Jenum PA & Vatten LJ (2011) Maternal concentrations of insulin-like growth factor I and insulin-like growth factor binding protein 1 during pregnancy and birth weight of offspring. *Am J Epidemiol* 174: 129–135.

Baird J, Jacob C, Barker M, Fall CHD, Hanson M, Harvey NC, Inskip HM, Kumaran K & Cooper C (2017) Developmental Origins of Health and Disease: A Lifecourse Approach to the Prevention of Non-Communicable Diseases. *Healthcare (Basel)* 5.

Baker JM, Al-Nakkash L & Herbst-Kralovetz MM (2017) Estrogen–gut microbiome axis: Physiological and clinical implications. *Maturitas* 103: 45–53.

Bergmann RL, Haschke-Becher E, Klassen-Wigger P, Bergmann KE, Richter R, Dudenhausen JW, Grathwohl D & Haschke F (2008) Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake and of their infants. *Ann Nutr Metab* 52: 157–166.

Bernhardt GV, Shivappa P, Bernhardt K, Bhat S, Pinto JRT, Jhancy M & Kumar S (2022) Markers of inflammation in obese pregnant women: Adenosine deaminase and high sensitive C - reactive protein. *Eur J Obstet Gynecol Reprod Biol X* 16.

Bisanz JE, Enos MK, PrayGod G, Seney S, Macklaim JM, Chilton S, Willner D, Knight R, Fusch C, Fusch G, *et al* (2015) Microbiota at Multiple Body Sites during Pregnancy in a Rural Tanzanian Population and Effects of Moringa-Supplemented Probiotic Yogurt. *Appl Environ Microbiol* 81: 4965–4975.

Boden G (2008) Obesity and free fatty acids. *Endocrinol Metab Clin North Am* 37: 635–646.

Bodnar LM, Ness RB, Harger GF & Roberts JM (2005) Inflammation and triglycerides partially mediate the effect of prepregnancy body mass index on the risk of preeclampsia. *Am J Epidemiol* 162: 1198–1206.

Bo S, Signorile A, Menato G, Gambino R, Bardelli C, Gallo ML, Cassader M, Massobrio M & Pagano GF (2005) C-reactive protein and tumor necrosis factor-alpha in gestational hyperglycemia. *J Endocrinol Invest* 28: 779–786.

Bray GA, Kim KK & Wilding JPH (2017) Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. *Obes Rev* 18: 715–723.

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, *et al* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56: 1761–1772.

Cani PD, Osto M, Geurts L & Everard A (2012) Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut Microbes* 3.

Chamorro R, Bascuñán KA, Barrera C, Sandoval J, Puigrredon C & Valenzuela R (2022) Reduced n-3 and n-6 PUFA (DHA and AA) Concentrations in Breast Milk and Erythrocytes Phospholipids during Pregnancy and Lactation in Women with Obesity. *Int J Environ Res Public Health* 19.

Chaparro A, Realini O, Hernández M, Albers D, Weber L, Ramírez V, Param F, Kusanovic JP, Sorsa T, Rice GE, *et al* (2021) Early pregnancy levels of gingival crevicular fluid matrix metalloproteinases-8 and -9 are associated with the severity of periodontitis and the development of gestational diabetes mellitus. *J Periodontol* 92: 205–215.

Chen L, Chen R, Wang H & Liang F (2015) Mechanisms Linking Inflammation to Insulin Resistance. *Int J Endocrinol* 2015.

Chiesa ST, Charakida M, Georgiopoulos G, Roberts JD, Stafford SJ, Park C, Mykkänen J, Kähönen M, Lehtimäki T, Ala-Korpela M, *et al* (2022) Glycoprotein Acetyls: A Novel Inflammatory Biomarker of Early Cardiovascular Risk in the Young. *J Am Heart Assoc* 11.

Choe SS, Huh JY, Hwang IJ, Kim JI & Kim JB (2016) Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne)* 7.

Christian LM & Porter K (2014) Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: Effects of maternal body mass index. *Cytokine* 70: 134–140.

Cinelli G, Fabrizi M, Ravà L, Atti MCD, Vernocchi P, Vallone C, Pietrantoni E, Lanciotti R, Signore F & Manco M (2016) Influence of Maternal Obesity and Gestational Weight Gain on Maternal and Foetal Lipid Profile. *Nutrients* 8.

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

Connelly MA, Otvos JD, Shalaurova I, Playford MP & Mehta NN (2017) GlycA, a novel biomarker of systemic inflammation and cardiovascular disease risk. *J Transl Med* 15.

Cortez R V., Taddei CR, Sparvoli LG, Ângelo AGS, Padilha M, Mattar R & Daher S (2019) Microbiome and its relation to gestational diabetes. *Endocrine* 64: 254–264.

Crovesy L, Masterson D & Rosado EL (2020) Profile of the gut microbiota of adults with obesity: a systematic review. *Eur J Clin Nutr* 74: 1251–1262.

Damsgaard CT, Harslof LBS, Andersen AD, Hellgren LI, Michaelsen KF & Lauritzen L (2016) Fish oil supplementation from 9 to 18 months of age affects the insulin-like growth factor axis in a sex-specific manner in Danish infants. *Br J Nutr* 115: 782–790.

Danielsson D, Teigen PK & Moi H (2011) The genital econiche: focus on microbiota and bacterial vaginosis. *Ann N Y Acad Sci* 1230: 48–58.

Dekker J, Collett M, Prasad J & Gopal P (2007) Functionality of probiotics - potential for product development. *Forum Nutr* 60: 196–208.

Delano FA & Schmid-Schönbein GW (2008) Proteinase activity and receptor cleavage: Mechanism for insulin resistance in the spontaneously hypertensive rat. *Hypertension* 52: 415–423.

Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, *et al* (2016) A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell* 167: 1339-1353.e21.

DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DSA, Wong RJ, Shawa G, *et al* (2015) Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A* 112: 11060–11065.

Diop K, Dufour JC, Levasseur A & Fenollar F (2019) Exhaustive repertoire of human vaginal microbiota. *Hum Microb J* 11: 100051.

Duan M, Wang Y, Zhang Q, Zou R, Guo M & Zheng H (2021) Characteristics of gut microbiota in people with obesity. *PLoS One* 16.

Dunstan JA, Mori TA, Barden A, Beilin LJ, Holt PG, Calder PC, Taylor AL & Prescott SL (2004) Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition. *Eur J Clin Nutr* 58: 429–437.

Duran M, Köşüş A, Köşüş N & Turhan N (2016) CRP, HbA1c, lipid, and biochemical parameters and their relation with maternal visceral adipose tissue and subcutaneous fat tissue thickness. *Turk J Med Sci* 46: 6–12.

Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MKJ, Biollaz G, *et al* (2007) Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* 56: 2356–2370.

Eivindson M, Grønbæk H, Nielsen JN, Frystyk J, Flyvbjerg A, Jørgensen L, Vind I, Munkholm P, Jensen S, Brandslund I, *et al* (2005) Insulin-like growth factors (IGFs) and IGF binding proteins in active Crohn's disease treated with omega-3 or omega-6 fatty acids and corticosteroids. *Scand J Gastroenterol* 40: 1214–1221.

Elvers KT, Wilson VJ, Hammond A, Duncan L, Huntley AL, Hay AD & van der Werf ET (2020) Antibiotic-induced changes in the human gut microbiota for the most commonly prescribed antibiotics in primary care in the UK: a systematic review. *BMJ Open* 10: e035677.

Farshbaf-Khalili A, Mohamad-Alizadeh S, Darabi M, Hematzadeh S, Mehdizadeh A, Shaaker M & Ostadrahimi A (2017) The effect of fish oil supplementation on serum phospholipid fatty acids profile during pregnancy: A double blind randomized controlled trial. *Women Health* 57: 137–153.

Faucher MA, Greathouse KL, Hastings-Tolsma M, Padgett RN, Sakovich K, Choudhury A, Sheikh A, Ajami NJ & Petrosino JF (2020) Exploration of the Vaginal and Gut Microbiome in African American Women by Body Mass Index, Class of Obesity, and Gestational Weight Gain: A Pilot Study. *Am J Perinatol* 37: 1160–1172.

Fekete K, Györei E, Lohner S, Verduci E, Agostoni C & Decsi T (2015) Long-chain polyunsaturated fatty acid status in obesity: a systematic review and meta-analysis. *Obes Rev* 16: 488–497.

Ferraro ZM, Qiu Q, Gruslin A & Adamo KB (2012) Characterization of the insulin-like growth factor axis in term pregnancies complicated by maternal obesity. *Hum Reprod* 27: 2467–2475.

Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, Huang B, Arodz TJ, Edupuganti L, Glascock AL, *et al* (2019) The vaginal microbiome and preterm birth. *Nat Med* 25: 1012–1021.

Fisk HL, Irvine M, Miles EA, Lietz G, Mathers JC, Packard CJ, Armah CK, Kofler BM, Curtis PJ, Minihane AM, *et al* (2018) Association of oily fish intake, sex, age, BMI and APOE genotype with plasma long-chain n-3 fatty acid composition. *Br J Nutr* 120: 23–32.

Foratori-Junior GA, Guennec A le, Fidalgo TK da S, Cleaver L, Buzalaf MAR, Carpenter GH & Sales-Peres SH de C (2022) Metabolomic Profiles Associated with Obesity and Periodontitis during Pregnancy: Cross-Sectional Study with Proton Nuclear Magnetic Resonance (1H-NMR)-Based Analysis. *Metabolites* 12: 1029.

Franssen R, Monajemi H, Stroes ESG & Kastelein JJP (2011) Obesity and dyslipidemia. *Med Clin North Am* 95: 893–902.

Friis CM, Roland MCP, Godang K, Ueland T, Tanbo T, Bollerslev J & Henriksen T (2013) Adiposity-related inflammation: effects of pregnancy. *Obesity (Silver Spring)* 21: E124–E130.

Fujimori M, França EL, Fiorin V, Morais TC, Honorio-França AC & de Abreu LC (2015) Changes in the biochemical and immunological components of serum and colostrum of overweight and obese mothers. *BMC Pregnancy Childbirth* 15.

Fujimori M, França EL, Morais TC, Fiorin V, de Abreu LC & Honório-França AC (2017) Cytokine and adipokine are biofactors can act in blood and colostrum of obese mothers. *Biofactors* 43: 243–250.

Gao L, Lin L, Shan N, Ren CY, Long X, Sun YH & Wang L (2020) The impact of omega-3 fatty acid supplementation on glycemic control in patients with gestational diabetes: a systematic review and meta-analysis of randomized controlled studies. *J Matern Fetal Neonatal Med* 33: 1767–1773.

García-Rodríguez CE, Olza J, Aguilera CM, Mesa MD, Miles EA, Noakes PS, Vlachava M, Kremmyda LS, Diaper ND, Godfrey KM, *et al* (2012) Plasma inflammatory and vascular homeostasis biomarkers increase during human pregnancy but are not affected by oily fish intake. *J Nutr* 142: 1191–1196.

Gázquez A, Giménez-Bañón MJ, Prieto-Sánchez MT, Martínez-Graciá C, Suárez C, Santaella-Pascual M, Galdo-Castiñeira L, Ballesteros-Meseguer C, Vioque J, Martínez-Villanueva M, *et al* (2021) Self-Reported DHA Supplementation during Pregnancy and Its Association with Obesity or Gestational Diabetes in Relation to DHA Concentration in Cord and Maternal Plasma: Results from NELA, a Prospective Mother-Offspring Cohort. *Nutrients* 13: 1–18.

Gholamhosseini S, Nematipour E, Djazayery A, Javanbakht MH, Koohdani F, Zareei M & Djalali M (2015) ω-3 fatty acid differentially modulated serum levels of IGF1 and IGFBP3 in men with CVD: a randomized, double-blind placebo-controlled study. *Nutrition* 31: 480–484.

Gille C, Böer B, Marschal M, Urschitz MS, Heinecke V, Hund V, Speidel S, Tarnow I, Mylonas I, Franz A, *et al* (2016) Effect of probiotics on vaginal health in pregnancy. EFFPRO, a randomized controlled trial. *Am J Obstet Gynecol* 215: 608.e1-608.e7.

Gillespie SL & Christian LM (2016) Body Mass Index as a Measure of Obesity: Racial Differences in Predictive Value for Health Parameters During Pregnancy. *J Womens Health (Larchmt)* 25: 1210–1218.

Gregor MF & Hotamisligil GS (2011) Inflammatory mechanisms in obesity. *Annu Rev Immunol* 29: 415–445.

Haghiac M, Yang XH, Presley L, Smith S, Dettelback S, Minium J, Belury MA, Catalano PM & Hauguel-De Mouzon S (2015) Dietary Omega-3 Fatty Acid Supplementation Reduces Inflammation in Obese Pregnant Women: A Randomized Double-Blind Controlled Clinical Trial. *PLoS One* 10.

Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Abbasi MM & Dolatkhah N (2018) Effect of probiotic supplements in women with gestational diabetes mellitus on

inflammation and oxidative stress biomarkers: a randomized clinical trial. *Asia Pac J Clin Nutr* 27: 581–591.

Hamaya R, Mora S, Lawler PR, Cook NR, Ridker PM, Buring JE, Lee IM, Manson JAE & Tobias DK (2021) Association of Plasma Branched-Chain Amino Acid With Biomarkers of Inflammation and Lipid Metabolism in Women. *Circ Genom Precis Med* 14: E003330.

Hauguel-de Mouzon S & Guerre-Millo M (2006) The placenta cytokine network and inflammatory signals. *Placenta* 27: 794–798.

Hautero U, Laakso P, Linderborg K, Niinivirta K, Poussa T, Isolauri E & Laitinen K (2013) Proportions and concentrations of serum n-3 fatty acids can be increased by dietary counseling during pregnancy. *Eur J Clin Nutr* 67: 1163–1168.

Heald AH, Anderson SG, Ivison F, Laing I, Gibson JM & Cruickshank K (2003) C-reactive protein and the insulin-like growth factor (IGF)-system in relation to risk of cardiovascular disease in different ethnic groups. *Atherosclerosis* 170: 79–86.

Heckman MG, Davis III JM & Crowson CS (2022) Expert Review Post Hoc Power Calculations: An Inappropriate Method for Interpreting the Findings of a Research Study..

Hellmuth C, Lindsay KL, Uhl O, Buss C, Wadhwa PD, Koletzko B & Entringer S (2017) Association of maternal prepregnancy BMI with metabolomic profile across gestation. *Int J Obes (Lond)* 41: 159–169.

Hjelmsø MH, Shah SA, Thorsen J, Rasmussen M, Vestergaard G, Mortensen MS, Brejnrod A, Brix S, Chawes B, Bønnelykke K, *et al* (2020) Prenatal dietary supplements influence the infant airway microbiota in a randomized factorial clinical trial. *Nat Commun* 11.

Holmes RP, Soothill PW & Holly JMP (2000) Maternal insulin-like growth factor binding protein-1, body mass index, and fetal growth. *Arch Dis Child Fetal Neonatal Ed* 82.

Hoppu U, Isolauri E, Laakso P, Matomäki J & Laitinen K (2012) Probiotics and dietary counselling targeting maternal dietary fat intake modifies breast milk fatty acids and cytokines. *Eur J Nutr* 51: 211–219.

Huhtala MS, Tertti K, Juhila J, Sorsa T & Rönnemaa T (2020) Metformin and insulin treatment of gestational diabetes: effects on inflammatory markers and IGF-binding protein-1 - secondary analysis of a randomized controlled trial. *BMC Pregnancy Childbirth* 20.

Hulander E, Bärebring L, Turesson Wadell A, Gjertsson I, Calder PC, Winkvist A & Lindqvist HM (2021) Diet intervention improves cardiovascular profile in patients with rheumatoid arthritis: results from the randomized controlled cross-over trial ADIRA. *Nutr J* 20: 1–12.

Husain S, Allotey J, Drymoussi Z, Wilks M, Fernandez-Felix BM, Whiley A, Dodds J, Thangaratinam S, McCourt C, Prosdocimi EM, *et al* (2020) Effects of oral probiotic supplements on vaginal microbiota during pregnancy: a randomised, double-blind, placebo-controlled trial with microbiome analysis. *BJOG* 127: 275–284.

Jääskeläinen T, Heinonen S, Hämäläinen E, Pulkki K, Romppanen J, Laivuori H, Laivuori H, Heinonen S, Kajantie E, Kere J, *et al* (2018) Impact of obesity on angiogenic and inflammatory markers in the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) cohort. *International Journal of Obesity 2018 43:5* 43: 1070–1081.

Jafarnejad S, Saremi S, Jafarnejad F & Arab A (2016) Effects of a Multispecies Probiotic Mixture on Glycemic Control and Inflammatory Status in Women with Gestational Diabetes: A Randomized Controlled Clinical Trial. *J Nutr Metab* 2016.

Jansson N, Nilsfelt A, Gellerstedt M, Wennergren M, Rossander-Hulthén L, Powell TL & Jansson T (2008) Maternal hormones linking maternal body mass index and dietary intake to birth weight. *Am J Clin Nutr* 87: 1743–1749.

Kac G, dos Santos Vaz J, Schlüssel MM & Moura AS (2011) C-reactive protein and hormones but not IL-6 are associated to body mass index in first trimester of pregnancy. *Arch Gynecol Obstet* 284: 567–573.

Kankaanpää P, Yang B, Kallio H, Isolauri E & Salminen S (2004) Effects of polyunsaturated fatty acids in growth medium on lipid composition and on physicochemical surface properties of lactobacilli. *Appl Environ Microbiol* 70: 129–136.

Kansu-Celik H, Seval Ozgu-Erdinc A, Kisa B, Findik RB, Yilmaz C & Tasci Y (2020) Prediction of gestational diabetes mellitus in the first trimester: comparison of maternal fetuin-A, N-terminal proatrial natriuretic peptide, high-sensitivity C-reactive protein, and fasting glucose levels. *Arch Endocrinol Metab* 64: 97.

Kaplas N, Isolauri E, Lampi AM, Ojala T & Laitinen K (2007) Dietary counseling and probiotic supplementation during pregnancy modify placental phospholipid fatty acids. *Lipids* 42: 865–870.

Kivelä J, Sormunen-Harju H, Girchenko P v., Huvinen E, Stach-Lempinen B, Kajantie E, Villa PM, Reynolds RM, Hämäläinen EK, Lahti-Pulkkinen M, *et al* (2021) Longitudinal Metabolic Profiling of Maternal Obesity, Gestational Diabetes, and Hypertensive Pregnancy Disorders. *J Clin Endocrinol Metab* 106: E4372–E4388.

Klein A, Friedrich U, Vogelsang H & Jahreis G (2008) Lactobacillus acidophilus 74-2 and Bifidobacterium animalis subsp lactis DGCC 420 modulate unspecific cellular immune response in healthy adults. *Eur J Clin Nutr* 62: 584–593.

Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Kling Bäckhed H, Gonzalez A, Werner JJ, Angenent LT, Knight R, *et al* (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 150: 470–480.

Krauss-Etschmann S, Hartl D, Rzehak P, Heinrich J, Shadid R, del Carmen Ramírez-Tortosa M, Campoy C, Pardillo S, Schendel DJ, Decsi T, *et al* (2008) Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women. *J Allergy Clin Immunol* 121.

Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jiménez M, Gil A, Rivero M, Veszprémi B, Decsi T, *et al* (2007) Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. *Am J Clin Nutr* 85: 1392–1400.

Kruit H, Heikinheimo O, Sorsa T, Juhila J, Paavonen J & Rahkonen L (2018) Cervical biomarkers as predictors of successful induction of labour by Foley catheter. *J Obstet Gynaecol* 38: 927–932.

Lahti L, Salonen A, Kekkonen RA, Salojärvi J, Jalanka-Tuovinen J, Palva A, Orešič M & de Vos WM (2013) Associations between the human intestinal microbiota, Lactobacillus rhamnosus GG and serum lipids indicated by integrated analysis of high-throughput profiling data. *PeerJ* 1.

Lain KY & Catalano PM (2007) Metabolic changes in pregnancy. *Clin Obstet Gynecol* 50: 938–948.

Laitinen K, Poussa T & Isolauri E (2009) Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. *Br J Nutr* 101: 1679–1687.

Larqué E, Gil-Sánchez A, Prieto-Sánchez MT & Koletzko B (2012) Omega 3 fatty acids, gestation and pregnancy outcomes. *Br J Nutr* 107 Suppl 2.

Larsson A, Palm M, Basu S & Axelsson O (2013) Insulin-like growth factor binding protein-1 (IGFBP-1) during normal pregnancy. *Gynecol Endocrinol* 29: 129–132.

Lauhio A, Färkkilä E, Pietiläinen KH, Åström P, Winkelmann A, Tervahartiala T, Pirilä E, Rissanen A, Kaprio J, Sorsa TA, *et al* (2016) Association of MMP-8 with obesity, smoking and insulin resistance. *Eur J Clin Invest* 46: 757–765.

Lewitt M, Dent M & Hall K (2014) The Insulin-Like Growth Factor System in Obesity, Insulin Resistance and Type 2 Diabetes Mellitus. *J Clin Med* 3: 1561–1574.

Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD & Gordon JI (2005) Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 102: 11070–11075.

Liang X, Li Z, Tye KD, Chen Y, Luo H & Xiao X (2021) The effect of probiotic supplementation during pregnancy on the interaction network of vaginal microbiome. *J Obstet Gynaecol Res* 47: 103–113.

Li K, Yang C, Fan J, Li X, Gu C & Liu H (2022) Prepregnancy body mass index, gestational weight gain, and maternal prepartum inflammation in normal pregnancies: findings from a Chinese cohort. *BMC Pregnancy Childbirth* 22.

Liu YJ, Tsushima T, Minei S, Sanaka M, Nagashima T, Yanagisawa K & Omori Y (1996) Insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBP-1, -2 and -3) in diabetic pregnancy: relationship to macrosomia. *Endocr J* 43: 221–231.

Loukovaara M, Leinonen P, Teramo K, Nurminen E, Andersson S & Rutanen EM (2005) Effect of maternal diabetes on phosphorylation of insulin-like growth factor binding protein-1 in cord serum. *Diabet Med* 22: 434–439.

Luoto R, Laitinen K, Nermes M & Isolauri E (2010) Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. *Br J Nutr* 103: 1792–1799.

Madan JC, Davis JM, Craig WY, Collins M, Allan W, Quinn R & Dammann O (2009) Maternal obesity and markers of inflammation in pregnancy. *Cytokine* 47: 61–64.

Maged AM, Moety GAF, Mostafa WA & Hamed DA (2014) Comparative study between different biomarkers for early prediction of gestational diabetes mellitus. *J Matern Fetal Neonatal Med* 27: 1108–1112.

McDade TW, Borja JB, Largado F, Adair LS & Kuzawa CW (2016) Adiposity and Chronic Inflammation in Young Women Predict Inflammation during Normal Pregnancy in the Philippines. *J Nutr* 146: 353–357.

McElwain CJ, McCarthy FP & McCarthy CM (2021) Gestational Diabetes Mellitus and Maternal Immune Dysregulation: What We Know So Far. *Int J Mol Sci* 22.

McMillan A, Rulisa S, Gloor GB, Macklaim JM, Sumarah M & Reid G (2018) Pilot assessment of probiotics for pregnant women in Rwanda. *PLoS One* 13.

Metzger BE, Lowe LP, Dyer AR, Chaovarindr U, Hospital R, Coustan DR, Hadden DR, McCance DR, Ireland N, Hod M, *et al* (2008) Hyperglycemia and Adverse Pregnancy Outcomes. *New England Journal of Medicine* 358: 1991–2002.

Miles EA, Noakes PS, Kremmyda LS, Vlachava M, Diaper ND, Rosenlund G, Urwin H, Yaqoob P, Rossary A, Farges MC, *et al* (2011) The Salmon in Pregnancy Study: study design, subject characteristics, maternal fish and marine n-3 fatty acid intake, and marine n-3 fatty acid status in maternal and umbilical cord blood. *Am J Clin Nutr* 94.

Mokkala K, Houttu N, Koivuniemi E, Sørensen N, Nielsen HB & Laitinen K (2020a) GlycA, a novel marker for low grade inflammation, reflects gut microbiome diversity and is more accurate than high sensitive CRP in reflecting metabolomic profile. *Metabolomics* 16.

Mokkala K, Paulin N, Houttu N, Koivuniemi E, Pellonperä O, Khan S, Pietilä S, Tertti K, Elo LL & Laitinen K (2021a) Metagenomics analysis of gut microbiota in response to diet intervention and gestational diabetes in overweight and obese women: a randomised, double-blind, placebo-controlled clinical trial. *Gut* 70: 309–318.

Mokkala K, Röytiö H, Ekblad U & Laitinen K (2017) Opportunities for probiotics and polyunsaturated fatty acids to improve metabolic health of overweight pregnant women. *Benef Microbes* 8: 3–15.

Mokkala K, Röytiö H, Munukka E, Pietilä S, Ekblad U, Rönnemaa T, Eerola E, Laiho A & Laitinen K (2016) Gut Microbiota Richness and Composition and Dietary Intake of Overweight Pregnant Women Are Related to Serum Zonulin Concentration, a Marker for Intestinal Permeability. *J Nutr* 146: 1694–1700.

Mokkala K, Vahlberg T, Houttu N, Koivuniemi E, Lahti L & Laitinen K (2021b) Impact of combined consumption of fish oil and probiotics on the serum metabolome in pregnant women with overweight or obesity. *EBioMedicine* 73.

Mokkala K, Vahlberg T, Pellonperä O, Houttu N, Koivuniemi E & Laitinen K (2020b) Distinct Metabolic Profile in Early Pregnancy of Overweight and Obese Women Developing Gestational Diabetes. *J Nutr* 150: 31–37.

Moludi J, Saiedi S, Ebrahimi B, Alizadeh M, Khajebishak Y & Ghadimi SS (2021) Probiotics Supplementation on Cardiac Remodeling Following Myocardial Infarction: a Single-Center Double-Blind Clinical Study. *J Cardiovasc Transl Res* 14: 299–307.

Mor G, Cardenas I, Abrahams V & Guller S (2011) Inflammation and pregnancy: the role of the immune system at the implantation site. *Ann N Y Acad Sci* 1221: 80–87.

Myntti T, Rahkonen L, Nupponen I, Pätäri-Sampo A, Tikkanen M, Sorsa T, Juhila J, Andersson S, Paavonen J & Stefanovic V (2017) Amniotic Fluid Infection in Preterm Pregnancies with Intact Membranes. *Dis Markers* 2017.

Myntti T, Rahkonen L, Pätäri-Sampo A, Tikkanen M, Sorsa T, Juhila J, Helve O, Andersson S, Paavonen J & Stefanovic V (2016) Comparison of amniotic fluid matrix metalloproteinase-8 and cathelicidin in the diagnosis of intra-amniotic infection. *J Perinatol* 36: 1049–1054.

Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK, Lee HC & Huh KB (1997) Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. *Int J Obes Relat Metab Disord* 21: 355–359.

National Institutes of Health (2012) NIH Human Microbiome Project defines normal bacterial makeup of the body.

Nielsen S & Karpe F (2012) Determinants of VLDL-triglycerides production. *Curr Opin Lipidol* 23: 321–326.

Nuutila M, Hiilesmaa V, Kärkkäinen T, Ylikorkala O & Rutanen EM (1999) Phosphorylated isoforms of insulin-like growth factor binding protein-1 in the cervix as a predictor of cervical ripeness. *Obstetrics and gynecology* 94: 243–249.

Official Statistics of Finland P statistics. THL (2020) Perinatal statistics - parturients, delivers and newborns. Statistical Report 48/2020, 20.11.2020.

Olausson H, Löf M, Brismar K, Forsum E & Sohlström A (2010) Maternal serum concentrations of insulin-like growth factor (IGF)-I and IGF binding protein-1 before and during pregnancy in relation to maternal body weight and composition and infant birth weight. *Br J Nutr* 104: 842–848.

Olivares P dos SG, Pacheco ABF, Aranha LN, Oliveira B da S, Santos AA, Santos PCM dos, Neto JFN, Rosa G & Oliveira GMM (2021) Gut microbiota of adults with different metabolic phenotypes. *Nutrition* 90: 111293.

de Oliveira LC, Franco-Sena AB, Rebelo F, Farias DR, Lepsch J, Lima N da S & Kac G (2015) Factors associated with maternal serum C-reactive protein throughout pregnancy: A longitudinal study in women of Rio de Janeiro, Brazil. *Nutrition* 31: 1103–1108.

Oren A & Garrity GM (2021) Valid publication of the names of forty-two phyla of prokaryotes. *Int J Syst Evol Microbiol* 71: 005056.

Otvos JD, Shalaurova I, Wolak-Dinsmore J, Connelly MA, Mackey RH, Stein JH & Tracy RP (2015) GlycA: A composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem* 61: 714–723.

Page MJ, Kell DB & Pretorius E (2022) The Role of Lipopolysaccharide-Induced Cell Signalling in Chronic Inflammation. *Chronic Stress (Thousand Oaks)* 6.

Pantham P, Aye ILMH & Powell TL (2015) Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta* 36: 709–715.

Payab M, Tayanloo-Beik A, Falahzadeh K, Mousavi M, Salehi S, Djalalinia S, Ebrahimpur M, Rezaei N, Rezaei-Tavirani M, Larijani B, *et al* (2021) Metabolomics prospect of obesity and metabolic syndrome; a systematic review. *J Diabetes Metab Disord* 21: 889–917.

Pellonperä O, Mokkala K, Houttu N, Vahlberg T, Koivuniemi E, Tertti K, Rönnemaa T & Laitinen K (2019) 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* 42: 1009–1017.

Pellonperä O, Vahlberg T, Mokkala K, Houttu N, Koivuniemi E, Tertti K, Rönnemaa T & Laitinen K (2021) Weight gain and body composition during pregnancy: a randomised pilot trial with probiotics and/or fish oil. *British Journal of Nutrition* 126: 541–551.

Penfield-Cyr A, Monthe-Dreze C, Smid MC & Sen S (2018) Maternal BMI, Mid-pregnancy Fatty Acid Concentrations, and Perinatal Outcomes. *Clin Ther* 40: 1659-1667.e1.

Pinto J, Barros AS, Domingues MRM, Goodfellow BJ, Galhano E, Pita C, Almeida MDC, Carreira IM & Gil AM (2015) Following healthy pregnancy by NMR metabolomics of plasma and correlation to urine. *J Proteome Res* 14: 1263–1274.

Plows JF, Stanley JL, Baker PN, Reynolds CM & Vickers MH (2018) The Pathophysiology of Gestational Diabetes Mellitus. *Int J Mol Sci* 19.

Plummer E, Vodstrcil L, Fairley C, Danielewski J, Murray G, Doyle M, Fehler G, Mcguinness C, Bulach D, Garland S, *et al* (2021) O02.3 Reclassification of Atopobium vaginae as three novel Fannyhessea species: implications for understanding their role in bacterial vaginosis. *Sex Transm Infect* 97: A18–A18.

Powe CE, Hivert MF & Udler MS (2020) Defining Heterogeneity Among Women With Gestational Diabetes Mellitus. *Diabetes* 69: 2064–2074.

Qiu C, Sorensen TK, Luthy DA & Williams MA (2004) A prospective study of maternal serum C-reactive protein (CRP) concentrations and risk of gestational diabetes mellitus. *Paediatr Perinat Epidemiol* 18: 377–384.

van Raaij JMA, Peek MEM, Vermaat-Miedema SH, Schonk CM & Hautvast JGAJ (1988) New equations for estimating body fat mass in pregnancy from body density or total body water. *Am J Clin Nutr* 48: 24–29.

Rafat D, Singh S, Nawab T, Khan F, Khan AU & Khalid S (2022) Association of vaginal dysbiosis and gestational diabetes mellitus with adverse perinatal outcomes. *Int J Gynaecol Obstet* 158: 70–78.

Raglan O, MacIntyre DA, Mitra A, Lee YS, Smith A, Assi N, Nautiyal J, Purkayastha S, Gunter MJ, Gabra H, *et al* (2021) The association between obesity and weight loss after bariatric surgery on the vaginal microbiota. *Microbiome* 9: 1–17.

Rahman A, Hammad MM, al Khairi I, Cherian P, Al-Sabah R, Al-Mulla F, Abu-Farha M & Abubaker J (2021) Profiling of Insulin-Like Growth Factor Binding Proteins (IGFBPs) in Obesity and Their Association With Ox-LDL and Hs-CRP in Adolescents. *Front Endocrinol (Lausanne)* 12.

Rajpathak SN, Gunter MJ, Wylie-Rosett J, Ho GYF, Kaplan RC, Muzumdar R, Rohan TE & Strickler HD (2009) The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. *Diabetes Metab Res Rev* 25: 3–12.

Ramsay JE, Ferrell WR, Crawford L, Michael Wallace A, Greer IA & Sattar N (2002) Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. *J Clin Endocrinol Metab* 87: 4231–4237.

Ranganathan P, Pramesh CS & Buyse M (2015) Common pitfalls in statistical analysis: Clinical versus statistical significance. *Perspect Clin Res* 6: 169.

Rauschert S, Gázquez A, Uhl O, Kirchberg FF, Demmelmair H, Ruíz-Palacios M, Prieto-Sánchez MT, Blanco-Carnero JE, Nieto A, Larqué E, *et al* (2019) Phospholipids in lipoproteins: compositional differences across VLDL, LDL, and HDL in pregnant women. *Lipids Health Dis* 18.

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, *et al* (2011) Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 108: 4680–4687.

Roessler A, Friedrich U, Vogelsang H, Bauer A, Kaatz M, Hipler UC, Schmidt I & Jahreis G (2008) The immune system in healthy adults and patients with atopic dermatitis seems to be affected differently by a probiotic intervention. *Clin Exp Allergy* 38: 93–102.

Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Bieda J, Chaemsaithong P, Miranda J, Chaiworapongsa T & Ravel J (2014a) The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term..

Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, Galuppi M, Lamont RF, Chaemsaithong P, Miranda J, *et al* (2014b) Correction: The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2.

Rosenberg E (2014) The family prevotellaceae. *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*: 825–827.

Ruebel ML, Gilley SP, Sims CR, Zhong Y, Turner D, Chintapalli S v., Piccolo BD, Andres A & Shankar K (2021) Associations between Maternal Diet, Body Composition and Gut Microbial Ecology in Pregnancy. *Nutrients* 13.

Sandler V, Reisetter AC, Bain JR, Muehlbauer MJ, Nodzenski M, Stevens RD, Ilkayeva O, Lowe LP, Metzger BE, Newgard CB, *et al* (2017) Associations of maternal BMI and insulin resistance with the maternal metabolome and newborn outcomes. *Diabetologia* 60: 518–530.

Santacruz A, Collado MC, García-Valdés L, Segura MT, Marítn-Lagos JA, Anjos T, Martí-Romero M, Lopez RM, Florido J, Campoy C, *et al* (2010) Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr* 104: 83–92.

Scifres CM, Catov JM & Simhan HN (2014) The impact of maternal obesity and gestational weight gain on early and mid-pregnancy lipid profiles. *Obesity (Silver Spring)* 22: 932–938.

Sender R, Fuchs S & Milo R (2016) Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 14.

Shearer J, Klein MS, Vogel HJ, Mohammad S, Bainbridge S & Adamo KB (2021) Maternal and Cord Blood Metabolite Associations with Gestational Weight Gain and Pregnancy Health Outcomes. *J Proteome Res* 20: 1630–1638.

Shin D, Hur J, Cho EH, Chung HK, Shivappa N, Wirth MD, Hébert JR & Lee KW (2017) Pre-Pregnancy Body Mass Index Is Associated with Dietary Inflammatory Index and C-Reactive Protein Concentrations during Pregnancy. *Nutrients 2017, Vol 9, Page 351* 9: 351.

Shokry E, Marchioro L, Uhl O, Bermúdez MG, García-Santos JA, Segura M [a]T, Campoy C & Koletzko B (2019) Impact of maternal BMI and gestational diabetes mellitus on maternal and cord blood metabolome: results from the PREOBE cohort study. *Acta Diabetol* 56: 421–430.

Sifnaios E, Mastorakos G, Psarra K, Panagopoulos ND, Panoulis K, Vitoratos N, Rizos D & Creatsas G (2019) Gestational Diabetes and T-cell (Th1/Th2/Th17/Treg) Immune Profile. *In Vivo* 33: 31–40.

Skjærbæk C, Frystyk J, Ørskov H & Flyvbjerg A (2004) Free IGF-I, IGFBP-1, and the binary complex of IGFBP-1 and IGF-I are increased during human pregnancy. *Horm Res* 62: 215–220.

Smirnakis K V., Plati A, Wolf M, Thadhani R & Ecker JL (2007) Predicting gestational diabetes: choosing the optimal early serum marker. *Am J Obstet Gynecol* 196: 410.e1-410.e7.

Smith SB & Ravel J (2017) The vaginal microbiota, host defence and reproductive physiology. *J Physiol* 595: 451–463.

Soininen P, Kangas AJ, Würtz P, Suna T & Ala-Korpela M (2015) Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* 8: 192–206.

Sparks JR, Ghildayal N, Hivert MF & Redman LM (2022) Lifestyle interventions in pregnancy targeting GDM prevention: looking ahead to precision medicine. *Diabetologia* 65: 1814–1824.

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

Stewart FM, Freeman DJ, Ramsay JE, Greer IA, Caslake M & Ferrell WR (2007) Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. *J Clin Endocrinol Metab* 92: 969–975.

Syngelaki A, Visser GHA, Krithinakis K, Wright A & Nicolaides KH (2016) First trimester screening for gestational diabetes mellitus by maternal factors and markers of inflammation. *Metabolism* 65: 131–137.

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

The Finnish Medical Society Duodecim (2021) Obesity in children, adolescents and adults. Current Care Guidelines. Working group appointed by the Finnish Medical Society Duodecim, the Finnish Association for the Study of Obesity, the Finnish Paediatric Society. Helsinki: The Finnish Medical Society Duodecim, 2021 (referred October 4, 2022). Available online at: www.kaypahoito.fi. *Obesity in children, adolescents and adults: The Finnish Medical Society Duodecim*.

The Finnish Medical Society Duodecim (2022) Gestational Diabetes Mellitus. Current Care Guidelines. Working group set by the Finnish Medical Society Duodecim, the Medical Advisory Board of the Finnish Diabetes Association and the Finnish Gynecological Association. Helsinki: The Finnish Medical Society Duodecim, 2022 (referred January 11, 2023). Available online at: www.kaypahoito.fi). *Gestational Diabetes Mellitus: The Finnish Medical Society Duodecim*.

Tomedi LE, Chang CCH, Newby PK, Evans RW, Luther JF, Wisner KL & Bodnar LM (2013) Pre-pregnancy obesity and maternal nutritional biomarker status during pregnancy: a factor analysis. *Public Health Nutr* 16: 1414–1418.

Tremaroli V & Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* 489: 242–249.

Tryggvadottir EA, Gunnarsdottir I, Birgisdottir BE, Hrolfsdottir L, Landberg R, Hreidarsdottir IT, Hardardottir H & Halldorsson TI (2021) Early pregnancy plasma fatty acid profiles of women later diagnosed with gestational diabetes. *BMJ Open Diabetes Res Care* 9.

Tuomainen AM, Nyyssönen K, Laukkanen JA, Tervahartiala T, Tuomainen TP, Salonen JT, Sorsa T & Pussinen PJ (2007) Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. *Arterioscler Thromb Vasc Biol* 27: 2722–2728.

Valentine CJ, Khan AQ, Brown AR, Sands SA, Defranco EA, Gajewski BJ, Carlson SE, Reber KM & Rogers LK (2021) Higher-Dose DHA Supplementation Modulates Immune Responses in Pregnancy and Is Associated with Decreased Preterm Birth. *Nutrients* 13.

Vanweert F, Schrauwen P & Phielix E (2022) Role of branched-chain amino acid metabolism in the pathogenesis of obesity and type 2 diabetes-related metabolic disturbances BCAA metabolism in type 2 diabetes. *Nutrition & Diabetes 2022 12:1* 12: 1–13.

Vasundhara D, Raju VN, Hemalatha R, Nagpal R & Kumar M (2021) Vaginal & gut microbiota diversity in pregnant women with bacterial vaginosis & effect of oral probiotics: An exploratory study. *Indian J Med Res* 153: 492–502.

Vetrani C, di Nisio A, Paschou SA, Barrea L, Muscogiuri G, Graziadio C, Savastano S & Colao A (2022) From Gut Microbiota through Low-Grade Inflammation to Obesity: Key Players and Potential Targets. *Nutrients 2022, Vol 14, Page 2103* 14: 2103.

Vidakovic AJ, Jaddoe VWV, Gishti O, Felix JF, Williams MA, Hofman A, Demmelmair H, Koletzko B, Tiemeier H & Gaillard R (2015) Body mass index, gestational weight gain and fatty acid concentrations during pregnancy: the Generation R Study. *Eur J Epidemiol* 30: 1175–1185.

Vilmi-Kerälä T, Lauhio A, Tervahartiala T, Palomäki O, Uotila J, Sorsa T & Palomäki A (2017) Subclinical inflammation associated with prolonged TIMP-1 upregulation and arterial stiffness after gestational diabetes mellitus: a hospital-based cohort study. *Cardiovasc Diabetol* 16.

Virtanen S, Rantsi T, Virtanen A, Kervinen K, Nieminen P, Kalliala I & Salonen A (2019) Vaginal Microbiota Composition Correlates Between Pap Smear Microscopy and Next Generation Sequencing and Associates to Socioeconomic Status. *Sci Rep* 9.

Vitali B, Cruciani F, Baldassarre ME, Capursi T, Spisni E, Valerii MC, Candela M, Turroni S & Brigidi P (2012) Dietary supplementation with probiotics during late pregnancy: outcome on vaginal microbiota and cytokine secretion. *BMC Microbiol* 12.

Wahab RJ, Jaddoe VWV, Voerman E, Ruijter GJG, Felix JF, Marchioro L, Uhl O, Shokry E, Koletzko B & Gaillard R (2022) Maternal Body Mass Index, Early-Pregnancy Metabolite Profile, and Birthweight. *J Clin Endocrinol Metab* 107: E315–E327.

Wallace TM, Levy JC & Matthews DR (2004) Use and abuse of HOMA modeling. *Diabetes Care* 27: 1487–1495.

Wang J, Zheng J, Shi W, Du N, Xu X, Zhang Y, Ji P, Zhang F, Jia Z, Wang Y, *et al* (2018) Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut* 67: 1614–1625.

Wang Q, Würtz P, Auro K, Mäkinen VP, Kangas AJ, Soininen P, Tiainen M, Tynkkynen T, Jokelainen J, Santalahti K, *et al* (2016) Metabolic profiling of pregnancy: cross-sectional and longitudinal evidence. *BMC Med* 14.

Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, *et al* (2011) Metabolite profiles and the risk of developing diabetes. *Nat Med* 17: 448–453.

Wang X & Chan CB (2015) n-3 polyunsaturated fatty acids and insulin secretion. *J Endocrinol* 224: R97–R106.

Wang XR, Wang WJ, Yu X, Hua X, Ouyang F & Luo ZC (2019) Insulin-Like Growth Factor Axis Biomarkers and Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne)* 10.

Warstedt K, Furuhjelm C, Duchen K, Fälth-Magnusson K & Facerás M (2009) The effects of omega-3 fatty acid supplementation in pregnancy on maternal eicosanoid, cytokine, and chemokine secretion. *Pediatr Res* 66: 212–217.

Wilson NA, Mantzioris E, Middleton PT & Muhlhausler BS (2019) Gestational age and maternal status of DHA and other polyunsaturated fatty acids in pregnancy: A systematic review. *Prostaglandins Leukot Essent Fatty Acids* 144: 16–31.

Witteveen AB, Henrichs J, Bellers M, van Oenen E, Verhoeven CJ & Vrijkotte TGM (2022) Mediating role of C-reactive protein in associations between pre-pregnancy BMI and adverse maternal and neonatal outcomes: the ABCD-study cohort. *J Matern Fetal Neonatal Med* 35: 2867–2875.

Wolf M, Sandler L, Hsu K, Vossen-Smirnakis K, Ecker JL & Thadhani R (2003) First-trimester C-reactive protein and subsequent gestational diabetes. *Diabetes Care* 26: 819–824.

Wu D, Dawson NAJ & Levings MK (2016) Obesity-Associated Adipose Tissue Inflammation and Transplantation. *Am J Transplant* 16: 743–750.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, *et al* (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821–1830.

Yamamoto JM, Kellett JE, Balsells M, García-Patterson A, Hadar E, Solà I, Gich I, van der Beek EM, Castañeda-Gutiérrez E, Heinonen S, *et al* (2018) Gestational Diabetes Mellitus and Diet: A Systematic Review and Meta-analysis of Randomized Controlled Trials Examining the Impact of Modified Dietary Interventions on Maternal Glucose Control and Neonatal Birth Weight. *Diabetes Care* 41: 1346–1361.

Yang S, Reid G, Challis JRG, Gloor GB, Asztalos E, Money D, Seney S & Bocking AD (2020) Effect of Oral Probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the Vaginal Microbiota, Cytokines and Chemokines in Pregnant Women. *Nutrients* 12.

Young LR, Kurzer MS, Thomas W, Redmon JB & Raatz SK (2013) Low-fat diet with omega-3 fatty acids increases plasma insulin-like growth factor concentration in healthy postmenopausal women. *Nutr Res* 33: 565–571.

Zacarías MF, Collado MC, Gómez-Gallego C, Flinck H, Aittoniemi J, Isolauri E & Salminen S (2018) Pregestational overweight and obesity are associated with differences in gut microbiota composition and systemic inflammation in the third trimester. *PLoS One* 13.

Zambon M, Mandò C, Lissoni A, Anelli GM, Novielli C, Cardellicchio M, Leone R, Monari MN, Massari M, Cetin I, *et al* (2018) Inflammatory and Oxidative Responses in Pregnancies With Obesity and Periodontal Disease. *Reprod Sci* 25: 1474–1484.

Zatterale F, Longo M, Naderi J, Raciti GA, Desiderio A, Miele C & Beguinot F (2020) Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Front Physiol* 10.

Zhang X, Liao Q, Wang F & Li D (2018) Association of gestational diabetes mellitus and abnormal vaginal flora with adverse pregnancy outcomes. *Medicine (United States)* 97.

Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, *et al* (2020) A taxonomic note on the genus Lactobacillus : Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae..

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