Heilbrigðisvísindasvið

Assessment of nutritional biomarkers in pregnant women and associations with gestational diabetes

Ellen Alma Tryggvadóttir

Thesis for the degree of Philosophiae Doctor

June 2022

HÁSKÓLI ÍSLANDS

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Ellen Alma Tryggvadóttir

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"But then science is nothing but a series of questions that lead to more questions."
Terry Pratchett

Ágrip

Bakgrunnur: Meðgöngusykursýki er einn algengasti sjúkdómur sem greinist á meðgöngu og getur hún haft slæmar afleiðingar fyrir bæði móðir og barn. Ýmsir þættir geta aukið hættuna á því að greinast með meðgöngusykursýki, bæði umhverfis og genatengdir. Hækkandi aldur, ofþyngd og offita, hreyfingarleysi, fyrri saga um sykursýki á meðgöngu eða fæðingu stórbura auk fjölskyldusögu um sykursýki eru meðal þekktra áhrifaþátta. Nýlegar rannsóknir hafa einnig sýnt að gæði mataræðis geti tengst hættu á meðgöngusykursýki, jafnvel óháð líkamsþyngdarstuðli (LÞS) og öðrum þekktum áhrifaþáttum. Í fyrri rannsóknum hefur fyrst og fremst verið stuðst við hefðbundnar aðferðir til að kanna mataræði, en þær ekki alltaf sannreyndar með mælingum á þekktum lífmerkjum í blóði. D-vítamínhagur barnshafandi kvenna á Íslandi hefur aldrei áður verið kannaður.

Markmið: Markmiðið doktorsverkefnisins í heild var að a) rannsaka fæðuval og bætiefnanotkun kvenna snemma á meðgöngu, með notkun á fæðutíðnispurningalista auk mælinga á lífmerkjum (greinar I-IV) og b) kanna tengsl við meðgöngusykursýki (greinar I-III). Auk þess var markmiðið að bera saman niðurstöður fæðutíðnispurningalista við niðurstöður lífefnamælinga (greinar I-IV).

Nánari markmið voru að: Kanna neyslu kvennanna á D-vítamín gjöfum, mæla gildi 25-hýdroxývítamíns (25OHD) í sermi kvennanna og rannsaka tengsl við meðgöngusykursýki. Kanna tengsl á milli neyslu heilkorna snemma á meðgöngu við hættu á meðgöngusykursýki, með því að mæla lífmerki heilkorna ásamt fæðutíðnispurningalista. Áætla fæðuval ásamt fitusýrusamsetningu í blóðvökva kvennanna snemma á meðgöngu og kanna tengsl við meðgöngusykursýkigreiningar síðar á meðgöngu. Auk þess var markmiðið að bera saman niðurstöður fæðutíðnispurningalista við niðurstöður mælinga á næringartengdim lífmerkjum.

Aðferðir: Þátttakendur voru barnshafandi konur sem mættu í fósturskimun á Landspítala við 11.-14. viku meðgöngu á tímabilinu október 2017 til mars 2018. Konurnar svöruðu fæðutíðnispurningalista auk almennra spurninga um aldur, hæð, þyngd og bakgrunn við þátttöku auk þess að veita þvag- og blóðsýni fyrir rannsókna. Blóðsýni voru notuð til mælinga á styrk 25OHD í sermi, lífmerki heilkorna og fitusýrum í blóðvökva. Upplýsingar um þyngd á meðgöngu og greiningu á meðgöngusykursýki var aflað úr sjúkraskrám.

Niðurstöður: Í heild greindust 127 konur (14,9%) með meðgöngusykursýki.

Einungis 14,9% þátttakenda í rannsókninni fylgdi ráðleggingum um neyslu tveggja skammta af heilkorni á dag (Grein II). Þátttakendur borðuðu magran fisk um það bil einu sinni í viku og feitan fisk að jafnaði mánaðarlega (Grein IV). Um þriðjungur þátttakenda mældust með ófullnægjandi styrk 25OHD <50 nmol/L í blóði, þar af voru 5% með D-vítamínskort (25OHD <30 nmól/L. Engin skýr tengsl sáust á milli styrk 25OHD í upphafi meðgöngu og hættu á því að greinast síðar með meðgöngusykursýki (Grein I). Heildargæði mataræðis snemma á meðgöngu virtust vera síðri hjá þeim konum sem greindust síðar með meðgöngusykursýki (Grein III). Hlutfallsleg áhætta (relative risk) þess að greinast með meðgöngusykursýki var 50% (95% CI: 0,27, 0,90) lægri hjá þeim konum sem tilheyrðu efsta fjórðungi í styrk alkylresorcinol (AR) í blóðvökva borið saman við þær sem tilheyrðu fjórðungnum með lægstu gildi AR eftir að leiðrétt hafði verið fyrir aldri, LÞS ≥25 kg/m^2, fjölda barna, menntun, reykingum á meðgöngu og fjölskyldusögu um meðgöngusykursýki og marktækt skammtaháð samband sást milli styrk AR og hættu á meðgöngusykursýki (Grein II).

Heildarstyrkur fitusýra var marktækt hærri hjá þeim konum sem síðar greindust með meðgöngusykursýki borið saman við þær sem greindust ekki. Sama átti við um heildarstyrk mismunandi flokka fitusýra, einómettaðra og fjölómettaðra, að undanskildum löngum ómega 3 fitusýrum (EPA og DHA). Leiðréttur meðalmunur á styrk heildarfitusýra í blóðvökva þátttakenda sem síðar greindust með meðgöngusykursýki og þeirra sem greindust ekki var 133 µg/ml (95% CI: 33 - 233) (Grein III).

Jákvæð fylgni sást á milli tíðni neyslu á fiski ásamt bætiefnum sem innihalda ómega 3 fitusýrur við styrk ómega 3 fitusýra í blóðvökva (r=0,34 P <0.001). Þær konur sem tóku inn bætiefni með ómega 3 daglega höfðu hærri gildi af EPA og DHA borið saman við þær sem notuðu aldrei slík bætiefni (miðgildi: 108 sbr. 91 µg/ml og 103 sbr. 90 µg/ml). Það var einnig jákvæð fylgni á inntöku D-vítamíns við styrk S-25OHD (r=0.31 P <0.001). Niðurstöður gáfu til kynna að hærri tíðni heilkornaneyslu endurspeglaðist í hærri styrk AR í blóðvökva (Greinar I-IV).

Samantekt: Niðurstöðurnar sýna að það eru fjölmörg tækifæri til að bæta fæðuval og D-vítamínstöðu barnshafandi kvenna á Íslandi. Heildargæði mataræðis kvenna virtust meiri meðal kvenna sem greindust ekki með meðgöngusykursýki. En hlutverk D-vítamíns var ekki eins skýrt í þessu samhengi. Tengsl heilkornaneyslu við áhættu á meðgöngusykursýki voru sannreynd með mælingum á styrk AR í blóðvökva, en það hafði ekki áður verið gert í rannsókn meðal barnshafandi kvenna. Niðustöður gefa einnig til kynna að unnt er að nota fæðutíðnispurningalistann til að áætla neyslu ákveðinna fæðuflokka og bætiefna.

Lykilorð:

Meðganga, Næring, Meðgöngusykursýki, Lífmerki, Fæðutíðnispurningalisti

Abstract

Background: Gestational diabetes mellitus (GDM) is one of the most common complications diagnosed in pregnancy and is associated with several adverse outcomes for mothers and their offspring. Several factors, both genetic and environmental, are thought to affect a person's risk of being diagnosed with GDM. Increased age, overweight and obesity, sedentary lifestyle, a previous history of GDM or giving birth to a child with macrosomia and family history of diabetes are among known risk factors. Recent studies have indicated that diet can also affect GDM risk, regardless of a women's body mass index (BMI) and other known risk factors.

Aim: This PhD thesis aimed to (a) examine dietary intake and nutritional status in early pregnancy by means of a subjective diet screening questionnaire as well as objective biomarkers (papers I-IV) and (b) explore associations with gestational diabetes (papers I-III). Further aim was to compare results from the diet screening questionnaire to biomarker results (papers I-IV).

Specifically, the aims were to: Determine the women´s intake of vitamin D, measure serum 25-hydroxyvitamin (25OHD) and investigate associations to risk of GDM diagnosis. Investigate associations between early pregnancy whole grain intake, with the use of a whole grain biomarker and diet screening questionnaire and explore associations to GDM diagnoses later in pregnancy. Determine dietary intake as well as plasma fatty acid profiles in early pregnancy and assess associations to later GDM diagnoses. Compare results of the diet screening questionnaire to those derived from biomarkers of consumption.

Methods: Subjects were women attending ultrasound screening at the Ultrasound Department at Landspitali - National University Hospital in their 11th to 14th week of pregnancy, from October 20178 to March 2018. During their visits, the women answered a diet screening questionnaire as well as questions regarding age, height, weight and background in addition to providing blood and spot urine samples. The samples were analysed for concentrations of plasma fatty acids, plasma alkylresorcinols (a biomarker for whole grain intake), and serum 25OHD. Data on weight and gestational diabetes diagnoses were later gathered from their medical records.

Results: GDM was diagnosed in 127 women (14.9%). Only 14.9% of the women adhered to the recommendation of consuming two portions of whole grains daily (Paper II). The women's median intake of lean fish was approximately once weekly, and their intake of fatty fish was approximately once monthly (Paper III). Approximately one-third of the cohort had S-25OHD concentrations below adequate levels (< 50 nmol/L) during the first trimester of pregnancy, thereof 5% had deficient concentrations (25OHD <30 nmol/L). However, no clear

association was observed between S-25OHD and GDM (Paper I). Overall diet quality in early pregnancy appeared to be lower among the women later diagnosed with GDM (Paper III). The relative risk (RR) of being diagnosed with GDM was 50% (95% CI: 0.27, 0.90) lower among individuals in the highest quartile compared to those in the lowest quartile of plasma ARs (P-trend = 0.01) after adjusting for age, pre-pregnancy BMI≥25 kg/m^2, parity, education, smoking during pregnancy, and family history of diabetes. There was a significant dose-response relationship between AR levels and GDM risk (Paper II).

The total concentration of fatty acids (FAs) was significantly higher in women diagnosed with GDM when compared to women without this diagnosis, as were the concentrations of all types of FA—except for long-chain n-3 fatty acids EPA+DHA. The mean adjusted difference for total FA between the women with and without GDM was 133 µg/mL (95% CI 33 to 233) (Paper III).

There was a positive correlation between the women's intake of all fish and total omega-3 supplements and the concentrations of plasma omega-3 FA (r=0.34 P <0.001). The women with a daily intake of cod liver oil or omega-3 oil supplements displayed higher concentrations of EPA and DHA compared to those who never used these supplements (median: 108 vs. 91 and 103 vs 90 µg/ml, respectively). A positive correlation was seen between reported intake of vitamin D supplements and S-25OHD concentrations (r=0.31 P <0.001), and higher reported frequency of whole grain intake was associated with a significant increase in plasma AR concentrations (Papers I-IV).

Conclusion: These results indicate several opportunities to improve the diet and vitamin D status of pregnant women in Iceland. The women who were not diagnosed with GDM appear to have a slightly better adherence to dietary recommendations overall. The role of vitamin D was not as clear in this context. The associations of whole grain intake to GDM risk were established by measuring plasma AR concentration, something that has not been done previously in a pregnancy cohort. Our findings also indicate that the diet screening questionnaire used in our study, is a tool that can be used to estimate food consumption of selected food groups and intake of specific nutrients during pregnancy.

Keywords:

Pregnancy, Nutrition, GDM, Biomarkers, FFQ.

Acknowledgements

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Above all, I want to thank my amazing supervisor, Ingibjörg Gunnarsdóttir, for allowing me to play a part in the work of PREWICE and the development of the dietary screening questionnaire. I feel blessed to have had the opportunity to receive your guidance and to learn so much from you ever since my master's project. You continue to inspire me. Thank you for everything. I also want to thank my supervisor Þórhallur Ingi Halldórsson for all the valuable assistance and insight he has provided during this project. You know your stuff and have taught me a lot along the way. Also, a big thank you to the wonderful Bryndís Eva Birgisdóttir for always being there for me and providing her unique BEB talks as needed. She also taught me the valuable lesson of: "when in doubt, go French". I would also like to thank the other members of my PhD committee, Helle Margrete Meltzer and Hildur Harðardóttir, for all of their input and helpful suggestions.

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A special shoutout to the women I sat next to the longest. In the beginning, it was Tinna Óðinsdóttir who brightened my days in the first half of my PhD work. Thank you for everything. In the later stages of my studies, the pandemic inadvertently brought me and Sigrún Sunna Skúladóttir together, which turned out to be a great blessing.

I am also eternally grateful to my amazing best friends in Gellz: Anna, Dísella, Dóra, Íris, Lára and Olga. I am so blessed to have you all in my life; you make everything better! The same goes for my friend, the champagne-loving Dr Áróra Rós Ingadóttir, to whom I can talk for hours. And my darling Bengta, you were

always so proud of me at every single graduation for making my dreams come true. I wish you could have been here for this one. I love you and miss you so much.

Finally, a huge thank you to my family. To my wonderful husband, love of my life, and best friend, Hörður Skúli Daníelsson: Thank you for everything. You are my other half, and I am so grateful for your love, endless support, sense of humour and our life together. I would also like to thank my amazing daughters, Helena and Rebekka, for their love and for making my life complete. I hope you will both strive to make your dreams come true <3.

I also want to thank my parents for all their love and constant support throughout my life. Also, a big thank you to my brothers for always being there for me. I especially appreciate everything you have done for me over the past few years, Ómar. And of course, to my almost sister Maddý: Thank you for being in my life.

Reykjavík, 2022

Ellen Alma Tryggvadóttir

Contents

List of abbreviations

ALA: Alpha-linolenic acid

AR: Alkylresorcinol

BMI: Body mass index

DHA: Docosahexaenoic acid

DHPPA: Dihydroxyphenyl Propanoic Acid

EPA: Eicosapentaenoic acid

FA: Fatty acid

FBDG: Food-based dietary guidelines

FFA: Free fatty acid

FFQ: Food frequency questionnaire

GDM: Gestational diabetes mellitus

IADPSG: The International Association of the Diabetes and Pregnancy Study Groups

ICEGUT: The Icelandic Gut Microbiota and Metabolome Project

IOM: Institute of Medicine

IR: Insulin resistance

MUFA: Monounsaturated fatty acids

OGGT: Oral glucose tolerance test

PREWICE: The Pregnant Women in Iceland Study

PUFA: Polyunsaturated fatty acids

SFA: Saturated fatty acids

T2D: Type II diabetes

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I–IV):

I. Magnúsdóttir KS, Tryggvadóttir EA, Magnúsdóttir OK, Hrólfsdóttir L, Halldórsson TI, Birgisdóttir BE, Hreiðarsdóttir IT, Harðardóttir H, Gunnarsdóttir I. Vitamin D status and association with gestational diabetes mellitus in a pregnant cohort in Iceland. Food & Nutrition Research. 2021 65. https://doi.org/10.29219/fnr.v65.5574.

II. Tryggvadóttir EA, Halldórsson TI, Landberg R, Hrólfsdóttir L, Birgisdóttir BE, Magnúsdóttir OK, Hreiðarsdoóttir IT, Harðardóttir H, Gunnarsdóttir I. Higher alkylresorcinol concentrations, a consequence of whole-grain intake, are inversely associated with gestational diabetes mellitus in Iceland. Journal of Nutrition 2021 May 11;151(5):1159–1166. doi: 10.1093/jn/nxaa449.

III. Tryggvadóttir EA, Gunnarsdóttir I, Birgisdóttir BE, Hrólfsdóttir L, Landberg R, Hreiðarsdóttir IT, Harðardóttir H, Halldórsson TI. Early pregnancy plasma fatty acid profiles of women later diagnosed with gestational diabetes. BMJ Open Diabetes Research & Care. 2021 Aug;9(1):e002326. doi: 10.1136/bmjdrc-2021-002326.

IV. Tryggvadóttir EA, Halldórsson TI, Birgisdóttir BE, Hrólfsdóttir L, Landberg R, Hreiðarsdóttir IT, Harðardóttir H, Gunnarsdóttir I. Correlation between intake of fish or supplements containing omega-3 fatty acids and early pregnancy plasma concentrations. The Icelandic Medical Journal 2022/108. doi 10.17992/lbl.2022.05.691.

Declaration of contribution

The data presented in this thesis originates from the prospective study PREgnant Women of ICEland II (PREWICE II; see Figure 1).

Under the supervision of the principal investigator, Professor Ingibjörg Gunnarsdóttir, the doctoral candidate, Ellen Alma Tryggvadóttir, took part in recruiting participants for the study at Landspitali National University Hospital. During recruitment, she assisted participants in answering the diet screening questionnaire, acquired spot urine samples, and requested blood samples for the study.

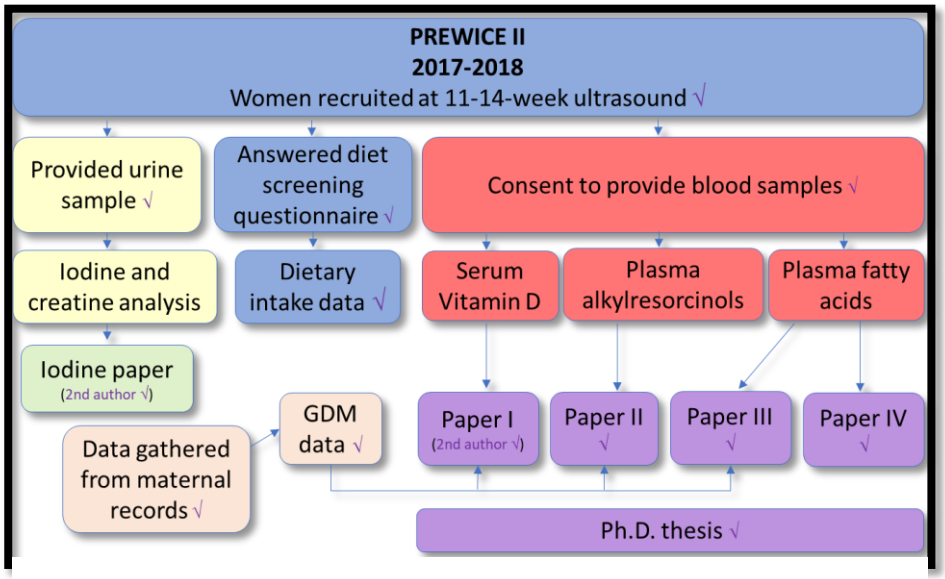

Figure 1 Flowchart of the PREWICE II study and the PhD student's contributions (√).

Ellen also participated in collecting data from maternal records and guided co-workers engaging in data collection. Ellen was responsible for merging data from the diet screening questionnaire, maternal records and all biochemical analyses. As such, Ellen has participated in and guided other students' work with the data. Ellen performed statistical analyses in papers II-IV, was responsible for the data interpretation, and prepared and drafted the data for presentations and publications. Ellen also contributed to the supervision of master's student Kristín Sigrún Magnúsdóttir (the first author of Paper I) and planned the research work

for Papers II–IV, with guidance and feedback from her supervisors and doctoral committee. She wrote the first draft of papers II–IV and had primary responsibility for the final content. During her studies, Ellen submitted abstracts and prepared and presented the results of research projects at several international and domestic conferences.

During her PhD studies, Ellen gained significant professional experience in academic research and university teaching. In recent years, Ellen has participated in teaching undergraduate and graduate students in Nutrition at the Faculty of Food Science and Nutrition, undergraduate and midwife students at the Faculty of Nursing, and supervised interdisciplinary cooperation in health sciences at the University of Iceland. Her main tasks included lecturing, leading workshops and grading assignments on maternal nutrition, the methods used in nutrition, public health nutrition, vitamins and minerals, literature searches and the management of references, special diets, food composition and the assessment of dietary intake, and discussions related to professional image and work ethic. She also organised a workshop for post-graduate students in nutrition.

During her PhD studies, she has worked as a project manager for the National Dietary Survey in Iceland (2019–2021) and assisted staff and students working on the research project ICEGUT (The Icelandic Gut Microbiota and Metabolome Project: Linking Diet with Infant Gut Microbiota Development). The ICEGUT study is a follow-up study of subjects participating in the PREWICE II study. Ellen also took an active role in promoting nutrition and healthy diets within projects by the Department of Communication at the University of Iceland, including the University for the Young and the University simulation.

1 Introduction

During pregnancy, all the nutrients necessary for foetal growth are provided by the mother. Therefore, her diet must be both sufficient and varied to be able to fuel a healthy foetal growth process. Not only is the maternal diet an important factor for a healthy pregnancy, but it is also a period that may impact the future health of the offspring [1]. When the foetus is either nutritionally deprived or exposed to nutritional excess, it may lead to the reprogramming of metabolic pathways and adaptation to its environment. This response may be irreversible and result in the increased risk of offspring metabolic disorders, obesity, and type II diabetes later in life [2-6].

Gestational diabetes mellitus (GDM) is one of the most common complications diagnosed in pregnancy and has been associated with adverse outcomes for both mother and child [7-10]. The rate of GDM diagnoses is said to be increasing, which may be related to a higher obesity rate and maternal age [11, 12]. Common risk factors for GDM include a family history of diabetes, higher maternal age, previous GDM diagnoses, high pre-pregnancy body mass index (BMI; kg/m^2), suboptimal nutrition and low levels of physical activity [13-17]. Several studies regarding diet during pregnancy have associated GDM with dietary patterns, with healthier patterns being associated with a decreased risk of GDM and unhealthier patterns being associated with increased risk [7-9].

Previous results from Icelandic studies have suggested that dietary quality during pregnancy could be improved as very few women were adhering to dietary recommendations [18, 19]. Most pregnant women in Iceland were not eating five portions of fruits and vegetables per day (87%) or eating two portions of whole grains or dairy daily (91 and 77%), respectively, which is recommended in the Icelandic dietary guidelines [18, 19]. Many pregnant women also appear to have a suboptimal intake of nutrients such as vitamin D and omega-3 fatty acids (FAs), which are mostly found in fish [11, 12]. Fruits, vegetables, whole grains and fish are nutrient-dense foods that have frequently been a part of healthy dietary patterns associated with a lower risk of GDM [13-17]. The rate of GDM diagnoses in Iceland is considered high when compared with other Nordic countries [20-27].

This knowledge, as well as the results from recent Icelandic studies suggesting that a healthy dietary pattern could minimise the risk of GDM regardless of the women's BMI [28] and that a short diet screening questionnaire could identify

those in need of dietary counselling [11], present us with an opportunity for prevention. In modern clinical practice, the need for nutritional intervention is often based on pre-pregnancy BMI. However, previous dietary studies in Iceland reported that women who had greater diet quality had a lower risk of excessive weight gain and gestational diabetes, suggesting that screening for diet quality could be a better method [11, 28]. Identifying those women who would benefit most from dietary counselling during pregnancy requires information on the women´s current diet.

However, obtaining reliable information on dietary intake can be a challenge [29]. Notably, all previous pregnancy studies performed in Iceland regarding diet have solely been based on dietary data obtained by subjective means, such as food frequency questionnaires (FFQs) or food records [11, 12, 28, 30-37].

It is widely known that using subjective methods to obtain information has its limitations [38]. Moreover, using objective methods such as biomarkers also has certain limitations. However, these limitations are not the same as in the case of subjective methods [39, 40]. Therefore, some studies have used biomarker analyses alongside subjective methods of obtaining information on dietary intake as a means of strengthening their results [41, 42].

This was the incentive for the 2017–2018 PREgnant Women in ICEland II (PREWICE II) study. In this study, pregnant women were asked to answer a diet screening questionnaire during early pregnancy (subjective) in addition to providing urine - and blood samples for biochemical analysis (objective) as well as permission to later obtain information on pregnancy and birth outcomes. This thesis is based on the part of the PREWICE II study that pertains to data from the diet screening questionnaire, blood sample analysis and information on GDM diagnoses during pregnancy.

2 Background

2.1 Gestational diabetes mellitus

GDM is one of the most common complications diagnosed in pregnancy and is associated with several adverse outcomes for mothers and their offspring [7-10]. GDM is a state of hyperglycaemia due to insulin resistance that is detected during pregnancy [43]. Although insulin resistance is a normal part of pregnancy and plays a role in supplying adequate nutrients to the foetus, it can progress to GDM in many cases [8]. This usually occurs between weeks 20 to 24 of gestation, when the growing placenta produces higher levels of hormones and insulin resistance increases. This can result in an increased flow of glucose across the placenta followed by a spike in insulin production in the foetus´s beta cells. The resulting hyperinsulinaemia in the foetus may lead to the excessive growth of fat and protein stores. Therefore, the children of GDM mothers are more likely to be born large for gestational age (over 90th percentile for gestational age) or macrosomic (>4000g) [10, 44, 45]. Having GDM increases the risk of further complications during birth, such as shoulder dystocia or having a caesarean section [10, 44, 46, 47]. There is also a further risk of the child having cardiometabolic disorders in later life [7] and the mother being diagnosed with type II diabetes later [43, 46, 47].

2.1.1Prevalence of GDM

The rate of GDM diagnoses can vary depending on the population and diagnostic criteria [48, 49]. Its prevalence is said to be increasing, possibly following a greater rate of obesity and older mothers [48, 50]. Recent rates of GDM reported in Iceland have been in the range of 11.8–19% [20, 21]. This is fairly high when compared to recent rates in the other Nordic countries, such as Sweden (1.4–2.6%) [22, 23], Denmark (2.3–2.9%) [24, 25] and Norway (5.2–7.4%) [24, 26]. Yet in Finland, the rates seem to be higher (10.5–11.3%) [26, 27] and closer to the rates in Iceland. However, this difference is largely due to a difference in diagnostic criteria between the countries. A recent meta-analysis estimated the worldwide GDM prevalence to be 4.4% when not discerning between populations, recruitment or diagnostic criteria, and 10.6% when only using the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria (utilised in Iceland) [51].

2.1.2 Known risk factors for GDM

Several genetic and environmental factors are thought to affect a person's risk of being diagnosed with GDM. The most common risk factors are family history of diabetes, higher maternal age, previous GDM diagnoses or the previous birth of a macrosomic child, ethnicity other than Caucasian or a high pre-pregnancy BMI (kg/m^2) [52]. These are mostly non-modifiable risk factors. However, some risk factors are modifiable, such as smoking, suboptimal nutrition and low levels of physical activity [8, 53]. Several studies regarding diet during pregnancy have presented associations between GDM and dietary patterns, where healthier patterns have been associated with a decreased risk and patterns deemed unhealthier have been associated with an increased risk [13, 28]. Following a healthy dietary pattern has also been associated with a decreased risk of developing type II diabetes for women with a previous GDM diagnosis [54]. Epidemiological studies have repeatedly verified the importance of a healthy and nutritious diet during pregnancy [2, 55-60], and nutrition interventions have been successful in decreasing the risk of excessive weight gain in pregnancy [61]. Evidence from intervention studies for pregnancy complications such as gestational diabetes mellitus is considered to be of low quality [62]

2.1.3 Diagnosis of GDM

The GDM diagnoses in Iceland are based on the IADPSG from 2010 [63]. During the first routine maternal care visit in Iceland (before 12 weeks of pregnancy), women are considered at risk of developing GDM due to one or more of the following factors: age (≥ 40 years), BMI (≥ 30 kg/m^2), ethnicity (non-Caucasian) and previous history of diabetes or macrosomia (≥ 4500 g in Iceland). Such women are invited to provide a fasting blood sample to measure fasting blood glucose. If results are ≥ 5.1 mmol/L, the women are diagnosed with GDM—or, in some cases, type 2 diabetes—if fasting blood sugar is > 7 mmol/L or HbA1c is ≥ 48 mmol/mol. The rate of women diagnosed with GDM during this early selective screening is unknown in Iceland. However, a study in France that also utilised selective screening and the IADPSG criteria reported an early hyperglycaemia rate of 2.3% in a cohort of almost 800000 women, corresponding to 26.9% of the women diagnosed with hyperglycaemia overall [64]. Later in pregnancy, usually at weeks 24–28, the women considered at risk of GDM that were negative in previous screenings undergo a 2-hour oral glucose tolerance test (OGTT). During the OGTT, blood sugar levels are measured during fasting, after 1 hour and after a second hour [65]. If a woman is diagnosed with GDM during maternal care, she is provided with instructions and the proper tools to monitor her own blood glucose levels. She is also

provided with some dietary guidelines to assist in maintaining fasting blood sugar between 3.5–5.9 mmol/L and below 7,8 mmol/L 60 minutes after meals [66]. If the woman is unable to maintain blood sugar levels within those guidelines in 85% of measurements, she is referred to more intensive observation at a hospital maternity department.

2.2 Assessment of diet and nutrient intake

2.2.1 Subjective methods

Obtaining reliable information on diet can be a challenge. The most common methods used to estimate dietary intake are subjective methods such as food records, FFQs or dietary recall [67]. For example, FFQs represent an easy and cost-effective method of obtaining dietary information over a longer period of time [67]. However, using subjective methods in dietary studies is known to have limitations [29, 68] since FFQs are usually meant to gather information regarding diet over a long period of time and answering them relies on memory and correct estimates of average intake. Additionally, people may underreport food intake thought to be "undesirable", and overestimate foods that are considered healthy [69]. Additionally, estimating the correct portion size and type of dietary intake can be a challenge due to variance in food composition data and self-reporting errors [70, 71]. Sometimes weighed food records, which are often considered more reliable [72], are used to validate the accuracy of FFQs despite also being a subjective method associated with certain limitations [73]. Using weighed food records to obtain diet information is time consuming and quite a burden for people, making them unsuitable for use in clinical settings [72].

2.2.2 Objective methods

The means of objectively obtaining information on diet are mostly based on biochemical analysis. However, while using biochemical analysis to assess the status of specific nutrients is helpful in a clinical setting, it does not necessarily provide any information on dietary intake. Often, the same nutrient can be provided by different food types and supplements, or can even be synthesised internally [74]. Recently, there has been an increase in the number of studies where biomarkers have been identified and associated with different food types [39]. Biomarkers can be objectively measured as markers of a biological process or a response to an intervention. Notably, biomarkers can reflect intake over various time periods based on different types of samples. Usually, samples taken from urine, plasma or serum reflect dietary intake over a short period of time

(days, for example) and samples taken from blood cells or tissue can reflect intake over several weeks [68].

In some cases, using biomarkers can provide more detailed information on specific nutrients than subjective methods. For instance, this can occur when food is influenced by external factors such as soil composition, feed types or sun exposure. For instance, differences in the amount of selenium found in soil can be a major factor influencing the selenium content of food grown. Therefore, using dietary databases to calculate intake could provide less reliable results. Another biomarker affected by the environment is serum 25-hydroxyvitamin-D as a biomarker of vitamin D. Since sunlight induces vitamin D synthesis, using 25-hydroxyvitamin-D as a biomarker of dietary vitamin D intake is more reliable in countries with little sun exposure [72].

Although using biochemical analysis can be considered desirable as a more reliable impartial method, it can be a more intrusive and costly method than most subjective methods. Moreover, differences in genetic variation, lifestyle factors, interactions between nutrients, and the type of biological sample can affect biomarker results [40, 72]. These differences can affect several internal factors such as absorption, metabolism, bioavailability, enzymes and hormonal activity [72]. Some studies have used objective biomarker analyses to validate subjective methods [41, 42] or increase the strength of results from questionnaires [75]. When validating the results for diet and biomarkers, a correlation of 0.3–0.4 is considered average, whilst a correlation of 0.4–0.7 or higher is considered good [72, 76]. The use of subjective dietary assessment in addition to objective biomarker methods can be useful when investigating a particular nutrient and its associations with health risks. By combining these methods, it may be possible to minimise the limitations associated with each method [39]. The biomarkers used in this thesis are detailed in the following chapters.

2.2.2.1 Serum 25 hydroxy vitamin D (S-25OHD)

Vitamin D can be synthesised in the skin following sun exposure or absorbed in the diet. However, the form of vitamin D absorbed or synthesised is not the active form that is used in biological functions [72]. The active form of vitamin D is called 1.25 dihydroxy vitamin D ($1.25(OH)_2D$) and is produced by the hydroxylation of vitamin D into 25 hydroxy vitamin D (25OHD), which is then hydroxylated into the active form. However, using the active form of $1.25(OH)_2D$ as a biomarker of vitamin D status is not considered reliable since levels of $1.25(OH)_2D$ are tightly regulated by levels of phosphate and calcium in addition to the parathyroid hormone. Therefore, 25OHD is considered a better alternative

as a biomarker for vitamin D since this form is not as regulated [72]. The results of a systematic review concluded that circulating 25OHD increased following vitamin D supplementation and could thus be used as a biomarker of vitamin D [77, 78]. The reference values for vitamin D in North America, the UK, the European Union and Nordic countries have been based on either plasma or serum 25-OHD as biomarkers for vitamin D status and associations with health [78, 79]. The reliability of using plasma or serum 25-OHD has been debated since different types of assays have been known to provide different results, even when the same samples are being analysed [80]. Furthermore, concentrations of 25-OHD can be affected by factors such as adiposity and exercise, resulting in further variations in concentrations that are difficult to adjust for [72, 80]. Despite this, S-25OHD is still considered the best available vitamin D biomarker [79].

Although the levels of S-25OHD that are considered optimal have been debated, a global consensus has not been reached to date. According to the Institute of Medicine, the European Food Safety Administration, and the Nordic Nutrition Recommendations, 50 nmol/L of 25OHD is considered sufficient, 30–49 nmol/L is insufficient, and <30 nmol/litre is deficient [79, 81, 82]. However, The Endocrine Society views concentrations of 75 nmol/L as sufficient, with concentrations of 50 nmol/L or less representing a deficiency [83].

2.2.2.2 Alkylresorcinols (AR)

Alkylresorcinols (ARs) are recently identified biomarkers that have been demonstrated as a valid method used to measure whole grain intake from wheat and rye [84, 85]. 5-n-alkylresorcinols are phenolic lipids that are mostly found in the bran of wheat and rye, and minimal amounts in barley. They are usually named based on their chain length and saturation [86]. They are derivatives of 1,3 dihydroxy benzene and have an odd-numbered alkyl chain at the 5th position in the benzene ring. Since the length of their saturation tail in wheat and rye is usually 15–27 carbons, their name represents their chain length (e.g., C17, C19, and so on). In wheat and rye, the main ARs are C17, C19, C21, C23 and C25; however, C25 appears to be most distinctive for barley. In wheat, the most common ARs are C21 and C19, whereas they are C17, C19 and C21 in rye [86, 87]. Since both wheat and rye contain the same ARs, it is difficult to determine whether they are derived from wheat or rye products. To this end, the ratio of C17/C21 has been used to estimate the source. Therefore, the origin is more likely to be increasingly wheat-based when the ratio is close to 0.1, and rye-based when it is closer to 1.0 [87]. Since ARs are present in the bran of cereals, they are not found in white flours. However, some amounts may linger in white

rye flour since the rye milling process is more complex and some of the bran layers remain in the rye flour [86].

ARs are absorbed in the lymphatic system of the small intestine and can be quantitively measured in plasma as well as their metabolites dihydroxy benzoic acid and dihydroxyphenyl propanoic acid (DHPPA) [88]. Their half-life is thought to be approximately 5 hours, with an absorption half-life of approximately 3–5 hours. In controlled interventions, participants consuming whole grains regularly had similar fasting plasma AR concentrations repeatedly (variance < 30%) [87]. When ARs were measured in non-controlled cohorts with 1 month to 3 years between analyses, the correlation of AR results was approximately 0.4–0.6 [89-91]. This suggests that a single plasma sample measurement may provide a reliable estimate of an individual's average whole grain intake and be used as a valid biomarker for whole grain wheat and rye intake [92].

2.2.2.3 Plasma fatty acids

Linoleic (LA) and α-linoleic acid (ALA) are essential FAs because the body is unable to produce them on its own. Therefore, their levels are viewed as a reliable reflection of dietary intake and a useful biomarker when validating methods of acquiring intake data [93-95]. However, when FA levels are measured, results may fluctuate between individuals due to differences in absorption, metabolism, lifestyle or genetic factors, for example [72, 96]. FA concentrations can represent the intake and absorption of both fatty acids and carbohydrates [97]. When FAs are synthesised endogenously (often from carbohydrates), it is referred to as *de novo* lipogenesis, which is stimulated by insulin and suppressed by the hormones glucagon and adrenaline [98, 99]. It can be difficult to distinguish between FAs that are derived from the intake of fat and those that are synthesised internally. Differences in metabolism and enzyme activity can also influence both the synthesis of FA and incorporation into cell membranes, which often makes it difficult to properly interpret FA results [72]. Therefore, it has been suggested that displaying FA as a relative concentration of total FA is preferable to absolute amounts due to the difficulty of distinguishing individual differences that can affect a person´s FA profile [72].

FA samples can be measured from several sources in the body, such as adipose tissue, blood cells, whole blood, erythrocyte membranes, plasma or serum fractions that also include triglycerides, free fatty acids (FFAs), phospholipids and cholesterol esters [72]. The composition of FA can be very different based on the source of the sample. This is due to many factors, such as endogenous synthesis and the different roles of FAs in function, structure, or transportation.

For the same reason, samples taken from various sources can indicate the dietary FA intake over different times frames [72]. For instance, samples from adipose tissue can represent long-term FA intake over 1–1.5 years, samples from erythrocytes can indicate intake over 120 days, and total plasma FA usually represents intake over the last few days up to a few weeks [93, 97, 100].

2.3 Nutrition during early life

Pregnancy is a time of rapid physiological growth during which the mother is the sole supplier of all energy and nutrients. Therefore, it is important to aim for an optimal diet to best meet these needs during this time. This is also a time at which the child's metabolic programming is said to take place, which impacts the rest of their life [1]. Nutrition in early life—sometimes referred to as the first 1000 days—plays a critical part in the child's future health [55, 101]. Gestation is a period of development that is affected by its environment. This means that nutrition, hormones and metabolism during pregnancy can influence how the offspring adapts and grows. When the foetus is either nutritionally deprived or exposed to nutritional excess, it may lead to metabolic pathway reprogramming and adaptation to its environment [55, 101]. Some studies have suggested that even moderately poor nutrition during pregnancy can induce adverse foetal programming [2]. Several micronutrient deficiencies during pregnancy have been associated with various adverse effects on the child [2]. For example, an iron deficiency may lead to anaemia, which has been associated with impaired development of the foetus during early gestation [2]. Folate deficiency has also been associated with an increased risk of anaemia as well as neural tube defects and cardiovascular disease [2, 102]. Iodine intake in pregnancy is important for physical and mental development of the foetus [103]. Vitamin D is important for healthy skeletal growth, and its deficiency during pregnancy can result in several bone disorders in offspring [104]. When women's diet quality is poor and their intake of nutrient-dense foods is low, the risk of multiple micronutrient deficiencies increases [2].

In Iceland, the guidelines for a healthy diet during pregnancy are the same as the public health dietary guidelines for Icelanders [19] with some additions [18]. The additional guidelines provide detailed information about the importance of hygiene in food preparation as well as what foods should be avoided during pregnancy (such as raw foods for example). The additional guidelines are also provided because the need for specific nutrients can change during pregnancy, and some are considered of such importance they need to be emphasised. For example, taking vitamin D supplements containing 15 µg daily is recommended for all Icelanders aged 10-70 years. This is emphasized in the additional

guidelines, where use of cod liver oil, omega-3 oil with vitamin D or vitamin D tablets is recommended. Omega-3 FAs are important for all individuals. However, during pregnancy, the need for DHA is increased due to the child´s developing nervous system [105]. Therefore, pregnant women are recommended to consume fatty and lean fish weekly. Alternatively, women could supplement daily with cod liver oil to meet the need for DHA, which is estimated at 200 mg per day [18].

2.4 Dietary intake and risk of GDM

Even in developed countries like Iceland, there is a risk of the maternal diet containing excessive amounts of energy whilst still providing little to no nutrients due to a low intake of nutrient-rich foods [2]. Energy-dense and nutrient-deficient diets in pregnancy that consist of high intakes of sugar and fat can increase the risks of adverse health outcomes for the child [106]. This can lead to an increased risk for chronic diseases later in life [1-6, 107, 108]. When a child is exposed to maternal diabetes in the womb, their own risk of disturbed glucose homeostasis later in life increases [109]. This is likely due to a combination of factors, such as the sensitivity of the developing pancreas to its environment in relation to glucose [109]. This may result in the decreased function of β-cells later in life. GDM can also negatively affect the placenta to various degrees depending on when it is diagnosed and how well it is treated [109]. This important period has been promoted as a window of opportunity to provide dietary interventions aimed at positively impacting future health.

Several studies on diet in pregnancy have focused on isolated food types. However, in recent years, the focus has shifted more towards dietary patterns [110]. A diet with a high intake of whole grains [106], vegetables, fruits, fish and legumes has been associated with overall improved pregnancy outcomes [106, 110]. Similarly, the results of a systematic review regarding dietary patterns that may reduce the risk of GDM suggested that a diet containing fruits, vegetables, whole grains, nuts, legumes, fish, and the decreased consumption of red and processed meats might be effective [15]. Worldwide, healthy eating and exercise are recommended during pregnancy to minimise the risk of excessive gestational weight gain. However, more detailed dietary guidelines and nutrient emphasis can somewhat differ between countries [111]. Although the results of dietary interventions in relation to GDM risk indicate that improving diet quality may be beneficial, but few studies exist and the quality of evidence is low [112].

2.4.1 Vitamin D

Insufficient concentrations of vitamin D during pregnancy appear to be a widespread problem in several countries, with reports ranging anywhere between 18 and 84% [113]. Taking vitamin D supplements is recommended for everyone in Iceland [19] and is emphasised in the guidelines for diet during pregnancy [18]. This is partially due to the limited sunshine in Iceland due to its northern latitude, especially during winter (October–March), making the possibility for vitamin D formation in the skin limited [79]. Vitamin D plays an important role in controlling blood calcium concentrations and maintaining bone health [81]. However, vitamin D deficiency has also been associated with an increased risk of diseases such as diabetes and GDM [114-116]. While the mechanism behind this association remains under investigation, it is considered manifold [117]. Several cells express vitamin D receptors, including pancreatic beta cells, which could play a part in glucose metabolism, insulin secretion or insulin resistance [118, 119]. Recent meta-analyses have indicated that vitamin D insufficiency is associated with increased GDM risk [120, 121] and that the women who had serum 25-hydroxyvitamin D (25(OH)D) levels of 40–90 nmol/L had reduced GDM risk [116]. A randomised trial tested the effect of supplementing vitamin D in a cohort of women at high risk of GDM (at least one risk factor). Their results demonstrated that the women who took 5000 IU of vitamin D daily until week 26 had lower rates of GDM when compared to the placebo group (11.4 vs 34.8%) [115].

A systematic review and meta-analysis of randomised control trials on vitamin D supplementation and glycaemic control in women with GDM reported that vitamin D supplementation in women with GDM could lead to improved glycaemic control [122]. Notably, in many of the studies on vitamin D during pregnancy, most of the women displayed low vitamin D concentrations despite the majority of them taking vitamin D supplements [113]. Since evaluating serum (25(OH)D) concentrations can be expensive, using common dietary assessment methods to screen populations is more cost effective and rapid [123]. Previous studies regarding vitamin D during pregnancy in Iceland have been based on subjective dietary assessment. Their results have indicated that many pregnant women in Iceland could be at risk of vitamin D deficiency [11, 12]. The vitamin D status of the pregnant population in Iceland has not been previously studied via biochemical analysis.

2.4.2 Whole grains and alkylresorcinols

Whole grain foods contain all parts of the grain and thus provide more nutrients and fibre than refined grains. Therefore, a diet rich in whole grains can result in positive health effects. For example, these include increased satiety and a slower digestion transit time, resulting in increased gut health and a slower glycaemic response [124]. Whole grain intake has been associated with decreased risk of obesity, type II diabetes, metabolic syndrome and some types of cancers [70, 86, 88]. On the other hand, the intake of refined grains has been linked to a greater risk of metabolic syndrome and increased adiposity in adults [7, 125].

Whole grains are also a part of healthy dietary patterns that have been associated with GDM prevention [13, 15, 126]. Whole grain intake is recommended during pregnancy due to its high nutritional content and as a possible means of reducing the risk of GDM [2]. The literature regarding the effect of refined grain intake during pregnancy on offspring is limited. However, results from an animal study suggest that refined carbohydrate exposure *in utero* may predispose offspring to exhibit an obese phenotype [127]. This was supported by another study suggesting that the intake of refined carbohydrates during GDM pregnancies increased the offspring's risk of being overweight or obese at 7 years old [7]. Therefore, increasing whole grain intake during pregnancy is a modifiable factor that could benefit both the mother and her offspring.

The few previous studies using AR concentrations to investigate associations between diabetes and whole grains appear to have conflicting results. One study found no associations between total AR concentrations and diabetes risk in a Scandinavian cohort of men and women but suggested that the increased ratio of rye to wheat could lower odds ratios for diabetes type II [71]. Another Scandinavian study showed an association between a higher AR indicator of rye whole grains and increased insulin sensitivity [128]. On the other hand, a different study demonstrated an association between the AR metabolite DHPPA and lower ORs of diabetes type II and impaired glucose regulation, respectively [88]. Notably, no previous studies regarding AR and GDM associations were found.

2.4.3 Fatty acids

2.4.3.1 Omega-3 fatty acids

Long omega-3 FAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important for healthy foetal development during pregnancy. Although the conversion of ALA to EPA and DHA is possible, it remains limited.

Therefore, intake of dietary sources of EPA and DHA is recommended [93-95]. The recommended DHA intake during pregnancy is 200 mg/day, which is equivalent to approximately three fish meals per week [129]. These recommendations are based on intervention studies regarding DHA during pregnancy and its associations with adverse outcomes during pregnancy or birth. According to those results, 200 mg/day is the minimum recommendation; however, an intake of up to 1 gram/day appeared to be safe [130].

2.4.3.2 Fatty acids and GDM

During pregnancy, FAs are transferred to the foetus through the placenta based on foetal need, which varies throughout pregnancy. The available FA stores of the mother are influenced by maternal intake, metabolism and storage. During early pregnancy, there is an increase in maternal fat storage in preparation for increased need later in pregnancy. During this time, the sensitivity to insulin increases, resulting in greater lipid formation and decreased lipid catabolism [131]. FA concentrations normally increase slightly throughout pregnancy, but for women with GDM, the increase appears to be greater [132]. This may be a result of abnormal insulin metabolism due to being overweight or obese, which leads to the ineffective suppression of lipolysis [132]. β-cell dysfunction and excessive insulin resistance (IR) are known to be the key components behind GDM development [133]. An increase in FFAs can result in the decreased uptake of glucose [134] and altered secretion of insulin and IR [97, 133, 135, 136]. Circulating maternal lipids normally increase during the third trimester due to the hormone oestrogen and increased IR. This results in the increased catabolism of fatty stores, which provides a greater supply of FAs for placental transfer. Fatty acid delivery to the foetus is controlled by the placenta [137]. This delivery system is thought to be impaired in women with GDM, which limits the levels of DHA transferred to the foetus [138, 139]. However, the reason behind the reported difference remains unknown [140] and there are no specific dietary recommendations for women with GDM regarding FA intake [97].

Previous studies have demonstrated that levels of saturated FAs (SFA) appear to be higher in women with GDM when compared to a control group [97], even in early pregnancy [141], and that the proportions of PUFA and SFA are associated with glucose tolerance and the risk of women being diagnosed with type 2 diabetes (T2D) after GDM pregnancies [142]. The concentrations of polyunsaturated FAs (PUFAs) have been reported as lower in women with GDM [138, 139]. However, in some instances, PUFA n-6 has been reported as significantly higher for women with GDM, whereas PUFA n-3 was lower [143]. Although a recent meta-analysis found that PUFA 6 and PUFA 3 were both

reduced for women with GDM, most analyses therein were performed after GDM diagnoses [97]. Another recent meta-analysis reported that women with GDM had higher total concentrations of FFAs in the second and third trimesters when compared to non-GDM women, and that the concentrations were greater in the second trimester [134].

A few of these studies investigating FA have also looked at diet where it was suggested that obese women diagnosed with GDM may consume more processed meat [144] and that total- and saturated fat intake may be higher—and PUFA intake lower—for women with GDM [97]. However, it remains unclear whether the differences in FA levels between GDM and non-GDM women are due to dietary or metabolic factors.

2.5 Diet and nutritional status during pregnancy in Iceland

Diet during pregnancy has been studied on several occasions in Iceland (see Table 1).

Table 1. Studies on diet during pregnancy in Iceland.

Year of study	Author	Study	Method and reference
1999–2001	Ólafsdóttir, AS. et al.	Combined effects of maternal smoking status and dietary intake related to weight gain and birth size parameters Maternal diet in early and late pregnancy in relation to weight gain.	FFQ [30, 31]
2007–2009	Gunnarsdóttir, I. et al.	Iodine status of pregnant women in a population changing from high to lower fish and milk consumption	FFQ and urine biomkarker [32]
2012–2013	Gunnarsdóttir, I. et al. Tryggvadóttir, EA. et al.	Diet and nutrient intake of pregnant women in the capital area of Iceland Association between healthy maternal dietary pattern and risk for gestational diabetes mellitus	Food records [10, 27]
2015–2016 PREWICE I	Hrólfsdóttir, L. et al.	Can a simple dietary screening in early pregnancy identify dietary habits associated with gestational diabetes? Development of a dietary screening questionnaire to predict excessive weight gain in pregnancy	FFQ [11, 33]
2017–2018[1] PREWICE II	Aðalsteinsdóttir, S. et al. Magnúsdóttir, KS. et al. Tryggvadóttir, EA et al.	Insufficient iodine status in pregnant women as a consequence of dietary changes Vitamin D status and association with gestational diabetes mellitus in a pregnant cohort in Iceland Higher alkylresorcinol concentrations, a consequence of whole grain intake, are inversely associated with gestational diabetes mellitus in Iceland Early pregnancy plasma fatty acid profiles of women later diagnosed with gestational diabetes	FFQ, urine and blood biomarkers [34-37]

[1] Current study

In an Icelandic study investigating early and late pregnancy diets during the 1999–2001 period, intake was assessed using an FFQ. The results indicated that the women had a low intake of vegetables (highest median 76 g/day) and a high intake of soft drinks (median 118 g/day) during early pregnancy [30]. During late pregnancy, the women had a high intake of dairy (median 500–700 g/day) [31]. Following results from an Icelandic population dietary survey in 2002, indicating a significant decrease in fish and dairy consumption [145], iodine status was assessed with an FFQ and urine iodine concentration in pregnant women over the 2007–2009 period [32]. The results showed that iodine status appeared to be within the optimal range, defined by the World Health Organization [32].

In 2012/2013, 4-day weighed food diaries were used for the first time in Iceland to estimate food and nutrient intake between the 19th to 24th weeks of pregnancy [12]. The results suggested that up to 25% of the participants had an intake of iodine, iron and vitamin D lower than the estimated average requirement. The intake of DHA reached 200 mg daily for only 35% of the women. The same study also reported that only 20% of the women reached the recommended minimum of 25 g/day of fibre [12]. Intake of these nutrients has also been shown to be low in other pregnant populations [146, 147]. Results from the same cohort reported a healthy dietary pattern that was associated with a decreased risk of GDM, independent of the women's BMI [28]. The pattern consisted of a higher intake of seafood, eggs, vegetables, fruits and berries, vegetable oils, nuts and seeds, pasta, breakfast cereals, coffee, tea and cocoa powder, and a lower intake of soft drinks and French fries [28].

In the 2015/2016 cohort, 1651 women answered a short diet screening questionnaire (FFQ) during gestational weeks 11–14 [11]. The results indicated that most of the women were not eating five portions of fruits and vegetables per day (87%) nor eating two portions of whole grains or dairy daily (91 and 77%, respectively), which is recommended in the Icelandic dietary guidelines [19, 148]. The proportion of pregnant women that reported drinking sugar or artificially sweetened drinks ≥5 times per week was 28%, while the proportion of those eating sweets, ice cream, cakes or cookies ≥2.5 times/week was 59%, and the proportion of those eating processed meat weekly or more was 31% [11, 33]. It was also reported that approximately one-third of the pregnant women consumed vitamin D supplements fewer than 5 times per week [11], which is one of the risk factors for an inadequate diet in the cohort [33]. These results suggest that diet quality during pregnancy in Iceland can be greatly improved. There is also a potential nutritional risk in part of the pregnant population, which warrants further research using objective measurements of nutritional status (i.e., biochemical analysis) [11, 12, 28].

2.6 Diet recommendations during pregnancy in Iceland

The Icelandic clinical guidelines for providing maternal care during pregnancy are based on guidelines from the National Institute for Clinical Excellence (NICE) [149, 150]. Maternal care takes place through primary health care at local health care centres throughout the country, with scheduled visits to a midwife. Primipara women and those considered in need of more frequent observations visit their midwife 10 times, while multiparas have 7 visits. The first visit is usually before 12 weeks gestational age for most women. During the first visit, the midwives obtain information regarding several health factors, such as height, weight, family history, overall health, blood pressure, a test for protein in urine and more. Additionally, they provide information on diet and should, according to clinical guidelines, direct the women towards pamphlets regarding Icelandic dietary recommendations [19] as well as other recommendations during pregnancy [18]. They also discuss tobacco and alcohol use during pregnancy, a healthy lifestyle, as well as all available tests, services and more. During the first visit, the midwives also perform a risk assessment for thyroid disease, gestational diabetes and the need for preventative acetylsalicylic acid [150, 151].

Previous studies have shown that Iceland has a high rate of gestational diabetes diagnoses [20, 21] and that it may be possible to identify dietary habits associated with the disease via a short diet screening questionnaire [11]. A healthier and more prudent dietary pattern during pregnancy has also been suggested as a means of GDM prevention [28]. Currently, Icelandic maternal healthcare does not provide any diet assessment or counsel from specialised dietitians regarding optimal diet in pregnancy. Most pregnant women meet their midwife about 6–10 times, during which all clinical measurements must be performed in addition to discussing the mother's physical and mental well-being. This allows for very little opportunity to explore dietary intake and assist the mother regarding an optimal diet. Considering the serious health impact that diet and lifestyle during pregnancy have on both mother and child, this situation holds potential for improvement. Diet is a modifiable factor, and it is vital to identify mothers who have poor quality diets or require personalised dietary counselling. By doing so, they can be guided into making the relevant changes needed to improve the future health of both mother and child.

The clinical guidelines refer to two healthy diet brochures that provide dietary information and emphasise guidelines regarding specific supplements [150]. One of the brochures contains the official Icelandic dietary recommendations for all adults [19] and the second contains additional guidelines for pregnant women in Iceland [18]. According to clinical guidelines, all pregnant women in Iceland should receive the brochure providing these specific recommendations during their visits to the midwife. However, when the source of dietary information was

investigated in 1009 pregnant women in Iceland in 2017/2018, only 44% had received it. Most of the women had obtained dietary information from websites and apps or family and friends. Over half of the women (55%) reported an interest in receiving more individualised dietary information [152].

2.7 Summery and purpose of the study

Overall, GDM can have negative consequences for both mother and child and the rate of diagnoses is high in Iceland [7-10, 20, 21]. Nutrition during pregnancy is important and healthy dietary patterns have previously been associated with a decreased risk of GDM [2, 15, 55-60]. However, according to previous studies in Iceland, pregnant women appear not to be adhering to dietary guidelines for a healthy diet [11, 12]. Previous studies during pregnancy in Iceland have for example indicated a low intake of vitamin D, wholegrains and fish, which have been associated with GDM risk [11, 12, 15, 120, 121]. However, aquiring reliable dietary information can be complicated both in studies as well as in a clinical setting [29, 68]. All previous studies on diet during pregnancy in Iceland have been based on subjective data and results have not been confirmed with objective biochemical analysis. If previous results are indeed accurate it highlights the need for means to identify pregnant women, who would benefit from dietary changes. The purpose of this study was to assess diet during pregnancy in Iceland, using both objective and subjective methods, and study associations to GDM risk. It was also to evaluate the use of a short diet screening questionnaire as a clinical tool to guide maternal care.

2.7.1 Study hypothesis

- The prevalence of insufficient vitamin D status in the pregnant population in Iceland is high.
- Lower S-25OHD during pregnancy is associated with an increased risk of GDM.
- Dietary intake of whole grains is low during pregnancy in Iceland.
- Lower intake of whole grains during pregnancy is associated with GDM risk.
- Plasma FA concentrations during early pregnancy are associated with GDM risk.
- A short dietary screening questionnaire can identify those in need of dietary changes.

The specific aims are presented in Chapter 3.

3 Aims

This PhD thesis intended to answer the following research questions:

- **a)** What is the prevalence of insufficient vitamin D status in the pregnant population in Iceland and it´s associations to GDM risk?
- **b)** Is the frequency of whole grain intake, estimated by a diet screening questionnaire in early pregnancy, associated with risk of GDM?
- **c)** Are plasma FA concentrations, measured in early pregnancy, associated with risk of GDM?
- **d)** Is dietary intake estimated by a diet screening questionnaire reflected in concentrations of corresponding biomarkers?

This PhD thesis aimed to examine dietary intake and nutritional status during early pregnancy by means of a short dietary screening questionnaire as well as biomarkers of consumption and explore their associations with gestational diabetes. Further aim was to compare results from the short dietary screening questionnaire to those from biochemical analysis.

More specifically, the aims were to:

- **a)** Assess the vitamin D status of pregnant women in Iceland during early pregnancy and its associations with gestational diabetes (Paper I).
- **b)** Explore associations between whole grain intake and gestational diabetes in early pregnancy and diagnoses later in pregnancy by utilising plasma alkylresorcinol concentrations (a biomarker for whole grain intake) together with results from a diet screening questionnaire (Paper II).
- **c)** Determine dietary intake as well as the plasma fatty acid concentrations of pregnant women in early pregnancy (Paper III and IV) and explore their associations with gestational diabetes diagnoses later in pregnancy (Paper III).
- **d)** Compare results of the diet screening questionnaire to those derived from nutritional biomarkers. (Paper I-IV)

4 Materials and methods

This PhD project is based on the PREWICE II study (PREgnant Women of ICEland). The study took place at the Foetal Ultrasound Department at Landspitali National University Hospital from October 2nd, 2017 to March 28th, 2018. The study protocol was approved by the National Bioethics Committee (VSN-17-057-S1) and the Medical Directorate of Landspitali National University Hospital (LSH 5-17).

4.1 Participants

All pregnant women who attended their first-trimester prenatal screening between October 2017 and March 2018 at Landspitali National University Hospital were invited to participate in the study. During the 6-month study period, 1,684 women were scheduled for their first ultrasound screening at the hospital, corresponding to approximately 77% of the pregnant population in Iceland in the study period.

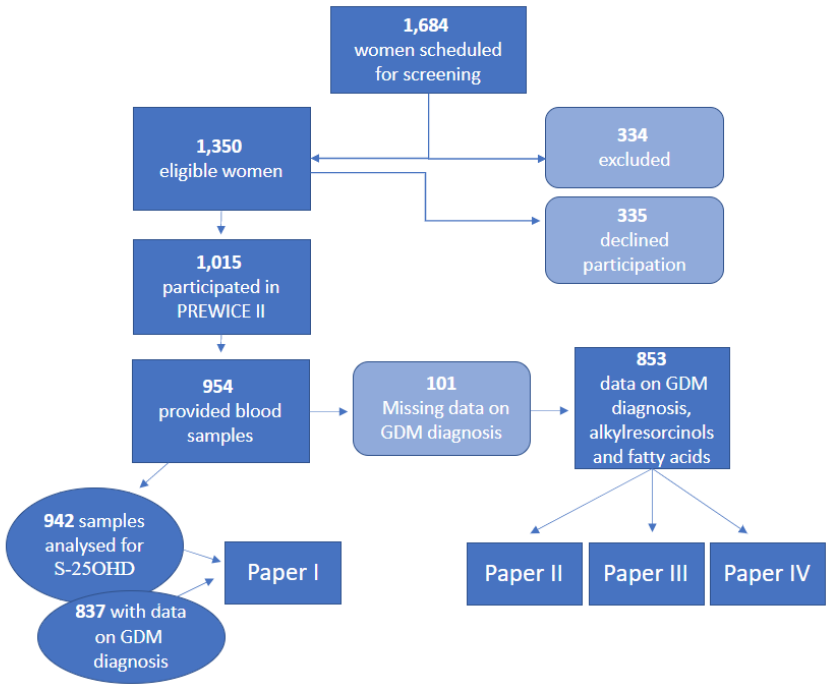

Figure 2 Participation flowchart, for which results are presented in Paper I-IV.

Of these 1,684 women, 244 (15%) were excluded from the study because they were not able to read Icelandic and could thus not answer the questionnaire, which was provided in Icelandic only. Other exclusion factors included falling short of or exceeding the 11- to 14-week gestational age, missing the scheduled appointment time, or miscarriage. This in total excluded 90 additional women, leaving 1,350 women eligible to participate in the study. Of these, 128 women declined due to personal time constraints and 207 declined without further explanation. Of the eligible women, 76% (n=1,015) agreed to participate in the study. The participation flowchart, for which results are presented in Papers I–IV, is shown in Figure 2.

4.2 Data collected

During the prenatal screening, visit subjects answered a diet screening questionnaire along with questions on height, pre-pregnancy weight, smoking, parity, education, marital status and residency. Subjects provided a spot urine sample for the analysis of urine iodine concentration [34] (an issue outside the scope of the present thesis) and allowed for additional blood samples to be drawn at their upcoming blood test. Samples taken at that time was used for biochemical analysis. The participants also gave study staff permission to collect data regarding their pregnancy and birth from medical records. The information from medical records includes variables of importance for the present thesis, such as information on weight throughout pregnancy as well as diagnosis of gestational and family history of diabetes mellitus. Information regarding height and measured weight were collected at the first and last maternal care visits. The dates of visits were gathered from maternal records. The information from medical records provided information on weight during pregnancy as well as gestational age. These data were used to calculate total weight gain (difference between last and first weight recorded) and weight gain per week by dividing total weight gain by the number of weeks between visits. Pre-pregnancy BMI (kg/m^2) was calculated based upon self-reported pre-pregnancy weight and height. We defined BMI <18.5 kg/m^2 as underweight, 18.5–24.9 kg/m^2 as normal weight, 25–29.9 kg/m^2 as overweight and ≥ 30.0 kg/m^2 as obese.

Other information gathered from medical records that was outside the scope of the present thesis included measurements of blood pressure, diagnosis of hypertension that starts in pregnancy or pre-eclampsia, as well as the mode of delivery and other complications during pregnancy or delivery.

4.3 Dietary intake and participants' characteristics

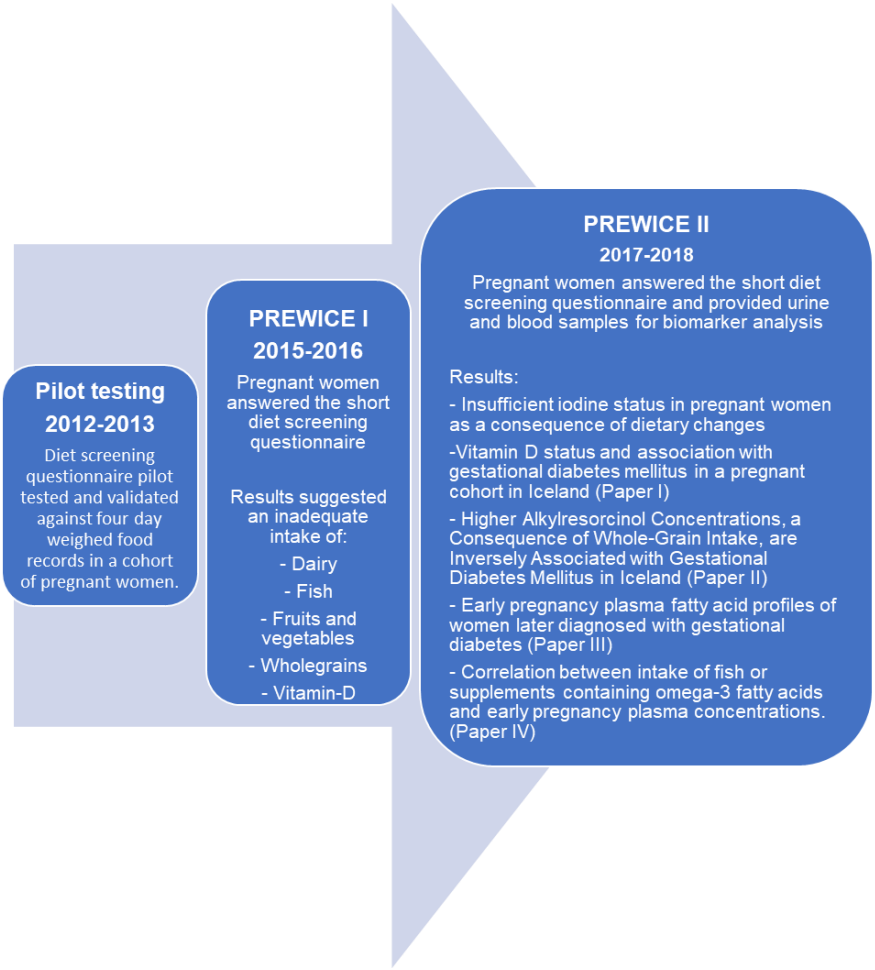

Figure 3. Timeline for the the development of the short diet screening questionnaire.

The diet screening questionnaire was applied in an electronic format and inquired about the frequency of consumption of 40 different food items and beverages in addition to dietary supplement intake. On average, it takes approximately 5–10 min to answer the questions in an electronic format. The questionnaire provides instructions on how to answer questions regarding dietary consumption based on the past 3 months (approximately from the beginning of pregnancy). It also provides a selection of 10 potential frequency responses ranging from "less than once a month" to "more than five times a day". The development of the diet screening questionnaire has previously been described in detail [11, 33, 34] and the timeline can be seen in Figure 3.

In brief, the questionnaire was pilot tested in a group of 25 pregnant women and results were compared to a 4-day weighed food record, with acceptable correlation (Spearman's correlation >+0.3) for most food groups/items [33]. Following this, the diet screening questionnaire was used in the PREWICE I study (2015–2016), a population-based observational study wherein a total of 2113 pregnant women answered the questionnaire [33]. The diet screening questionnaire used in the PREWICE studies (I and II) was designed to capture the total healthiness of the diet according to current food-based dietary guidelines at that time, and sources of nutrients known to be of importance for foetal development [18, 19]. As an example, according to results from the 2010/2011 dietary survey, the contribution of fish and dairy to total iodine intake in the Icelandic diet is 68% (47 and 21%, respectively). Together, fish liver oil (48%) and fish (19%) provide 67% of the vitamin D in the Icelandic diet. Furthermore, fish liver oil and fish provide a large proportion of long-chain omega-3 in the Icelandic diet. A similar short questionnaire has been validated against a reference method in different age groups and was shown to be capable of ranking individuals according to their intake of food such as vegetables, fruits, dairy etc., and be indicative of the consumption of added sugar, dietary fibre from the diet and fat quality [153].

4.4 Biochemical analysis

The voluntary ultrasound assessment provided at Landspitali National University Hospital in gestational weeks 11–14 involved blood samples for genetic testing. On this occasion, extra tubes were drawn from women consenting to participate in the present study. Blood samples were processed within 1 hour after collection to separate plasma from red blood cells and buffy coats through centrifugation for 10 min.

4.4.1 Serum 25OHD

Serum samples were stored at −80°C until the analysis of serum 25OHD (S-25OHD) with an electro-chemiluminescence immunoassay at the Clinical Core Laboratory, Landspitali National University Hospital in the spring of 2019. Control samples measured daily at the laboratory have shown that the Coefficient of Variation % of the method is approximately 4–5%. Concentrations of S-25OHD that were ≥50 nmol/L were considered sufficient, while 30–49.9 nmol/L were insufficient and those below 30 nmol/L were defined as deficient [79, 81].

4.4.2 Plasma

Plasma was aliquoted into cryotubes and stored in a freezer at –80°C until being shipped for AR and FA analysis at the Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden.

4.4.2.1 Plasma AR

Plasma AR concentrations of the homologues C17:0, C19:0, C21:0, C23:0 and C25:0 were measured with normal-phase liquid chromatography-tandem mass spectrometry as described in detail in Paper II, with some modifications for instrumentation. Firstly, 100 µl of plasma was loaded on HybridSPEPlus phospholipid removal 96-well plates (Sigma-Aldrich, St. Louis, MO) and recovered with acetone. The eluted samples were evaporated and resuspended in heptane-ethanol (95:5 volume/volume). Thereafter, the extracts were transferred to chromatographic vials and analysed by liquid chromatography–tandem mass spectrometry (QTRAP 6500+, AB SCIEX, Framingham, MA). The analytes were separated by using a 50 × 2.1-mm amino column with 1.8 mm particles (Blue Orchid, Knauer, Berlin, Germany) using a gradient programme with heptane and ethanol (99.7%) solvents. The liquid chromatography column was kept at 30°C. Atmospheric pressure chemical ionisation in positive mode was used for ionisation. For individual AR homologues, optimal conditions were set for multiple reaction monitoring modes. To assess intra- and inter-batch variation, each batch included quality control samples (<15% for all homologues).

4.4.2.2 Plasma FA

In total, 24 FAs ranging from C12:0 to C24:1 were quantified in plasma samples. An internal standard solution (100 µL of 0.1 mg C23:0 methyl ester/mL toluene) was added to 50 µL of thawed out plasma samples, and 1.8 mL of acetyl chloride-MeOH solution (10% v/v) fortified with BHT (2.78 µg/mL) was added. Samples were incubated at 70 °C for 60 min in a water bath with shaking. Single extractions of fatty acid methyl esters (FAMEs) were separated using the GC-FID system equipped with a Zebron ZB-FAME column. Single extraction of FAMEs was performed by adding 1.5 mL of hexane. The extraction solvent was evaporated using a vacuum concentrator (125 mbar, 30 °C, 30 min). FAME was dissolved in 200 µL of hexane before injection into the GC-FID for analysis. Two water blanks (Millipore-purified water) and six quality controls (pooled plasma) were prepared and run together with the study samples for each batch of a maximum of 50 study samples. FAMEs were separated using the GC-FID system

(Thermo Scientific Focus GC, FID detector, Pal GC-xt autosampler, AD-100 H2 generator, MicroClip XT hydrogen gas alert and a ZA 1500 zero air generator) equipped with a Zebron ZB-FAME column (20 m x 0.18 µm ID x 0.15 µm). The oven programme was performed as follows: initial 80 °C with a 1.5 min hold; ramp: 40 °C/min to 160 °C, 5 °C/min to 185 °C with a 0 min hold; 30 °C/min to 260 °C with a 0 min hold. The instrumental conditions were as follows: nitrogen as the carrier gas, constant flow, carrier flow 1.25 mL/min, inlet temp. 260 °C, split flow 12.5 mL/min, split ratio 15, detector temp. 260 °C; gas flow: air 450 mL/min, hydrogen 35 mL/min, makeup gas 10 mL/min. The injection volume was 1 µL. The concentration of fatty acids in samples was quantified against external standard calibrations made from GLC-462 mixed FAMEs (Nu-Check Prep, Elysian, MN, USA) dissolved in toluene. The external standard included 24 FAMEs ranging from C12:0 to C24:1. An equal amount of internal standard (C23:0 methyl ester) was added to the external standards as added to the study samples, and all analyte peaks were normalised with the peak of the internal standard before calibration.

4.5 Gestational diabetes diagnoses

Information on GDM diagnoses (based on IADPSG [63]) was gathered from medical records. If results after the OGTT were ≥ 5.1 mmol/L when fasting, ≥ 10.0 mmol/L after 60 minutes or ≥ 8.5 mmol/L after 120 minutes, the women were diagnosed with GDM. Since it was not always obvious in the records whether the diagnoses were GDMA1 (controlled with diet) or GDMA2 (medication needed), all diagnoses were combined (yes/no). We were only able to gather information regarding GDM diagnoses for the women who gave birth at Landspitali National University Hospital, or 84% of eligible women (n=853). The reason was that the ethical approval only covered medical records in the possession of Landspitali National University Hospital.

4.6 Statistical analysis

Data from the dietary questionnaire and maternal hospital records were compiled in Microsoft Excel and then imported into Statistical Package for Social Sciences (SPSS). Both IBM SPSS for Windows (version 24.0/26.0; Armonk, NY) and Statistical Analysis Software (SAS; version 9.2) were used to analyse the data. The level of significance was accepted as $P < 0.05$.

Overall, the data are presented as mean ± standard deviation for normally distributed variables, median and interquartile range/10th–90th percentile for skewed variables, as well as frequencies and percentages for dichotomous

variables. For continuous variables, the independent sample T-test for equality of means was used to formally test for differences between two groups for normally distributed variables, while the Mann–Whitney U test for two independent samples was used to compare differences for skewed variables. The chi-square test was used to test for differences across groups for dichotomous variables.

In Paper I, we used logistic regression to examine the association between categories of serum 25OHD status (<30, 30–49.9, 50–74.9 and ≥75 nmol/L) and GDM. Results were presented as odds ratios with 95% confidence intervals before and after adjustment for covariates using 25OHD status (<30 nmol/L) as the referent category. The covariates included in the adjusted models were maternal age, pre-pregnancy BMI, parity and smoking during pregnancy. The chi-square test was used as a formal test for association, based on modelling categorical variables as a continuous term in the regression model.

In Paper II, we used multivariate log-binomial regression to evaluate the RR of GDM across quartiles of dietary whole grain consumption and serum AR concentrations. By using the median value in each quartile and modelling the whole grain and serum AR variables as continuous in the regression model, a P value for trend was evaluated. Results from the regression model were presented as both univariate- and multivariate-adjusted. The covariates included in the adjusted models were age, pre-pregnancy BMI, parity, education, maternal smoking during pregnancy and family history of diabetes. In cases when missing values for covariates were low (<5%) or missing, they were imputed using the median or the most probable value. This was the case for pre-pregnancy BMI. Missing values for family history of diabetes (15%) were accounted for by using a missing category for covariate adjustment.

In Paper III, the concentrations of different types of FA were combined to determine the total for SFA, MUFA, PUFA n-6, PUFA n-3 as well as EPA and DHA. Ratios of individuals and groups of FAs were calculated by dividing their concentration by the total FA concentration. To compare means between the women who were later diagnosed with GDM and those who were not, a linear regression model was used to evaluate adjusted differences in FA types. Multivariate binary logistic regression was used to evaluate the odds ratio of GDM across quartiles of FA types as well as relative FA. P value for trend was evaluated by using the median value in each quartile and modelling the plasma FA and relative FA variables as continuous in the regression model. Spearman correlation was used to assess correlations between dietary intake and FA.

In Paper IV, the results of EPA and DHA concentrations are described as total and relative amounts of plasma FA. Spearman correlation was used to assess correlations.

5 Results

In the following sections, the main findings from the PhD studies are presented, starting with a description of the GDM diagnosis and characteristics of the participants. This is followed by a presentation of the main results from the four papers. More detailed results can be found in Papers I–IV.

5.1 Diagnosis of GDM and characteristics

In our cohort, 127 women (14.9%) were diagnosed with GDM. The highest rates of GDM diagnosis were among women with a BMI ≥30 kg/m^2. Among these women, the rate of GDM was 26.5%—compared to 14,2% in the overweight group and 11,1% in the normal weight group. The characteristics of all the participants in PREWICE II who provided blood samples and had available data on GDM diagnoses are summarised in Table 2. The mean age of all participants was 30 years, and 44% were nulliparae. In total, 59% had a university-level or higher academic education, and 14% smoked before pregnancy. The rate of GDM was 14.9% (*n* = 127), and the women in the GDM group were more likely to be older and have a higher pre-pregnancy BMI. Women diagnosed with GDM were more likely to be overweight or obese (74.8%) when compared to non-GDM women (43.0%). Information on pre-pregnancy BMI was missing for 10 (7.9%) women with GDM.

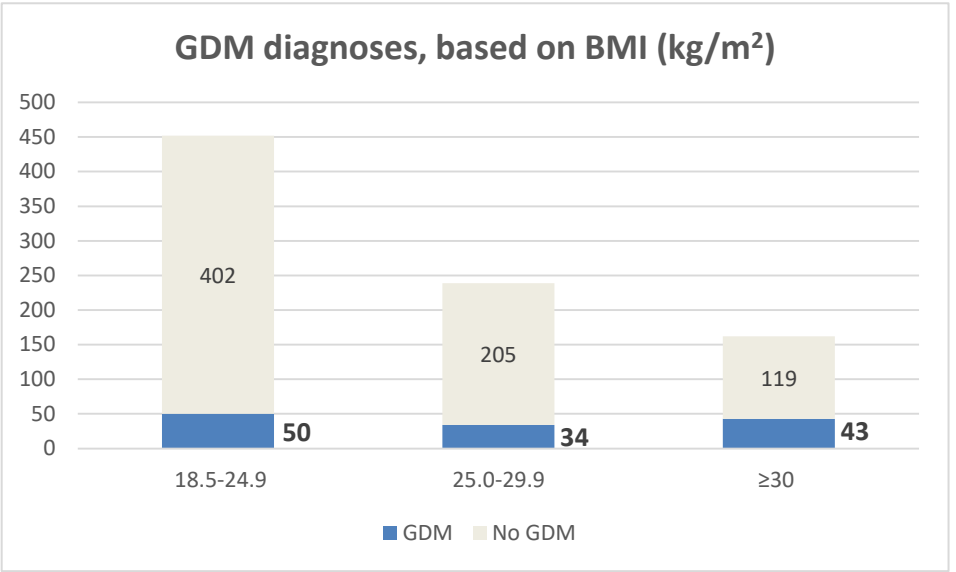

Figure 4. Number of gestational diabetes diagnoses, based on Body Mass Index.

Table 2. Characteristics of the women in PREWICE II that did or did not have Gestational diabetes[1].

		All (n = 853)	Non-GDM (n = 726)	GDM (n = 127)	p^2
Age, years		30.3 ± 4.9	29.9 ± 4.8	32.4 ± 5.5	<0.01
Pre-pregnancy BMI[3], kg/m^2		25.8 ± 5.7	25.4 ± 5.4	28.4 ± 6.8	<0.01
Total weight gain[4], kg		12.3 ± 5.5	12.8 ± 5.2	9.6 ± 6.1	<0.01
Weight gain, kg/week[5]		0.49 ± 0.2	0.50 ± 0.2	0.39 ± 0.2	<0.01
Parity[6], %					
	0	44	45	41	
	1	36	35	40	
	≥ 2	20	20	19	0.60
Education[7], %					
	Elementary school	11	11	13	
	Technical/High school	30	29	31	
	University education	35	36	28	
	Higher academic	24	24	28	0.36
Marital status[8], %					
	Married	24	23	26	
	Living together	71	72	69	
	Single	5	5	5	0.77
Smoking[9], %					
before pregnancy	Yes	14	14	17	0.39
during pregnancy	Yes	5	4	6	0.55
Family history of diabetes (Yes)[10], %		7	6	14	<0.01

[1]Data are presented as means ± std. deviation or ratios.
[2] Differences between Non-GDM and GDM using *T*-test for equality of means, Pearson's chi-square test and the Mann–Whitney *U* test for two independent samples.
[3] Information on pre-pregnancy BMI is missing for 22 women.
[4] Information on weight gain is missing for 45 women. Total weight gain is the difference between measured weight at the first and last maternal care visit.
[5] Weekly weight gain is the total weight gain divided by of weeks between the first and last maternal care visit.
[6] Information on parity is missing for 6 women.
[7] Information on education is missing for 5 women.
[8] Information on marital status is missing for 21 women.
[9] Information on smoking is missing for 6 women.
[10] Information on family history of diabetes is missing for 128 women.
BMI: Body mass index. GDM: Gestational diabetes mellitus. PREWICE II: Pregnant women in Iceland II.

5.2 Vitamin D status of pregnant women in Iceland and associations with GDM (Paper I)

The first aim of this thesis was to assess the vitamin D status of pregnant women in Iceland during early pregnancy and its associations with gestational diabetes.

The mean ± standard deviation of the serum 25OHD (S-25OHD) concentration in this cohort was 63±24 nmol/L (Table 3). The proportion of women with an S-25OHD concentration considered adequate (≥50 nmol/L) was 70%, while 25% had a concentration considered insufficient (30–49.9 nmol/L) and 5% had a concentration considered deficient (<30 nmol/L).

Table 3. S-25OHD concentration (nmol/L) in all subjects (*n*=938) and their reported use of supplements containing vitamin D (*n*=935).

	n	**Mean ± SD**	**<30 nmol/L, *n* (%)**	**30–49.9 nmol/L, *n* (%)**	**50–74.9 nmol/L, *n* (%)**	**≥75 nmol/L, *n* (%)**
All subjects	938	63.0 ± 24.4	51 (5.4)	234 (24.9)	398 (42.4)	255 (27.2)
Not taking any supplements containing vitamin D	104	44.6 ± 17.5	19 (18.3)	50 (48.1)	29 (27.9)	6 (5.8)
Irregular use of supplements containing vitamin D[1]	65	55.1 ± 21.1	6 (9.2)	24 (36.9)	25 (38.5)	10 (15.4)
Daily vitamin D supplementation	766	65.9 ± 24.1	26 (3.4)	160 (20.9)	342 (44.6)	238 (31.1)

[1] Subjects reported use of vitamin D supplements from 1–2 times per month up to 4–6 times per week.

Most of the women (*n*=766, 82%) used supplements containing vitamin D daily, with an S-25OHD concentration (mean ± SD) of 66 ± 24 nmol/L. Interestingly, approximately 24% of the women using vitamin D supplements daily still had insufficient status. Women that reported never using supplements containing vitamin D (*n*=104, 11%) had an S-25OHD concentration (mean ± SD) of 45 ± 18 nmol/L. In this group, approximately 66% were defined as having insufficient S-25OHD concentration (<50 nmol/L), of which 18% were defined as deficient (<30 nmol/L).

Both non-users and irregular users of vitamin D supplements had significantly lower S-25OHD when compared to those taking supplements containing vitamin D daily (P <0.01). Most of the women (89%) who had sufficient vitamin D status (≥50 nmol/L) used vitamin D supplements daily. Approximately one-third of this cohort had S-25OHD concentrations below adequate levels (< 50 nmol/L) during the first trimester of pregnancy.

As seen in Table 4, there was a modest non-significant decrease in the proportion of women diagnosed with GDM alongside increased S-25OHD concentrations: from 17.8% in the group with <30 nmol/L S-25OHD to 12.8% in the group with ≥75 nmol/L (P for trend = 0.17). After adjustment for covariates, the association was somewhat strengthened but still non-significant (P for trend = 0.11). In terms of effect size, the OR for GDM among those with ≥75 nmol/L compared to those with <30 nmol/L was 0.60 (95% CI: 0.25, 1.45). Therefore, no clear association was observed between vitamin D status and GDM.

Table 4. Vitamin D status in women with and without GDM (n=837).

	Serum 25OHD (nmol/L)				
	<30	**30–49.9**	**50–74.9**	**≥75**	***P* for trend[1]**
No cases (%)/*n*	8 (17.8%)/45	36 (17.2%)/209	53 (14.9%)/356	29 (12.8%)/227	
Unadjusted OR (95% CI)	1.00	0.96 (0.41, 2.24)	0.81 (0.36, 1.83)	0.68 (0.29, 1.60)	0.17
Adjusted OR (95% CI)[2]	1.00	0.90 (0.38, 2.12)	0.77 (0.33, 1.76)	0.60 (0.25, 1.45)	0.11

[1]Chi-square test.

[2]Adjusted for maternal age, parity, maternal pre-pregnancy body mass index and smoking during pregnancy.

5.3 Whole grain intake early in pregnancy and associations with GDM (Paper II)

The second aim of this thesis was to explore associations between whole grain consumption in early pregnancy, plasma alkylresorcinol (AR) concentration (a whole grain consumption biomarker) and later GDM diagnoses.

In total, 14.9% of the women adhered to the national food-based dietary guidelines (n = 127), which recommend two portions of whole grains daily. The frequency of whole grain consumption was lower in women who were later diagnosed with GDM when compared to women without GDM (median: five portions/week vs. six portions/week, respectively; P = 0.02). This difference was reflected in the lower median concentration of total AR among women diagnosed with GDM (163 vs. 209 nmol/L, respectively; P < 0.01).

The associations between the frequencies of both whole grain consumption and AR concentrations with GDM, respectively, were assessed in a multivariate model while adjusting for age, pre-pregnancy BMI≥25 kg/m^2, parity, education, smoking during pregnancy and family history of diabetes. The results are presented for unadjusted and adjusted models (Table 5). The median frequency of whole grain consumption was 7.8 times per week in the highest quartile of AR concentrations and 3.6 times per week in the lowest. The RR of being diagnosed with GDM was 50% (95% CI: 0.27, 0.90) lower among individuals in the highest quartile when compared to those in the lowest quartile of plasma ARs (*P*-trend = 0.01). There was a significant dose response related to GDM risk with higher AR levels.

Regarding whole grain consumption, similar risk estimates were observed for both quartiles 3 and 4, where the two quartiles with the highest frequency of whole grain consumption were significantly associated with a lower risk of being diagnosed with GDM in comparison to the lowest quartile RR (95% CI: 0.26, 0.83) and (95% CI: 0.27, 0.86), respectively. Moreover, a test for dose response was significant (*P*-trend > 0.01).

Additionally, we calculated the median ratio of the ARs C17/C21, which was 0.09 (0.03–0.27) (not shown in a table).

The findings from this paper suggest that the higher consumption of whole grains, reflected both by reported consumption according to the diet screening questionnaire and AR biomarkers, is associated with a decreased risk of receiving a GDM diagnosis.

Table 5. Associations of quartiles of plasma alkylresorcinols and FFQ-reported weekly consumption of whole grains with GDM.

		Crude	**Adjusted**[1]
Total plasma AR quartile[2] (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
n = 853			
AR Quartile 1 (66)	40 (19.0)/210	1.00 -	1.00 -
AR Quartile 2 (140)	36 (17.0)/212	0.87 (0.53, 1.43)	1.00 (0.59, 1.70)
AR Quartile 3 (279)	30 (13.8)/217	0.68 (0.41, 1.14)	0.71 (0.41, 1.23)
AR Quartile 4 (706)	21 (9.8)/214	0.46 (0.26, 0.82)	0.50 (0.27, 0.90)
P-trend		0.006	0.01
FFQ weekly whole grain consumption (median, times/week)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
n = 834[3]			
FFQ Quartile 1 (1.2)	40 (19.9)/201	1.00 -	1.00 -
FFQ Quartile 2 (3.8)	31 (14.5)/214	0.68 (0.41, 1.14)	0.71 (0.41, 1.22)
FFQ Quartile 3 (7.6)	25 (12.3)/204	0.56 (0.33, 0.97)	0.47 (0.26, 0.83)
FFQ Quartile 4 (14.5)	25 (11.6)/215	0.53 (0.31, 0.91)	0.48 (0.27, 0.86)
P-trend		0.03	0.01

[1]Adjusted for age, pre-pregnancy BMI kg/m², parity, smoking during pregnancy, family history of diabetes, and reported intake of beans, nuts, seeds, fruit juice and coffee.
[2] The medians of FFQ weekly whole grain consumption for each AR quartile are: Q1=3.6, Q2=5.3, Q3=6.5 and Q4=7.8.
[3] FFQ data on whole grain intake is missing for 19 women.
BMI: Body mass index. CI: Confidence Interval. GDM: Gestational diabetes mellitus.
RR: Relative risk.

5.4 Fatty acid profiles during early pregnancy and associations with later GDM diagnoses (Paper III)

The third aim of this thesis was to determine the fatty acid concentrations of pregnant women at the 11th–14th week of pregnancy and explore associations to GDM diagnoses later in pregnancy.

The concentrations of total SFA, MUFA, and PUFA n-6 and n-3 at gestational weeks 11–14, as well as the ratio of different groups of FA, are presented in Table 6. Differences in FA profile in early pregnancy between women who were later diagnosed with GDM and those who were not were evaluated using the Mann–Whitney U test. The total concentration of FA was significantly higher in women diagnosed with GDM, as were the concentrations of all types of FA, except for long chain n-3 fatty acids EPA+DHA. The median concentration of total FA in early pregnancy was 2898 µg/mL for women who were later diagnosed with GDM and 2681 µg/mL for those without GDM. The mean adjusted difference for total FA between the groups was 133 µg/mL (95% CI 33 to 233).

When stratified by pre-pregnancy BMI (<25 vs ≥25 kg/m^2), the same tendency towards a higher concentration of total FA and MUFA in women who were later diagnosed with GDM was observed in both groups. When comparing the relative FA concentrations of women later diagnosed with GDM to those who were not, MUFA was significantly higher and PUFA n-6 was significantly lower for the women who later developed GDM. This difference in relative FA concentrations also remained after stratifying by pre-pregnancy BMI (<25 vs ≥25 kg/m^2).

The results we observed in pre-pregnancy normal weight women and overweight women/women with obesity were similar. Therefore, the findings from this paper suggest that plasma FA profiles in early pregnancy are different for women later diagnosed with GDM compared to those who were not, independent of the women's BMI.

Table 6. Fatty acid concentrations at gestational weeks 11–14 in women with and without GDM diagnosis later in pregnancy (also stratified by BMI[1]).

	Non-GDM	**GDM**		**Mean adjusted difference**	**Non-GDM**	**GDM**	
	Median (10th–90th percentile)				Median (10th–90th percentile)		
	Total µg/ml		*P*	*µg/ml (95%CI)[2]*	*Ratio %[3]*		*P*
All	*(n=726)*	*(n=127)*			*(n=726)*	*(n=127)*	
SFA	906 (712 – 1166)	966 (747 – 1230)	**<0.01**	37 (1, 75)	34 (32 – 36)	33 (31 – 36)	0.39
MUFA	688 (521 – 928)	784 (562 – 1041)	**<0.01**	75 (40, 110)	26 (23 – 29)	27 (24 – 31)	**<0.01**
PUFA n-6	955 (793 – 1178)	989 (813 – 1188)	**0.02**	21 (–11, 52)	36 (32 – 40)	35 (31 – 38)	**<0.01**
PUFA n-3	128 (94 – 177)	134 (99 – 180)	**0.05**	-0,3 (-8, 7)	4.7 (3.7 – 6.2)	4.6 (3.8 – 5.9)	0.38
EPA + DHA	93 (66 – 135)	97 (73 – 133)	0.11	-2 (-8, 5)	3.4 (2.5 – 4.8)	3.4 (2.6 – 4.5)	0.40
Total FAs	2681 (2174 – 3392)	2898 (2287 – 3632)	**<0.01**	133 (33, 233)	-	-	
BMI <25 kg/m²	*(n=396)*	*(n=44)*			*(n=396)*	*(n=44)*	
SFA	887 (705 – 1153)	941 (730 – 1282)	0.17	45 (-11, 100)	34 (32 – 36)	34 (31 – 36)	0.54
MUFA	660 (516 – 906)	753 (522 – 1034)	**<0.01**	88 (38, 138)	25 (22 – 29)	27 (23 – 30)	**<0.01**
PUFA n-6	947 (789 – 1178)	969 (769 – 1203)	0.18	24 (-23, 72)	36 (32 – 40)	35 (32 – 38)	**0.02**
PUFA n-3	126 (93 – 178)	133 (99 – 176)	0.14	3 (-8, 15)	4.7 (3.8 – 6.2)	4.7 (3.8 – 6.0)	0.99
EPA + DHA	92 (64 – 133)	98 (73 – 136)	0.14	2 (-7, 12)	3.4 (2.5 – 4.8)	3.5 (2.6 – 5.0)	0.87
Total fatty acids	2615 (2127 – 3340)	2773 (2178 – 3701)	**0.04**	161 (14, 307)	-	-	
BMI >25 kg/m²	*(n=324)*	*(n=77)*			*(n=324)*	*(n=77)*	
SFA	918 (727 – 1186)	973 (766 – 1231)	**0.01**	30 (-23, 83)	34 (31 – 36)	33 (31 – 36)	0.68
MUFA	717 (535 – 962)	822 (570 – 1092)	**<0.01**	60 (12, 109)	26 (23 – 30)	27 (24 – 32)	**<0.01**
PUFA n-6	963 (796 – 1180)	990 (814 – 1191)	0.07	16 (-27, 59)	36 (32 – 39)	35 (30 – 38)	**0.01**
PUFA n-3	131 (95 – 181)	134 (104 – 182)	0.34	-3 (-13, 6)	4.7 (3.7 – 6.2)	4.6 (3.8 – 5.9)	0.30
EPA + DHA	96 (67 – 136)	95 (74 – 132)	0.52	-5 (-12, 3)	3.5 (2.6 – 4.7)	3.3 (2.5 – 4.5)	0.27
Total fatty acids	2769 (2210 – 3412)	2944 (2323 – 3632)	**<0.01**	103 (-35, 240)	-	-	

[1] BMI information is missing for 12 women.
[2] Adjusted for age, pre-pregnancy BMI, weekly weight gain and smoking during pregnancy when all women are included. No adjustment for pre-pregnancy BMI when stratifying for BMI.
[3] Relative FA concentrations as a ratio of total FA.
BMI: Body mass index. FA: Fatty acids. GDM: Gestational diabetes mellitus

5.5 Results of the diet screening questionnaire and related biomarkers. (Paper I-IV)

The fourth aim of this thesis was to compare results of the diet screening questionnaire to those derived from nutritional biomarkers.

In this chapter the emphasis will be results from paper IV in addition to results of reported intake of vitamin D supplements and whole grain intake to related biomarkers.

The intake of fish and/or the supplements containing omega-3 FAs available on the Icelandic market is shown in Table 7. The median intake of lean fish was approximately once weekly, and fatty fish was consumed approximately once monthly. There was a positive correlation between the intake of lean and fatty fish (r=0.39 P<0,001) (not shown in a table).

Table 7. FFQ-reported weekly intake of foods at 11th–14th week of pregnancy and correlations1 with total (µg/ml) and relative (%) EPA and DHA concentrations. Data presented as medians and percentiles (10th–90th

FFQ, frequency per week (n=853)	Median (10^{th}–90^{th} percentile)	Total EPA+DHA correlation	P	Ratio[2] EPA+DHA correlation	P
All fish and omega-3 supplements	7.5 (1.0–16.3)	0.34	<0.001	0.41	<0.001
All fish, cod liver oil and omega-3[3]	3.3 (0.9–14.7)	0.37	<0.001	0.46	<0.001
All fish	1.3 (0.4–3.0)	0.24	<0.001	0.28	<0.001
Fish, lean	1.0 (0.1–2.5)	0.18	<0.001	0.23	<0.001
Fish, fatty	0.3 (0.1–1.0)	0.24	<0.001	0.28	<0.001
Any omega-3 supplements	0.7 (0.3–14.0)	0.31	<0.001	0.40	<0.001
Cod liver oil[4]	0.1 (0.1–7.0)	0.21	<0.001	0.27	<0.001
Omega-3 oil[5]	0.2 (0.2–7.1)	0.19	<0.001	0.25	<0.001
Maternal supplement[6]	0.1 (0.1–7.0)	0.01	0.835	0.001	0.977

[1] Spearman correlation.
[2] Relative FA concentrations as a ratio of total fatty acids.
[3] Does not contain the maternal multivitamin.
[4] Recommended daily intake provides 114 mg EPA and 150 mg DHA according to the manufacturer.
[5] Recommended daily intake provides 160 mg EPA and 100 mg DHA according to the manufacturer.
[6] Recommended daily intake provides 150 mg EPA and 100 mg DHA according to the manufacturer.
FFQ: Food frequency questionnaire.

There was a positive Spearman correlation between the women's intake of all fish and total omega-3 supplements and the total (r=0.34) and relative (r=0.41) concentrations of plasma omega-3 FA.

Similarly, a positive correlation was observed between intake of cod liver oil/capsule and omega-3 oil/capsule supplements and concentrations of EPA and DHA (r=0.21 and r=0.19, respectively). However, when assessing the correlation between the frequency of intake of a popular pregnancy multivitamin supplement (tablets) containing omega-3 and concentrations of plasma EPA and DHA, no correlations were observed (r=0.01).

The rate of women taking various supplements containing omega-3 daily, less than daily or never is presented in Table 8 alongside total and relative FA concentrations. Overall, approximately half of the women were taking omega-3-containing supplements daily and some used more than one product.

When comparing the women´s total and relative values of EPA and DHA stratified by frequency of intake, we observed that women with a daily intake of cod liver oil/capsules or omega-3 oil/capsules had higher concentrations of EPA + DHA when compared to those who never used the supplements (median: 108 vs. 91 µg/ml and 103 vs 90 µg/ml).

The rate of women taking maternal multivitamins daily was 17.1%. Moreover, there were no significant differences between total or relative EPA + DHA values for those who had a daily intake of the multivitamin when compared to those who never took it.

Table 8. Median and relative values of EPA and DHA for women taking different omega-3 supplements daily or more compared to those taking them less than daily or never.

Median (10th–90th percentile)		%	EPA+DHA, µg/ml	EPA+DHA, %[1]
Total omega-3 supplements	≥ Daily	50.4	102 (70–148)	3.7 (2.7–5.3)
	< Daily	12.5	89 (64–120)	3.3 (2.4–4.2)
	never	37.0	86 (62–118)	3.1 (2.4–4.0)
	P[2]		**<0.01**	**<0.01**
Cod liver and omega-3 oil/capsules	≥ Daily	39.7	105 (72–151)	3.9 (2.8–5.5)
	< Daily	12.1	91 (66–128)	3.4 (2.5–4.5)
	never	48.2	87 (62–118)	3.1 (2.4–4.0)
	P[2]		**<0.01**	**<0.01**
Cod liver oil/capsules	≥ Daily	18.8	108 (76–157)	4.0 (3.0–5.6)
	< Daily	10.4	93 (66–128)	3.4 (2.6–4.7)
	never	70.8	91 (64–129)	3.3 (2.5–4.5)
	P[2]		**<0.01**	**<0.01**
Omega-3 oil/capsules	≥ Daily	27.5	103 (70–145)	3.8 (2.8–5.2)
	< Daily	6.8	92 (71–144)	3.5 (2.5–4.7)
	never	65.7	90 (64–130)	3.3 (2.5–4.4)
	P[2]		**<0.01**	**<0.01**
Maternal multivitamin	≥ Daily	17.1	98 (65–134)	3.4 (2.6–0.5)
	< Daily	5.3	88 (65–125)	3.2 (2.4–4.3)
	never	77.6	93 (66–136)	3.4 (2.5–4.8)
	P[2]		0.25	0.41

[1] Relative FA concentrations as a ratio of total FAs.
[2] Kruskal Wallis test used to compare differences.
EPA: Eicosapentaenoic acid. DHA: Docosahexaenoic acid.

Results of S-25OHD concentrations stratified by reported use of supplements containing vitamin D are presented in Table 9. The women who reported taking supplements containing vitamin D had higher concentrations of S-25OHD.

Reported intake of fish as well as use of supplements containing vitamin D correlated positively with S-25OHD. The strongest correlations were seen for intake of vitamin D supplements (r=0.31 P <0.001) and consumption of fish together with any vitamin D containing supplements (r=0.24 P <0.001).

Table 9. S-25OHD concentration (nmol/L) in all subjects (*n*=938) and their reported use of supplements containing vitamin D (*n*=935).

		Serum 25OHD (nmol/L)				
	n	**Mean ± SD**	**<30, *n* (%)**	**30–49.9, *n* (%)**	**50–74.9, *n* (%)**	**≥75, *n* (%)**
Not taking any supplements containing vitamin D	104	44.6 ± 17.5	19 (18.3)	50 (48.1)	29 (27.9)	6 (5.8)
Irregular use of supplements containing vitamin D[1]	65	55.1 ± 21.1	6 (9.2)	24 (36.9)	25 (38.5)	10 (15.4)
Daily vitamin D supplementation	766	65.9 ± 24.1	26 (3.4)	160 (20.9)	342 (44.6)	238 (31.1)
P for trend[2]		<0.001				

[1] Subjects reported use of vitamin D supplements from 1–2 times per month up to 4–6 times per week.
2 Kruskal Wallis test used to compare differences.

Frequency quartiles for reported consumption of whole grains and corresponding concentrations of alkylresorcinols are are shown in table 10.

There was a significant increase in plasma AR concentrations along with a higher reported frequency of whole grain intake.

Table 10 Frequency quartiles of reported whole grain intake and plasma alkylresorcinol concentrations (nmol/L).

n = 834 FFQ weekly whole grain consumption (median, times/week)	*n*	Plasma AR Median (10^{th}–90^{th} percentile)
FFQ Quartile 1 (1.2)	201	129 (43-476)
FFQ Quartile 2 (3.8)	214	207 (53-794)
FFQ Quartile 3 (7.6)	204	208 (62-1004)
FFQ Quartile 4 (14.5)	215	294 (90-1047)
P-trend[1]		<0.001

[1] Kruskal Wallis test used to compare differences.

Results of correlation analysis between reported consumption of whole grain foods and total plasma concentrations of alkylrecorcinols were also significant (r=0.26 P <0.001).

5.6 Dietary intake

Although the assessment of overall diet was not one of the main aims of the present thesis, additional information on the median consumption of food from different food groups in early pregnancy is provided in this chapter.

The comparison of reported weekly intake of foods groups between the women who were later diagnosed with GDM and those who were not is shown in table 11.

The main differences observed when comparing intake frequency between the groups, are regarding consumption of fatty fish, fruit juice, coffee and wholegrains. The women who were later diagnosed with GDM reported a significantly lower intake frequency for these food groups. Intake frequencies of skimmed milk as well as beans, nuts and seeds were also significantly different between the groups, with a tendency of frequency being skewed towards a lower intake among the women later diagnosed with GDM. The medians, however, were the same for both groups.

Reported intake of omega-3 supplements and vegetable oil for cooking also tended to be lower for the women later diagnosed with GDM, with differences being close to significance.

Table 11. Reported weekly median intake of foods groups for women with and without GDM.

FFQ, frequency per week[3]	Non-GDM (*n* = 724)	GDM (*n* = 123)	*p*[2]
Fish, fatty	0.5 (0.1-1.0)	0.1 (0.1-1.0)	**<0.01**
Fish, lean	1.0 (0.1-2.5)	1.0 (0.1-2.5)	0.39
Omega-3 supplements	1.0 (0.3-14.1)	0.3 (0.3-10.9)	0.09
Red meat	1.0 (0.1-2.5)	1.0 (0.1-2.5)	0.94
Poultry	1.0 (0.1-2.5)	1.0 (0.1-2.5)	0.41
Processed meat	0.5 (0.1-2.5)	0.5 (0.1-1.3)	0.39
Whole milk	0.1 (0.1-5.0)	0.1 (0.1-7.0)	0.68
Low fat milk	1.0 (0.2-7.1)	0.6 (0.2-14.1)	0.55
Skimmed milk	0.2 (0.2-1.7)	0.2 (0.2-1.1)	**0.02**
Sour dairy	2.5 (0.1-7.0)	2.5 (0.1-7.0)	0.18
Cheese	5.0 (1.0-14.0)	5.0 (1.0-14.0)	0.11
Butter on bread	5.0 (0.5-14.0)	5.0 (0.3-7.0)	0.42
Butter for cooking	1.0 (0.1-5.0)	2.5 (0.1-5.4)	0.76
Vegetable oil for cooking	5.0 (1.0-7.0)	2.5 (0.3-7.0)	0.07
French fries and chips	0.5 (0.3-2.5)	0.5 (0.1-2.5)	0.53
Cakes, sweets, Ice cream and cookies	3.5 (1.0-8.0)	3.0 (0.6-7.5)	0.11
Soft drinks	1.5 (0.2-7.1)	2.6 (0.2-10.8)	0.44
Fruit juice	1.0 (0.1-7.0)	0.5 (0.1-7.0)	**0.01**
Coffee	0.5 (0.10-14.0)	0.1 (0.1-7.0)	**0.01**
Fruits and vegetables	14.0 (5.0-39.0)	14.0 (3.4-39.0)	0.12
Beans, nuts and seeds	0.5 (0.1-5.0)	0.5 (0.1-2.5)	**0.01**
Whole grain products	6.0 (1.2-15.0)	4.0 (0.5-19.2)	**<0.01**
White bread	2.5 (0.1-7.0)	2.5 (0.1-7.0)	0.97

[1]Data presented as medians and percentiles (10th–90th).

[2]Differences between Non-GDM and GDM using the Mann–Whitney *U* test for two independent samples.

[3]FFQ information on intake is missing for 6 participants.

FFQ: Food frequency questionnaire. GDM: Gestational diabetes mellitus.

6 Discussion

6.1 GDM diagnoses and Characteristics

The rate of women diagnosed with GDM in our cohort was 14.9% which can be considered fairly high when compared to a worldwide estimate of 10.6% when only using the IADPSG criteria which is used in Iceland [51]. This rate is however similar to those seen in previous Icelandic studies such as 11.8 - 16% [154, 155]. When comparing characteristics between the women who were and were not diagnosed with GDM we saw some differences between the groups. The women who were diagnosed with GDM were older, had on average a higher BMI and a higher rate of family history of diabetes. These are all known risk factors for GDM. However, gestational weight gain for the women diagnosed with GDM was lower overall as well as weight gain per week. This is to be expected since the women diagnosed with GDM tended to have a higher BMI and recommended gestational weight gain is based on BMI ranges.

The guidelines from the Institute of Medicine recommend a certain range of optimal GWG based on four classes of pre-pregnancy BMI (underweight, normal weight, overweight and obese) [156]. According to the guidelines, women with a higher pre-pregnancy BMI should gain less weight than those with a lower BMI. In Iceland the GWG recommendations used by the maternal healthcare [157] are based on a cohort from 1998 and are divided to two BMI classes, where underweight/normal weight women should aim at a weight gain of 12-18 kg [148, 158], which is a broader range than proposed by the IOM (11.5-16 kg). According to the Icelandic recommendations an optimal weight gain of overweight/obese women is considered to be 7-12 kg, whereas the Institute of medicine recommends a weight gain of 7–11.5 kg for overweight women and 5-9 kg for those who are obese.

6.2 Paper I: Vitamin D

The first aim of this thesis was to assess the vitamin D status of pregnant women in Iceland early in pregnancy and its associations with dietary and supplemental intake as well as gestational diabetes.

In this study, the vitamin D status of pregnant women in Iceland was studied for the first time. The results indicated that approximately one-third of the pregnant

women had S-25OHD concentrations below adequate levels during the first trimester of pregnancy, despite taking vitamin D supplements regularly.

Overall, most of the women (70%) had 25OHD levels that are considered sufficient (≥50 nmol/L). However, 25% had concentrations considered insufficient (30–49.9 nmol/L) and 5% had deficient concentrations (<30 nmol/L). Interestingly approximately 24% of the women who reported taking vitamin D supplements daily were among those classified as having insufficient vitamin D. Some of these women used multivitamin supplements that contain about 5–10 µg of vitamin D, which does not meet the recommended daily intake of 15 µg [18, 19].

Therefore, it is possible that the women were not taking enough vitamin D, or that the women only recently began taking vitamin D supplements. This could imply that they already had low concentrations of 25OHD when they became pregnant. Since the recommended intake of vitamin D is not meant to correct deficiency [159, 160], it may be necessary for vitamin D-deficient women to temporarily take higher dosages to correct a previous deficiency. A practical method used to calculate the dose of vitamin D required to correct vitamin D deficiency in individuals was developed by Van Groningen et al. [161]. According to this method, if a woman (72 kg) is taking 10 µg of vitamin D and has a 25OHD concentration of 24 nmol/L prior to pregnancy, it would take her over 6 months to reach the sufficient concentration of 50 nmol/L. Thus, if the goal was to reach this concentration of 25OHD before week 20 of pregnancy, the dose would need to be up to 45 µg/day. In response to these results, the Icelandic Directorate of Health has now updated its guidelines regarding vitamin D during pregnancy. Women who have not taken vitamin D supplements prior to pregnancy are recommended to take supplements containing 25–50 µg daily for a few weeks [162].

We found no significant difference in vitamin D status between women with and without later GDM diagnoses. Moreover, we found no significant relationship between low vitamin D levels in early pregnancy and the increased risk of GDM. The proportion of women with vitamin D deficiency (S-25OHD <30 nmol/L) was relatively low in our cohort, and the number of women taking vitamin D-containing supplements might have been higher in this study compared to previous studies (data is not always shown). Clinical trials on vitamin D supplementation in GDM patients have yielded unclear results [163], however a recent meta-analysis has shown that vitamin D supplementation in women with GDM could lead to improved glycaemic control [122]. Notably, researchers have identified several possible factors that might lead to misleading results. For

example, low vitamin D concentration might not be a contributing factor to GDM since it might be causal the other way around. Also, this could be due to the study design, whether the subjects were at high risk for developing GDM or whether they were vitamin D deficient at baseline. Doses, supplementation periods and methods to assess serum 25OHD can also vary since the measurements are done at different weeks of gestation. In some studies, all participants were supplemented with vitamin D due to ethical reasons, which can affect the results [164-166]. The fact that 25OHD levels were measured during the first trimester and that most GDM diagnoses occur during the second trimester may also be a factor in our study. Since some of the women may have recently started taking vitamin D (recommended during the first maternal care visit), they may have had higher levels of 25OHD at the time of the OGTT.

6.3 Paper II: Alkylresorcinols and whole grains

The second aim of this thesis was to explore the associations between whole grain intake early in pregnancy and later gestational diabetes mellitus diagnoses by utilising plasma alkylresorcinol concentrations as well as the results of an FFQ.

We used a novel objective method to measure whole grain consumption during early pregnancy. To the best of our knowledge, ours is the first study to investigate the association between AR concentrations and GDM. Similarly, we found that the frequency of whole grain consumption was lower in women who were later diagnosed with GDM compared to the women without GDM.

We reported that women with greater consumption of whole grains in early pregnancy reflected a higher plasma concentration of AR and had a decreased risk of developing GDM. Furthermore, we observed a significant decrease in GDM risk with higher AR levels. Several factors affect GDM risk, such as age, ethnicity, smoking and family history of GDM [167]. However, obesity in pregnancy and suboptimal nutrition are also associated with GDM risk [8]. Therefore, our results emphasise the importance of further investigating the impact that diet can have on GDM risk.

Whole grain foods contain all parts of the grain and thus provide more nutrients, fibre and phytochemicals than refined grains [168]. A whole grain-rich diet can result in increased satiety, a slower digestion transit time, increased gut health and a slower glycaemic response [124]. Dietary patterns that contain whole grains have repeatedly been presented as possible means of GDM prevention [13, 15, 126]. GDM has been associated with increased pro inflammatory cytokines and lower levels of anti-inflammatory adiponectin which can lead to IR and damaged β cells [169, 170]. Previous studies have suggested that women

who are diagnosed with GDM may have entered pregnancy with some level of insulin resistance and β cell dysfunction, making it difficult for them to handle the metabolic changes brought on by pregnancy [171]. Inflammation and oxidative stress are interconnected, as inflammation can cause oxidative stress and oxidative stress can induce inflammation [172]. Several nutrients have been presented as beneficial in lowering oxidative stress, such as selenium, vitamins E, - C, - D, omega-3 fatty acids and plant flavonoids [172]. It has been suggested that a diet rich with micronutrients may lower the risk of GDM, as the nutrients provide a positive protective effect [173]. Several healthy dietary patterns have been associated with a decreased risk of GDM. These dietary patterns usually consist of foods such as vegetables, fruits, whole grains, fish and a decreased consumption of red- and processed meat [171]. Most of these recommended healthy foods are nutrient dense foods containing fibre, vitamins, micronutrients, antioxidants and phenolic compounds [171]. The combination of these factors may promote health and protect against free radicals and oxidative stress [171]. A meta-analysis showed that an increased intake of fibre rich foods resulted in lowered blood lipids, - inflammation and improved glycaemic control [174]. It was also suggested that increasing the daily intake of fibre of about 15 - 35 g, could be an important part of dietary management for people with diabetes [174].

In contrast, the consumption of refined grains has been linked to a greater risk of metabolic syndrome and increased adiposity in adults [7, 175], as well as an increased risk of GDM [13]. Therefore, increasing whole grain consumption during pregnancy is a modifiable factor that could benefit both the mother and her offspring.

In a recent pregnancy cohort (2013/2014) in Iceland, only 20% of the women reportedly reached the recommended minimum of 25 g/day of fibre (34). In our study, only approximately 15% of the women adhered to the food-based dietary guidelines (FBDG) for whole grains, which recommends two portions daily, while 29% consumed at least one portion of whole grains daily. Use of the same diet screening questionnaire as that utilised in the previous PREWICE study found that the rate of women reaching a minimum whole grain consumption of two portions daily to be 9%. This indicates that whole grain consumption might have increased somewhat among pregnant women in Iceland since 2016 [11]. This positive change may stem from the increased availability of whole grain breads and other whole grain food sources in Iceland.

The AR homologue elimination half-life is approximately 5 hours [176]. However, since the absorption half-life of AR is approximately 3–5 hours, plasma

concentrations remain more stable than expected. During interventions where whole grains rich in AR were consumed regularly, the fasting plasma AR concentrations had similar variations within and between individuals [177]. In normal conditions where AR was measured 1 month to 3 years apart, the intra-class correlation was 0.4–0.6 [89-91]. This suggests that an AR measurement in a single plasma sample can provide a fair estimate of an individual's AR concentrations—and thus their average long-term whole grain consumption. Moreover, several studies have shown positive correlations between estimated whole grain wheat and rye consumption in adults and plasma AR concentrations (r=0.3–0.6) [92], suggesting that AR are valid as biomarkers of whole grain wheat and rye consumption in those with stable and frequent whole grain consumption.

However, since the participants were not fasting in our study, the median total plasma AR was high in our cohort in comparison to a similar study on pregnant women in Singapore. In that study, the fasting median was extremely low (9 nmol/L). To our knowledge, this is the only other study that has measured AR in pregnant women; however, it did not investigate associations with GDM risk [70]. Usually, when diets are whole grain-free under controlled circumstances in non-pregnant studies, the fasting median is below 60 nmol [178]. For instance, the fasting medians for total AR were 20 nmol/L in an elderly US cohort [179], 43 nmol/L in a Scandinavian cohort [71] and 87.7 nmol/L in the WHOLE heart study [180].

Previous non-pregnancy studies that have used AR concentrations to investigate associations between whole grains and T2D have shown conflicting results. One Scandinavian cohort study found no associations between total AR concentrations and diabetes risk in men and women but suggested that a higher ratio of rye to wheat—as measured by the plasma C17:0/C21:0 homologue ratio—was associated with a lower T2D risk [71]. In contrast, another study showed results similar to ours, with an inverse association between an AR metabolite and T2D risk [88]. To the best of our knowledge, ours is the first study to investigate the association between AR concentrations and GDM.

The ratio of the ARs C17/C21 has previously been used to determine whether the dietary source of whole grains is more wheat- or rye-based. The calculated median ratio of C17/C21 in our cohort was 0.09 (0.03–0.27), suggesting that the whole grain origin is more likely to be increasingly wheat-based for the women in PREWICE II since the ratio is closer to 0.1 [87].

6.4 Paper III: Fatty acids and GDM

The third aim of this thesis was to determine fatty acid concentrations of pregnant women in early pregnancy and explore associations with GDM diagnoses later.

We reported both total and relative concentrations of all FA subgroups and stratified our FA results by BMI, a process that, to our knowledge, has not been previously done in a pregnancy cohort. Our findings suggest that plasma FA profiles in early pregnancy are different for women later diagnosed with GDM when compared to those who were not, independent of the women's BMI.

We found that women who were later diagnosed with GDM had a higher concentration of total plasma FAs, total MUFA and MUFA ratios, as well as lower PUFA n-6 ratios in early pregnancy independent of the women's BMI, when compared to women who remained free of GDM. The fact that stratifying by BMI did not alter our results is important to note because obesity, IR and fatty acid profiles in GDM are strongly interrelated [97]. In most previous studies regarding FA profiles in pregnancy, FA analysis was performed either during or after GDM diagnoses [97]. The results of these studies indicated that SFA concentrations were higher in women diagnosed with GDM when compared to controls and that PUFA n-6 and PUFA n-3 concentrations were lower in women with GDM [97]. Similarly, a recent meta-analysis reported higher total concentrations of FFAs in the second and third trimesters in women with GDM when compared to women without GDM, and those concentrations decreased as pregnancy progressed [134].

The few studies that have investigated FA in early pregnancy as we did showed similar results to ours: a higher concentration of total SFA [141, 144, 181] and MUFA [144, 181] as well as PUFA n-6 [143] and PUFA n-3 [181] in women who were later diagnosed with GDM compared to those who were not. However, some studies reported lower PUFA n-6 [144, 181] and PUFA n-3 [143, 144, 182] concentrations in women who later received a GDM diagnosis.

These results suggest that fatty acid biomarkers in early pregnancy may predict GDM. However, further studies are required to confirm this hypothesis. FA profiles have been proposed as a means of predicting later T2D diagnosis in non-pregnant populations, where higher relative concentrations of FFA [183], PUFA n-6 [100], MUFA [100, 183] and SFA [100, 183] have been associated with an increased risk of impaired glucose tolerance and T2D risk. These studies have reported similar results to those observed for circulating FA in the pregnant population in our study.

FA concentrations are influenced by differences in the intake or absorption of both carbohydrates and fat [97]. Plasma MUFA and SFA concentrations represent both dietary intake and synthesis since FAs can be endogenously synthesised, mainly from carbohydrates (lipogenesis) [98, 99]. Lipogenesis is stimulated by insulin and suppressed by hormones glucagon and adrenaline. Some studies have suggested that higher free FA can alter insulin signalling, secretion and glucose production [184, 185]. An abnormal increase of insulin in the blood may thus lead to higher FA concentrations and vice versa. Red- and processed meat contains saturated fat, cholesterol, heme-iron and nitrosamines, and sometimes trans-fat, which have all been associated with increased oxidative stress, β cells damage. insulin resistance and a higher GDM risk [16, 171]. A high intake of meat has also previously been associated with insulin resistance and T2D [16]. Saturated fat can induce insulin resistance as the accumulation of lipids may possibly lead to inflammation resulting in diabetes development [186]. Higher dietary MUFA intake has on the other hand been shown to decrease insulin resistance [186] and higher intake of fish has been inversely associated with GDM risk, possibly as a result of inflammation inhibition [16].

The women who were not diagnosed with GDM tended to have better diet quality in early pregnancy. It remains unclear how differences found in food consumption in the first trimester between women with and without GDM diagnoses, might be reflected in the plasma FA profile. It could have been expected to see a difference in EPA and DHA concentrations between the two groups because women who later were diagnosed with GDM had a less frequent intake of both fatty fish and omega-3 supplements. However, since FA can be synthesised endogenously from excess carbohydrates, the overall quality of the diet—including carbohydrate quality and the amount consumed—might explain some of the differences observed in FA concentration between the two groups, overall carbohydrate quality being one [11, 28, 36].

6.5 Results of the dietary screening questionnaire

The fourth aim of this thesis was to compare results of the use the short diet screening questionnaire to results of related biomarkers.

Throughout all of the papers presented in this thesis, we have compared the results of the PREWICE II diet screening questionnaire to the results of biochemical analyses of Vitamin D, whole grains and alkylresorcinols, as well as plasma FAs. A previous paper from the PREWICE II study group additionally investigated results regarding the dietary intake of iodine and its biochemical analysis [34]. In that paper the median of Urinary Iodine Concentrations

increased along with higher frequency of dairy intake, ranging from a median of 55µg/L for women who reported consuming dairy products <1 time per week to 124µg g/L in the group who reported consuming dairy products >2 times per day (p for trend <0.01) [34].

Dietary intake of the most common omega-3-containing foods and supplements was reflected in EPA and DHA plasma levels, apart from a popular Icelandic maternal multivitamin that should contain omega-3. This may be due to insufficient absorption since the omega-3 FA were in the form of ethyl esters, which have been associated with decreased uptake [187]. The multivitamin also contains other nutrients that might interfere with FA uptake.

The median intake of lean fish was approximately once weekly, whilst fatty fish was consumed approximately once monthly. There was a positive correlation between the intake of lean and fatty fish. Overall, approximately half of the women were taking omega-3 containing supplements daily. These results indicate that many pregnant women in Iceland do not have a sufficient intake of long chain omega-3 FA. This suggests a need to obtain information regarding fish consumption in early pregnancy to evaluate whether omega 3 supplements are required.

For vitamin D, we saw an increase in S-25OHD concentrations along with higher reported intake frequencies of vitamin D containing supplements. The intake also correlated positively with S-25OHD ($r=0.31$, $p<0.001$), which is considered an average correlation for dietary data [72, 76]. However, we do not have information on quantity of supplementation and supplements do provide varying amounts of vitamin D. Vitamin D is also derived from alternative sources besides supplements such as vitamin fortified foods and sun exposure [72].

Regarding whole grain intake, we saw that the median concentrations of total AR were significantly higher in the women who reported a more frequent consumption of whole grains. The correlation between all reported consumption of whole grains and AR was $r=0.26$ $P<0.001$, which is considered below average [72, 76]. These low correlation values may be explained by the women reporting all whole grain consumption and AR concentrations represent mostly whole grains from wheat and rye [86]. This excludes other dietary sources of whole grains such as oats, brown rice, quinoa etc. Concentrations of AR also represent recent whole grain intake while the diet screening questionnaire is based on intake during the previous month [87].

Our findings indicate that the diet screening questionnaire used in our study is a tool that can be used to estimate the dietary and nutrient intake of pregnant

women as a means of identifying women in need of dietary intervention. Therefore, our findings have provided important insights into the existing gaps in maternal nutrition and may help to guide maternal care, hopefully resulting in more healthy pregnancies. This was the ultimate aim of the PREWICE diet screening questionnaire. Fortunately, following these results indicating its use as a clinical screening tool, the Icelandic health care system is working on incorporating it into the website National Citizen Health Portal (Heilsuvera) in Iceland [188]. The portal is a centralized web-application where all citizens have secure, digital access to their own health information (e.g., maternal records) and official eHealth services currently available in the country. When completed, it will be made available to midwives for use in maternal care. To begin with, the diet screening questionnaire will aim to identify factors associated with the risk of nutrient deficiency or excessive intake. However, in the future, the hope is to incorporate the means of identifying overall dietary patterns associated with the risk of GDM and excessive weight gain. This would provide a great opportunity to investigate the effects of dietary intervention during pregnancy based on dietary patterns.

By changing *in utero* exposure, such as by aiming for optimal maternal nutrition and recommended weight gain levels, it may be possible to minimise the risk of adverse metabolic programming [189]. Our overall results underline the importance of assessing dietary intake during early pregnancy to identify which women require dietary counselling. This illustrates the importance of introducing prevention strategies during pregnancy to reduce the future risk to offspring [5]. Early dietary interventions could be beneficial in reducing unfavourable outcomes during pregnancy [190] and increasing diet variety [191]. Some intervention studies have also reported a decrease in GDM rates in addition to improved maternal and neonatal outcomes [192]. However, approaches to prevent GDM are still needed [193]. Many previous interventions during pregnancy have recruited participants based on their BMI. However, not all women with a higher BMI have low diet quality [28]. By using the diet screening questionnaire to screen for pregnancy diet quality first and foremost, it may be possible to recruit participants in true need of intervention. This method could determine whether providing dietary intervention during pregnancy has a positive effect on the GDM rate when compared to standard maternal care. Since Iceland is a small country with established and frequently used prenatal care, we believe that it is a suitable place to test the feasibility of diet screening and interventions in early pregnancy.

Screening diets using the questionnaire provides an opportunity to identify the women in need of improved diet and vitamin D status.

6.6 Diet during pregnancy in Iceland

Both the Icelandic dietary guidelines and the specific guidelines for diet during pregnancy emphasize the importance of taking vitamin D supplements [19, 150]. Despite this, there were 104 women (11%) in this cohort who were not taking any vitamin D supplements. Many of the women who reported taking vitamin D supplements also demonstrated surprisingly low concentrations of S-25OHD, suggesting that they may not be meeting their requirements or have started taking the supplements recently. This suggests that the message regarding the importance of vitamin D supplements is not reaching everyone successfully, which needs to be addressed. Vitamin-D is important for calcium uptake and dietary sources are limited to fatty fish, egg yolks and some fortified foods [106].

Overall, the women´s adherence to dietary recommendations regarding whole grains, unsaturated fats and fish was inadequate. In total, 85.1% (n = 726), of the women were not adhering to the recommended consumption of two portions of whole grains daily. Even though this is a high rate, consumption of whole grains does appear to be greater when compared to results from PREWICE I (2015-2016)[194]. There the median weekly intake of whole grains was 2.8 times/week in PREWICE I to 5.6 times/week in our study. This is a positive change which could possibly be explained by an increase in availability of wholegrain breads and other wholegrain products in Iceland. However, this is still a low rate, especially when considering the vast nutritional and health benefits that the consumption of these foods provides in pregnancy [71].

The use of vegetable oil for cooking also appears to have increased since 2015-2016 going from a median of 2.5 times/week to 5.0 times/week in 2017-2018. Only 35% of the women consumed fish at least twice a week (as recommended) [18, 19] and they were more likely to have lean fish as opposed to fatty fish. However, there was a correlation between the intake of lean and fatty fish. Therefore, it was likely the same women who were consuming both fatty and lean fish. The median fish intake was 1.3 times per week, which is very similar to results from previous Icelandic studies [12, 194], suggesting that consumption of fish has not increased over the past decade. Fish is one of the main dietary sources of long chain omega-3 FA and has few dietary alternatives. However, the internal synthesis of EPA and DHA from ALA is possible [195]. This process requires enzymes to elongate ALA from 18:3 into 20:5 (EPA), which can subsequently be elongated to 22:6 (DHA) [196]. This process can be affected by several factors, such as the dietary intake of fat, genetics, age and health status [197]. Overall, it does appear that the amount of ALA elongated into DHA is quite limited [130, 198].

Overall, 50% of the women used supplements containing omega-3 FA daily, which was reflected in their plasma levels. However, of the 554 women (65%) who were not eating fish at least twice per week, only approximately half were taking any supplements containing omega-3. Fish and dairy are the main dietary sources of iodine in Iceland. Therefore, pregnant women are recommended to ensure regular consumption of these foods. Substituting these with seaweed or seaweed containing supplements is not recommended as they may contain harmful substances for the fetus [18]. Omega-3 fatty acids are necessary for all individuals but during pregnancy the need for DHA is especially important for the normal development of the child´s nervous system. To meet this requirement the pregnant women are recommended to consume fatty fand lean fish weekly. Alternatively the women could supplement with cod liver oil daily, to meet the need for DHA which is estimated at 200 mg per day [18]. These results indicate that many pregnant women in Iceland do not have an intake of long omega-3 FA that can be considered sufficient. This suggests the need to obtain information regarding fish consumption in early pregnancy to evaluate whether omega-3 supplements are required. It also appears to be important to consider the form of recommended omega-3 supplements.

When we compared dietary intake of foods groups between the women who were later diagnosed with GDM and those who were not we saw few differences. The main differences observed are regarding consumption of fatty fish, fruit juice, coffee and whole grains. The women who were later diagnosed with GDM reported a significantly lower intake frequency for these food groups. Intake frequencies of skimmed milk as well as beans, nuts and seeds were also significantly different between the groups, with a tendency of frequency being skewed towards a lower intake among the women later diagnosed with GDM. The medians, however, were the same for both groups. Reported intake of omega-3 supplements and vegetable oil for cooking also tended to be lower for the women later diagnosed with GDM, with differences being close to significance.

Interestingly, four of the food groups that women, who were not diagnosed with GDM, consumed more of (fish, coffee, vegetable oil, nuts and seeds) were also part of an Icelandic prudent dietary pattern associated with a decreased risk of GDM [28]. This prudent pattern also contains breakfast cereals and pasta/cous cous, without discerning between whole grain versions or not.

6.7 Strengths and limitations

The main strengths of the study this thesis is based on are that it features a large sample size, high participation rate and data that were collected prospectively. Results are based on a combination of objective biochemical analysis and a subjective questionnaire.

In the current study, vitamin D status of pregnant women in Iceland was studied for the first time. Therefore, it has provided important new information. Further strengths include our use of a novel objective method to measure whole grain consumption and analysis of plasma FA during early pregnancy. We reported both total and relative concentrations of all FA subgroups and stratified our FA results by BMI, a process that, to our knowledge, has not been previously performed in a pregnancy cohort.

Recruitment was limited to the capital of Iceland (Reykjavik), which might be considered a limitation. However, according to Statistics Iceland, approximately 70% of all women in Iceland live in the capital area. However, we cannot exclude the possibility that the dietary and supplemental intake of women living outside the capital area could be different from those who could participate in the study.

The limitations regarding blood samples are that the women were not fasting at the time of sampling. This factor, as well as the time of day and individual absorption, may have affected the results of biochemical analyses. However, we assessed the rate of women later diagnosed with GDM based on whether they participated in the morning or afternoon, and the rates were similar. Other limitations are that we did not have information on physical activity nor exact information on the date of GDM diagnosis.

7 Conclusion

Previous results suggest that there is a potential nutritional risk in part of the pregnant population and that the nutritional quality of diet during pregnancy in Iceland can be greatly improved. In our studies we have provided valuable information on early pregnancy dietary intake, nutritional status, and associations to risk of gestational diabetes. Approximately one third of pregnant women in Iceland have vitamin-D concentrations below adequate levels during the first trimester of pregnancy, indicating that necessary actions must be taken to remedy this. The findings also suggest that increasing the overall nutritional quality of the diet, especially regarding wholegrain intake, during pregnancy may lower the risk of gestational diabetes. In our study of early pregnancy plasma fatty acids, a difference in the fatty acid profile for the women who were diagnosed with gestational diabetes later in pregnancy was observed, independent of the women's body mass index. This suggests that fatty acid profiles could be a possible gestational diabetes predicter, although this requires further research. Overall intake of fish was low and about half of the women were not taking any omega-3 containing supplements daily. These results indicate that many pregnant women in Iceland do not have a sufficient intake of long omega-3 FA. This suggests a need for obtaining information regarding fish consumption in early pregnancy to evaluate if omega-3 supplements are required. Our findings also indicate that the diet screening questionnaire used in our study, is a tool that can be used to estimate food consumption of selected food groups and intake of specific nutrients during pregnancy. Its use may therefore indicate those women who need dietary intervention. These findings have therefore provided important information into the existing gaps of maternal nutrition and may contribute a tool to guide maternal care.

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Paper I

food & nutrition research

ORIGINAL ARTICLE

Vitamin D status and association with gestational diabetes mellitus in a pregnant cohort in Iceland

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Popular scientific summary

- Vitamin D supplementation is encouraged by the Icelandic authorities and was quite widespread (80%) in the population studied.
- Approximately one-third of this cohort reported S-25OHD concentrations below adequate levels (< 50 nmol/L) during the first trimester of pregnancy.
- No clear association was seen between vitamin D status and GDM in the population studied.

Abstract

***Background*:** Vitamin D deficiency has been associated with an increased risk of gestational diabetes mellitus (GDM), one of the most common pregnancy complications. The vitamin D status has never previously been studied in pregnant women in Iceland.
***Objective*:** The aim of this research study was to evaluate the vitamin D status of an Icelandic cohort of pregnant women and the association between the vitamin D status and the GDM incidence.
***Design*:** Subjects included pregnant women (n = 938) who attended their first ultrasound appointment, during gestational weeks 11–14, between October 2017 and March 2018. The use of supplements containing vitamin D over the previous 3 months, height, pre-pregnancy weight, and social status were assessed using a questionnaire, and blood samples were drawn for analyzing the serum 25-hydroxyvitamin D (25OHD) concentration. Information regarding the incidence of GDM later in pregnancy was collected from medical records.
***Results*:** The mean ± standard deviation of the serum 25OHD (S-25OHD) concentration in this cohort was 63±24 nmol/L. The proportion of women with an S-25OHD concentration of ≥ 50 nmol/L (which is considered adequate) was 70%, whereas 25% had concentrations between 30 and 49.9 nmol/L (insufficient) and 5% had concentrations < 30 nmol/L (deficient). The majority of women (n = 766, 82%) used supplements containing vitamin D on a daily basis. A gradual decrease in the proportion of women diagnosed with GDM was reported with increasing S-25OHD concentrations, going from 17.8% in the group with S-25OHD concentrations < 30 nmol/L to 12.8% in the group with S-25OHD concentrations ≥75 nmol/L; however, the association was not significant (P *for trend* = 0.11).
***Conclusion*:** Approximately one-third of this cohort had S-25OHD concentrations below adequate levels (< 50 nmol/L) during the first trimester of pregnancy, which may suggest that necessary action must be taken to increase their vitamin D levels. No clear association was observed between the vitamin D status and GDM in this study.

Keywords: *vitamin D*; *pregnancy*; *supplements*; *gestational diabetes mellitus; nutritional status; cod liver oil*

To access the supplementary material, please visit the article landing page

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Vitamin D deficiency or insufficiency during pregnancy is believed to be a common problem reported worldwide (1). However, the vitamin D status of pregnant women has never been studied in Iceland. In countries that receive limited sunshine, such as Iceland, vitamin D formation in the skin can be very limited, especially during the winter months (from October to March) (2, 3). A very few natural sources of vitamin D exist, and all Icelanders are, therefore, encouraged to consume vitamin D in the form of supplements or cod liver oil, especially during the winter season (4).

Although the most well-established role of vitamin D involves the control of blood calcium concentrations and the maintenance of healthy bones (5), vitamin D deficiency has also been associated with several other conditions and diseases, including gestational diabetes mellitus (GDM) (1, 6). The mechanisms that underlie this association are not fully understood and are thought to be manifold (7). Vitamin D receptors are expressed by many different cells, including muscle cells and pancreatic beta cells (8), and may influence glucose metabolism, insulin secretion, and insulin resistance (9, 10). Studies that have investigated the association between the vitamin D status and the increased gestational diabetes risk have been somewhat conflicting, with some studies showing that vitamin D is associated with an increased risk of GDM, and others reporting no relationship between these two factors (11).

The primary aim of this study was to assess the vitamin D status of pregnant women in Iceland and to determine whether the intake of dietary supplements containing vitamin D was associated with 25-hydroxyvitamin D (25OHD) concentrations in the serum. The secondary aim of this study was to investigate the association between the vitamin D status and the gestational diabetes incidence.

Methods

Subjects

All women who visited the Prenatal Diagnostic Unit at Landspitali National University Hospital, Reykjavik, Iceland during gestational weeks 11–14, between 2 October 2017 and 28 March 2018, were invited to participate in this study. During the study period (6 months), 1,684 women were scheduled to undergo their first ultrasound screening at Landspitali, corresponding to approximately 77% of the total pregnant population in Iceland. Of these 1,684 women, 244 women (15%) were excluded from the study because they did not speak Icelandic and could, therefore, not respond to the questionnaire. Other exclusion criteria included women outside of the gestational weeks 11–14 defined in this study, women who failed to appear at their scheduled appointment times, and women who experienced miscarriage, which resulted in the exclusion of an additional 90 women. Of the remaining 1,350 women deemed to be eligible for participation, 329 declined to participate for various reasons. Of the 1,015 women who were enrolled in this study, blood samples were obtained from 942 women. Information regarding GDM diagnosis later in pregnancy was retrieved from the medical records of 837 women who also had their blood drawn for the assessment of vitamin D status. The study was approved by the National Bioethics Committee and the Medical Directorate of Landspitali University Hospital. Written informed consent was obtained from all participants.

Assessment of vitamin D status

Blood samples were obtained from subjects during gestational weeks 11–14. Serum samples were stored at −80°C until analysis of serum 25OHD (S-25OHD) concentration using an electrochemiluminescence immunoassay at the Clinical Core Laboratory, Landspitali University Hospital, which was performed during spring 2019. Control samples, which are measured daily by the laboratory, have shown that the CV% of this analysis method is approximately 4–5%. S-25OHD concentrations ≥ 50 nmol/L were considered adequate, 30–49.9 nmol/L were considered insufficient, and < 30 nmol/L was defined as deficient (2, 5). We also report the number and rate of women with S-25OHD concentrations ≥ 75 nmol/L, as no consensus exists regarding optimal S-25OHD levels, and some researchers use a cut-off value of 75 nmol/L as an indicator of adequate vitamin D status (12, 13).

Dietary and supplement intake and background variables

Women who agreed to participate in this study answered a questionnaire presented in an electronic format. The questionnaire included questions regarding background information, including maternal age, education, smoking habits, parity, nausea in pregnancy, pre-pregnancy weight, and height. Information regarding weight and height was used to calculate the pre-pregnancy body mass index (BMI, in kg/m^2). BMI < 18.5 kg/m^2 was defined as underweight, 18.5–24.9 kg/m^2 as normal weight, 25–29.9 kg/m^2 as overweight, and ≥ 30.0 kg/m^2 as obese.

Supplement intake during the previous 3 months (starting at approximately the onset of pregnancy) was assessed by a short food frequency questionnaire (FFQ), which included questions regarding the frequency of cod liver oil (a traditional source of vitamin D in Iceland), vitamin D supplement, and multivitamin consumption. Information regarding the consumption of other potential dietary sources of vitamin D, such as vitamin D-enriched milk and oily fish, was also assessed through the FFQ. The development of the FFQ has previously been described in detail (14–16).

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GDM

Information regarding the occurrence of GDM was retrieved from maternal hospital records (ICD-10 codes O24.4 and O24.9, but O24.9 is used at Landspitali GDM treated with medications). The criteria for GDM diagnoses were based on the recommendations of the 2010 International Association of Diabetes and Pregnancy Study Groups (IADPSG) Consensus Panel (17). Other information gathered from medical records included gestational age, family history of diabetes mellitus, and measured weight at the first and last maternal care visits, and were used to calculate the total weight gain during pregnancy.

Statistical analysis

Data from the dietary questionnaire and maternal hospital records were entered in SPSS (IBM SPSS Statistics, version 26), where all statistical analysis was conducted. Data are expressed as mean ± standard deviation (SD) for normally distributed variables and as the median and interquartile range (IQR) for skewed variables. Dichotomous variables are reported as frequencies and percentages (%). For continuous variables, an independent sample T-test was used to formally test the significance of differences between the two groups of normally distributed variables, and the Mann-Whitney U test was used to assess the differences among skewed variables. The Chi-square test was used to test differences in dichotomous variables across groups.

Logistic regression analysis was used to examine associations between categories of S-25OHD status (< 30, 30–49.9, 50–74.9, and ≥ 75 nmol/L) and GDM occurrence. The results are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs), both before and after adjustment for covariates, using the deficient S-25OHD status (< 30 nmol/L) as the reference category. The covariates included in our adjusted models were maternal age, pre-pregnancy BMI, parity, and smoking during pregnancy. As a formal test for an association, we used the Chi-square test by modeling categorical variables as continuous terms in the regression model and using the median S-25OHD value for each category. $P < 0.05$ was considered to be statistically significant. All reported *P*-values are two-sided.

Results

The characteristics of the study participants are presented in Table 1, both for the whole cohort and for subgroups of women with and without a later GDM diagnosis. Maternal age was 25–34 years for 66% of cases, and the median (IQR) pre-pregnancy BMI was 24.4 (6.3) kg/m^2. Approximately 93% of participants were married or lived with a partner. Women who later developed GDM were more likely to be primi/multiparous and had higher pre-pregnancy BMI [median (IQR) 26.6 (8.8) kg/m^2 vs. 24.2 (5.9) kg/m^2] but lower gestational weight gain [mean (SD) 9.7 (6.1) kg vs. 12.7 (5.2) kg] than those who did not develop GDM. No association was found between the vitamin D status and BMI or social status, other than marital status. Single mothers tended to have lower S-25OHD concentrations than married or partnered mothers ($P < 0.05$).

Table 2 shows the vitamin D status of the whole cohort, as well as the use of vitamin D supplements. The proportion of women with S-25OHD concentrations ≥ 50 nmol/L (adequate) was 70%, whereas 25% of them had S-25OHD concentrations of 30–49.9 nmol/L (insufficient) and 5% had concentrations < 30 nmol/L (deficient). The majority of women ($n = 766$, 82%) who consumed supplements of vitamin D daily had a mean ± SD S-25OHD concentration of 66 ± 24 nmol/L. Interestingly, approximately 24% of women who took vitamin D supplements daily were classified as having an insufficient vitamin D level (< 50 nmol/L). Women who reported never using vitamin D supplements ($n = 104$, 11%) had a mean ± SD S-25OHD concentration of 45 ± 18 nmol/L. In this group, approximately 66% of women were defined as having an insufficient S-25OHD concentration (< 50 nmol/L), including 18% who were defined as deficient (< 30 nmol/L). Both non-users and irregular users of vitamin D supplements had significantly reduced S-25OHD concentrations compared with those taking daily supplements containing vitamin D ($P < 0.01$). A majority of the women (89%) who had sufficient vitamin D status (≥ 50 nmol/L) used vitamin D supplements on a daily basis.

The amounts of vitamin D found in the most commonly used supplements available in Icelandic markets are shown in Supplementary file. The number (%) of subjects taking various daily supplements containing vitamin D, their 25OHD concentrations, and the number (%) of subjects categorized as having deficient (< 30 nmol/L), insufficient (< 50 nmol/L), and sufficient (≥ 50 nmol/L) vitamin D status are also shown in the same Supplementary file. Relatively few women used vitamin D (1 µg/100 g) enriched milk daily ($n = 100$). A large majority of women who consumed vitamin D-enriched milk daily also consumed daily supplements containing vitamin D ($n = 81$). The average frequency of oily fish consumption was < 0.5 times per week. The consumption of neither vitamin D-enriched milk nor oily fish was associated with vitamin D status.

Medical records were obtained for 837 women, including 126 women (15%), who were eventually diagnosed with GDM (Table 3). The mean ± SD S-25OHD concentration of the GDM group was 60 ± 24 nmol/L, compared with 63 ± 24 nmol/L in the non-GDM group ($P > 0.05$). Approximately 35% of subjects with GDM and 30% of non-GDM subjects had S-25OHD concentrations < 50

Table 1. Characteristics of the subjects divided according to the diagnosis of gestational diabetes

Characteristics	All (*n* = 938)		GDM (*n* = 126)		Non-GDM (*n* = 711)		Pa
Maternal age (year), *n* (%)	*n*	%	*n*	%	*n*	%	0.06[b]
18–24	144	15.3	14	11.1	114	16.0	
25–29	341	36.2	48	38.1	261	36.7	
30–34	281	29.8	33	26.2	218	30.7	
35–39	138	14.6	24	19.0	95	13.4	
40–45	27	2.9	7	5.6	19	2.7	
Parity, *n* (%)							0.03[b]
Nulliparous	411	43.6	51	40.5	316	44.4	
Primi/multiparous	524	55.6	73	57.9	394	55.4	
Marital status, *n* (%)							0.76[b]
Married/cohabitant	874	92.7	106	95.1	661	95.0	
Single	42	4.5	6	4.9	35	5.0	
Smoking in pregnancy, *n* (%)	43	4.6	7	5.6	31	4.4	0.12[b]
Education level, *n* (%)							0.34[b]
Less than elementary school	5	0.5	0	0.0	5	0.7	
Elementary school	104	11.0	16	12.9	73	10.3	
High school and technical school	273	29.0	38	30.6	208	29.3	
Bachelor's degree	317	24.9	35	28.2	257	36.2	
Master's or doctorate degree	235	24.9	35	28.2	167	23.5	
Height (cm), mean ± SD	167.5 ± 7.4		168.1 ± 5.2		167.3 ± 7.8		0.34[c]
Pre-pregnancy weight (kg), mean ± SD	72.3 ± 15.7		79.2 ± 19.0		71.3 ± 15.0		<0.01[d]
Pre-pregnancy body mass index (BMI), median (IQR)	24.4	6.3	26.5	8.8	24.2	5.9	<0.01[d]
Pre-pregnancy BMI (groups), *n* (%)							<0.01[b]
<18.5 kg/m^2	18	1.9	2	1.6	13	1.8	
18.5–24.99 kg/m^2	498	52.9	50	41.0	388	55.0	
25–29.99 kg/m^2	240	25.5	30	24.6	186	26.4	
≥30 kg/m^2	171	18.2	40	32.8	118	16.7	
Gestational weight gain (kg), mean ± SD	12.3 ± 6.9		9.7 ± 6.1		12.7 ± 5.2		<0.01[c]
Gestational week at delivery, median (interquartile range)	39.7	3.3	39.6	2.3	39.9	2.7	0.76[d]
Family history of type 2 diabetes, *n* (%)	11	1.2	2	1.8	6	1.0	<0.01[b]

Missing data in the total group: maternal age *n* = 7; height *n* = 7; pre-pregnancy weight *n* = 11; pre-pregnancy BMI *n* = 11; pre-pregnancy BMI (groups) *n* = 11; parity *n* = 3; marital status *n* = 22; prenatal smoking *n* = 8; education level *n* = 4. Information on GDM diagnosis was available for 837 subjects.
[a]Differences between GDM and non-GDM.
[b]Chi-square test for differences among groups.
[c]T-test for differences among groups.
[d]Mann-Whitney U test for difference among groups.

Table 2. S-25OHD concentration (nmol/L) in all subjects (*n* = 938) and according to the use of supplements containing vitamin D (*n* = 935)

	n	mean ± SD	<30 nmol/L		30–49.9 nmol/L		50–74.9 nmol/L		≥ 75 nmol/L	
			n	%	*n*	%	*n*	%	*n*	%
All subjects	938	63.0 ± 24.4	51	5.4	234	24.9	398	42.4	255	27.2
Not taking any supplements containing vitamin D	104	44.6 ± 17.5	19	18.3	50	48.1	29	27.9	6	5.8
Irregular use of supplements containing vitamin D*	65	55.1 ± 21.1	6	9.2	24	36.9	25	38.5	10	15.4
Daily vitamin D supplementation	766	65.9 ± 24.1	26	3.4	160	20.9	342	44.6	238	31.1

*Subjects reporting use of vitamin D supplements from 1–2 times per month up to 4–6 times per week.

nmol/L. A gradual decrease in the proportion of women diagnosed with GDM was found to occur with increasing S-25OHD concentrations, going from 17.8% in the group with S-25OHD concentrations < 30 nmol/L to 12.8% in the group with S-25OHD concentrations ≥ 75 nmol/L (P for trend = 0.17). After adjustment for covariates, as presented in Table 3, the association was somewhat strengthened but remained non-significant (P = 0.11). The OR

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Table 3. Vitamin D status in subjects who later were diagnosed with gestational diabetes mellitus (n = 126) or not (total number of subjects included in the analysis n = 837)

	Serum 25OHD (nmol/L)				*P* for trend[a]
	<30	30–49.9	50–74.9	≥75	
No cases (%)/*n*	8 (17.8%)/45	36 (17.2%)/209	53 (14.9%)/356	29 (12.8%)/227	
Unadjusted OR (95% CI)	1.00	0.96 (0.41, 2.24)	0.81 (0.36, 1.83)	0.68 (0.29, 1.60)	0.17
Adjusted OR (95% CI)[b]	1.00	0.90 (0.38, 2.12)	0.77 (0.33, 1.76)	0.60 (0.25, 1.45)	0.11

[a]Chi-square test.
[b]Adjusted for maternal age, parity and maternal pre-pregnancy body mass index, and smoking during pregnancy.

for GDM among women with S-25OHD concentrations ≥ 75 nmol/L compared with those with S-25OHD concentrations < 30 nmol/L was 0.60 (95%CI: 0.25, 1.45).

Discussion

In this study, 70% of the women (n = 942) had S-25OHD concentrations ≥ 50 nmol/L, which is considered adequate. The remaining 30% had insufficient S-25OHD concentrations, with 25% presenting S-25OHD concentrations between 30 and 49.9 nmol/L (insufficient) and 5% presenting S-25OHD concentrations < 30 nmol/L (deficient). Approximately 27% of the women had S-25OHD concentrations ≥ 75 nmol/L. No clear association was observed between the vitamin D status and GDM in this study.

A majority of subjects in this study (approximately 82%) used supplements containing vitamin D daily, in line with guidelines established by Icelandic health authorities (18). The frequency of vitamin D supplement intake in Iceland appears to be higher in this study than has been reported in other countries (19). The most common type of vitamin D supplements used in this study included vitamin D tablets (51%), whereas fewer than 20% of participants reported using the traditional Icelandic source of vitamin D, cod liver oil. A concerning finding was that 25% of those who claimed they used supplements daily had inadequate vitamin D status. Some but not all of these participants reported the use of multivitamin supplements, which typically contain 5–10 µg (200–400 IU) vitamin D in a daily dose. This dose is lower than the current 15 µg/day (600 IU) recommended daily intake (RDI) for adults, including pregnant women, established by Icelandic health authorities (18). In comparison, most single-nutrient vitamin D supplements available on the Icelandic market contain 25–50 µg (1,000–2,000 IU) vitamin D per daily serving. Some women may have only recently begun to take supplements containing vitamin D, and may have entered pregnancy with low S-25OHD concentrations. The RDI for vitamin D is the required amount necessary to maintain an adequate vitamin D status, and may not be high enough to correct an insufficient status (20, 21). Van Groningen et al. (22) developed a practical method for calculating the vitamin D dose required to rapidly correct vitamin D deficiency in individuals using the following equation to estimate the necessary loading dose to increase vitamin D levels to 50 nmol/L: dose (IU) = 40 × [50 − serum 25-OHD (nmol/L)] × [body weight (kg)]. According to this equation, a 72-kg woman with a pre-pregnancy S-25OHD concentration of 24 nmol/L would require 6 months to achieve an S-25OHD concentration of 50 nmol/L when consuming 10 µg vitamin D daily, which is the amount of vitamin D provided in many of the most commonly used multivitamins on the Icelandic market. If the goal is to achieve an S-25OHD concentration > 50 nmol/L before week 20, the dose would need to be increased to as high as 45 µg/day (1,800 IU). In a recent study, a dose of 30 µg/day (1,200 IU) was suggested to be necessary to maintain a sufficient vitamin D status (50 nmol/L) for the majority of pregnant, white-skinned women at northern latitudes, which would also maintain an umbilical cord S-25OHD concentration of ≥ 25–30 nmol/L for almost all newborns (23). Other studies have suggested that doses greater than the current RDI dose are necessary to maintain adequate vitamin D levels in pregnancy (24–26).

Previous studies conducted to examine the association between the vitamin D status and GDM risk have reported somewhat conflicting results. Recent systematic reviews and meta-analyses found a relationship between low vitamin D status (insufficient or deficient) and an increased risk of GDM (11–13, 27–29). These studies also reported that vitamin D levels were lower in women with GDM than in those with normal glucose levels. However, in this study, no significant difference in vitamin D status was observed between women who developed GDM later in pregnancy and those who did not, and no significant relationship was identified between low vitamin D levels and an increased risk of GDM. The proportion of women with vitamin D deficiency (S-25OHD < 30 nmol/L) was relatively low in our cohort, and the number of women taking supplements containing vitamin D may have been higher than in previous studies (these data are not always reported). Clinical trials examining the effect of vitamin D supplements on GDM patients have yielded

unclear results (30). Researchers have identified several possible factors that might confound results. For example, low vitamin D concentrations might not be a contributing factor for GDM, or a causal relationship may exist in the opposite direction. In addition, factors associated with study design and whether the subjects were at high risk of developing GDM or vitamin D deficiency at baseline could also affect the outcomes. The supplementation doses, supplementation periods, and methods used to assess S-25OHD concentrations can vary, and measurements may be performed at different pregnancy stages. In some studies, all participants were supplemented with vitamin D for ethical reasons, which could also affect the results (24, 31, 32).

The primary strengths of this study are the inclusion of a large sample size and a high participation rate. According to Statistics Iceland, 2,188 infants were born in Iceland between April 2018 and September 2018 (which corresponds with the expected delivery dates for women attending 11–14-week ultrasound examinations during the study period). This study, therefore, includes 45% of the total population of pregnant women in Iceland. One of the limitations may be that recruitment was limited to the capital of Reykjavik. According to Statistics Iceland, approximately 70% of women in Iceland live in the capital region. However, we cannot exclude the possibility that the vitamin D status of women living outside the capital area may be different from that of the women included in this study. Furthermore, the number of women that were excluded for not speaking Icelandic indicates the importance of including English versions of the questionnaires for future studies performed in Iceland. The vitamin D status of pregnant women in Iceland has never been studied before; therefore, this study provides new and important information regarding the vitamin D status of this sensitive group during the winter months when skin-based vitamin D production is limited in Iceland due to limited sunlight.

This study also features some limitations. Although we obtained the estimated vitamin D levels from various types of supplements available on the Icelandic market, additional details would be preferable, including the total vitamin D intake from food. However, the results of this study suggested that the contributions of dietary vitamin D sources to total vitamin D intake and status are minimal. Although the Roche method used to analyze serum 25OHD concentration in this study has been successfully applied over the years, a recent issue was identified that Roche has not yet been able to solve, in which the method occasionally falsely reports high measurements (33). Because of this issue, vitamin D results are particularly closely monitored, and all samples that report high levels are repeated for confirmation.

Conclusion

Vitamin D supplementation is encouraged by Icelandic authorities and was widely used (approximately 80%) in the studied population. However, approximately one-third of our cohort had serum S-25OHD concentrations below adequate levels (< 50 nmol/L) during the first trimester of pregnancy, which suggests the necessary actions to increase vitamin D levels to be taken among this population. No clear association was observed between the vitamin D status and GDM in this study.

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Supplement table 1. Number (%) of subjects taking different types of supplements containing vitamin D daily, concentration of 25OHD and number (%) of subjects defined with deficient, insufficient, and sufficient vitamin D status.

Cod liver oil (15–20 µg D_3)*	*n* (%)**	175 (18.7)
	25OHD nmol/L, mean ± SD	66.1 ± 25.6
	25OHD <30 nmol/L, *n* (%)	4 (2.3)
	25OHD 30–49.9 nmol/L, *n* (%)	39 (22.3)
	25OHD 50–74.9 nmol/L, *n* (%)	76 (43.2)
	25OHD ≥75 nmol/L, *n* (%)	56 (32.0)
Vitamin D supplement (25–50 µg D_3)*	*n* (%)**	477 (51.0)
	25OHD nmol/L, mean ± SD	70.2 ± 25.5
	25OHD <30 nmol/L, *n* (%)	12 (2.5)
	25OHD 30–49.9 nmol/L, *n* (%)	76 (15.9)
	25OHD 50–74.9 nmol/L, *n* (%)	211 (44.2)
	25OHD ≥75 nmol/L, *n* (%)	178 (37.3)
Omega-3 with vitamin D (20 µg D_3)*	*n* (%)**	164 (17.5)
	25OHD nmol/L, mean ± SD	65.4 ± 20.3
	25OHD <30 nmol/L, *n* (%)	4 (2.5)
	25OHD 30–49.9 nmol/L, *n* (%)	28 (17.1)
	25OHD 50–74.9 nmol/L, *n* (%)	80 (48,8)
	25OHD ≥75 nmol/L, *n* (%)	52 (31.7)
Multivit with vitamin D (5–10 µg D_3)*	*n* (%)**	264 (28.2)
	25OHD nmol/L, mean ± SD	62.5 ± 23.5
	25OHD <30 nmol/L, *n* (%)	12 (4.5)
	25OHD 30–49.9 nmol/L, *n* (%)	71 (26.9)
	25OHD 50–74.9 nmol/L, *n* (%)	111 (42.0)
	25OHD ≥75 nmol/L, *n* (%)	70 (26.5)

*Vitamin D_3 content of the most popular brands in each category, available in the Icelandic market.

** Some subjects reported daily use of more than one product containing vitamin D.

Paper II

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Higher Alkylresorcinol Concentrations, a Consequence of Whole-Grain Intake, are Inversely Associated with Gestational Diabetes Mellitus in Iceland

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ABSTRACT

Background: A diet rich in whole grains may provide benefits for pregnant women due to whole grains' high nutritional value and dietary fiber content.

Objectives: To study the associations of whole-grain consumption, as well as the plasma alkylresorcinol concentration, a whole-grain consumption biomarker, in early pregnancy with gestational diabetes mellitus (GDM) diagnoses.

Methods: Subjects were women from the prospective study Pregnant Women in Iceland II (PREWICE II; $n = 853$) who attended their ultrasound appointment in gestational weeks 11–14 during the period from October 2017 to March 2018. During that visit, whole-grain consumption was estimated using a diet screening questionnaire, and blood samples were collected for analysis of plasma alkylresorcinols (ARs). Information on GDM diagnoses was later extracted from medical records. Multivariate log-binomial regression was used to evaluate the association of dietary whole-grain and AR concentrations with GDM.

Results: In total, 14.9% of the women adhered to the national food-based dietary guidelines ($n = 127$), which recommend 2 portions of whole grains daily. GDM was diagnosed in 127 women (14.9%). The frequency of whole-grain consumption was lower in women who were later diagnosed with GDM compared to the women without GDM (median, 5 times/week vs. 6 times/week, respectively; $P = 0.02$). This difference was reflected in the lower median concentration of total AR in women diagnosed with GDM (163 nmol/L vs. 209 nmol/L, respectively; $P < 0.01$). The quartile with the highest concentrations of AR had a RR of 0.50 (95% CI: 0.27–0.90) of being diagnosed with GDM, in comparison to the lowest quartile. There was a significant dose response in the GDM risk with higher AR levels.

Conclusions: We found that a higher consumption of whole grains, reflected both by reported consumption according to the FFQ and AR biomarkers, was associated with a decreased risk of receiving a GDM diagnosis. *J Nutr* 2021;0:1–8.

Keywords: alkylresorcinol, biomarkers, pregnancy, whole grains, diet, gestational diabetes

Introduction

Gestational diabetes mellitus (GDM), a state of hyperglycemia due to insulin resistance (1), is among the most common complications diagnosed in pregnancy. It is associated with several adverse outcomes for both mother and offspring (2–4). The rates of GDM diagnoses differ among studies and have been demonstrated to vary depending on populations and diagnostic criteria (5, 6). Even though some level of insulin resistance is a normal part of a pregnancy that plays a role in supplying adequate nutrients to the fetus, it can progress to GDM in many cases (3). This usually occurs between weeks 20 and 24 of gestation, when the growing placenta produces higher levels of hormones and the insulin resistance of the mother increases. The result can be an increased flow of glucose across the placenta, followed by a spike of insulin production in the fetus' beta cells. The resulting hyperinsulinemia in the fetus may lead to excessive growth of fat and protein stores in late gestation. Therefore, children of mothers with GDM are more likely to be

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born large for their gestational age (over the 90th percentile for their gestational age) or macrosomic (>4000 g) (7, 8), which increases the risk of further complications, such as shoulder dystocia or caesarean section (7, 9, 10). Moreover, both the child and the mother are at greater risk of developing type 2 diabetes and cardiometabolic disorders later in life (1, 2, 9, 10).

Consumption of whole grains is recommended during pregnancy, as they provide more nutrients, fiber, and phytochemicals than refined grains. Consumption of whole grains also leads to a lower glycemic response than habitual consumption engenders, which might reduce the risk of developing GDM (11); this pattern is further supported by studies that demonstrate that high whole-grain consumption is a central component of healthy dietary patterns, which have been associated with GDM prevention (12–14).

Obtaining reliable information on diet can be challenging due to the fact that subjective methods, such as food records, FFQs, or dietary recalls, are known to be prone to relatively large measurement errors (15), while objective methods, which incorporate analyses of blood or urine, are sometimes limited due to cost and other factors, such as proper sample storage (16). Additionally, estimating the correct portion size and type of whole-grain consumption can prove to be a challenge due to variance in food composition data and self-reporting errors (17, 18). Metabolomic studies, which identify biomarkers associated with different food items (19, 20), are therefore valuable. Alkylresorcinols (ARs) are an example of such biomarkers for whole-grain consumption: they are phenolic compounds that are found mostly in the bran of wheat and rye among commonly consumed foods and are usually named based on their chain length and saturation (21). ARs measured in plasma samples have been demonstrated as a valid method by which to reflect whole-grain wheat and rye consumption, with sufficient validity and reproducibility to render the data useful for epidemiological investigations (22, 23). The aim of this study was to examine the associations between the frequency of whole-grain consumption, as well as plasma AR concentrations in early pregnancy, and the risk of developing GDM.

Methods

Subjects

All women who attended first trimester screenings, at 11–14 weeks of gestation, at the Prenatal Diagnostic Unit at The National University Hospital, Reykjavik, Iceland, during a 6-month period between October 2017 and March 2018 were invited to participate in the study (**Supplemental Figure 1**). During the study period, 1684 women were scheduled for first trimester screenings with ultrasounds and biomarker measurements at Landspitali, which corresponded to approximately 77% of the pregnant population in Iceland. Of these 1684 women, 244 women (15%) were excluded from the study because they did not speak Icelandic, and therefore could not fill out the questionnaire. Other exclusion factors included not being within the 11–14-week pregnancy range, missing the scheduled appointment time, or miscarriage, which in total excluded an additional 90 women. This left 1350 women eligible to participate in the study. Of these, 128 women declined due to personal time constraints, and 207 declined without further explanation. Therefore, 75% of eligible women ($n = 1015$) agreed to participate in the study. Blood samples for plasma AR concentrations were provided by 954 of the 1015 participating women. We were only able to gather information regarding GDM diagnoses for the women who gave birth at Landspitali University hospital, or 84% of study participants ($n = 853$). When comparing characteristics between the 162 women with unavailable GDM data to our final cohort of 853 women (**Supplemental Table 1**), we found no significant differences between the groups except in BMI, which was higher for the women with unavailable GDM data. However, information on BMI was only available for 52 of the 162 women with unavailable GDM data.

The study was approved by the National Bioethics Committee (VSN-17–057-S1) and the Medical Directorate of Landspitali University Hospital (LSH 5–17). Written consent was obtained from the participants.

Dietary intake and background

Subjects answered a short questionnaire in an electronic format on their dietary intake (FFQ), maternal age, education, smoking habits, parity, nausea in pregnancy, pre-pregnancy weight, and height.

The questionnaire was pilot tested in a group of 25 pregnant women and compared with a 4-day weighed food record prior to its use in Pregnant Women in Iceland I (PREWICE I; 2015–2016), with acceptable correlations (Spearman's correlation > +0.3) for most food groups/items (24). A dose-dependent association has previously been described in the present cohort between consumption of dairy (a main source of dietary iodine in Iceland) and urine iodine concentration (25).

The FFQ assessed dietary habits through inquiring about the frequency of consumption of 40 different food items and beverages, as well as dietary supplement intake. The instructions were to record dietary consumption reflecting the past 3 months (approximately from the beginning of pregnancy). Women selected between 10 potential frequency responses, ranging from "less than once a month" to "more than 5 times a day." The development of the FFQ has previously been described in detail (24–26). The frequency responses for whole-grain breads (labeled with the Nordic keyhole or similar labels, which are most commonly whole wheat), rye breads, and other whole-grain products (such as pasta, oatmeal, barley, and whole-grain products other than bread) were used to categorize women to compare rates of whole-grain consumption.

Information on GDM diagnoses, based on The International Association of the Diabetes and Pregnancy Study Groups (IADPSG) (27), was gathered from medical records. As it was not always obvious in the records if the diagnoses were GDM A1 (controlled with diet) or GDM A2 (medication needed), all diagnoses were combined (yes/no). We also gathered information on height and measured weight at the first and last maternal care visits, in addition to other visits. The weight information was used to calculate total weight gain during pregnancy. The number of weeks between visits was calculated based on visitation dates, since the amount of time that passed between the first and last maternal visits varied between women. Total weight gain was subsequently divided by the number of weeks passed to acquire information on the rate of weight gain per week. The pre-pregnancy BMI (kg/m^2) was calculated based upon self-reported pre-pregnancy weight and height. A BMI <18.5 kg/m^2 was defined as underweight, a BMI of 18.5–24.9 kg/m^2 was defined as normal weight, a BMI of 25–29.9 kg/m^2 was defined as overweight, and a BMI ≥30.0 kg/m^2 was defined as obese.

Measurement of plasma alkylresorcinols

The voluntary ultrasound assessment provided at Landspitali University Hospital in weeks 11–14 involved blood samples for genetic testing. At this assessment, an extra tube of blood was drawn from women consenting to participate in the present study. Blood samples were processed within 1 hour after collection to separate plasma from red

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Supplemental Figures 1 and 2 and Supplemental Tables 1–7 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: AR, alkylresorcinol; FBDG, food-based dietary guidelines; GDM, gestational diabetes mellitus; IADPSG, The International Association of the Diabetes and Pregnancy Study Groups; PREWICE, Pregnant Women in Iceland; T2D, type 2 diabetes.

TABLE 1 Characteristics of the women in Pregnant Women in Iceland II that did or did not have gestational diabetes mellitus

Characteristics	All $n = 853$	Non-GDM $n = 726$	GDM $n = 127$	P[1]
Age, y	30.3 ± 4.9	29.9 ± 4.8	32.4 ± 5.5	<0.01
Pre-pregnancy BMI,[2] kg/m^2	25.8 ± 5.7	25.4 ± 5.4	28.4 ± 6.8	<0.01
Total weight gain,[3] kg	12.3 ± 5.5	12.8 ± 5.2	9.6 ± 6.1	<0.01
Weight gain,[4] kg/week	0.49 ± 0.2	0.50 ± 0.2	0.39 ± 0.2	<0.01
Parity,[5] %				
0	44	45	41	
1	36	35	40	
≥2	20	20	19	0.60
Education,[6] %				
Elementary school	11	11	13	
Technical/high school	30	29	31	
University education	35	36	28	
Higher academic	24	24	28	0.36
Marital status,[7] %				
Married	24	23	26	
Living together	71	72	69	
Single	5	5	5	0.77
Smoking,[8] %				
Before pregnancy	14	14	17	0.39
During pregnancy	5	4	6	0.55
Family history of diabetes, yes,[9] %	7	6	14	<0.01

Data are presented as means ± SDs or ratios. Abbreviation: GDM, gestational diabetes mellitus.
[1] Differences between non-GDM and GDM using a *t*-test for equality of means and a Pearson's chi-squared test and the Mann-Whitney U test for 2 independent samples.
[2] Information on pre-pregnancy BMI is missing for 10 women.
[3] Information on weight gain is missing for 45 women. Total weight gain is the difference between the measured weights at the first and last maternal care visits.
[4] Weekly weight gain is the total weight gain divided by number of weeks between first and last maternal care visit.
[5] Information on parity is missing for 6 women.
[6] Information on education is missing for 5 women.
[7] Information on marital status is missing for 21 women.
[8] Information on smoking is missing for 6 women.
[9] Information on family history of diabetes is missing for 128 women.

blood cells and the buffy coat through centrifugation for 10 min. Plasma was aliquoted into cryotubes and stored in a freezer at −80°C until shipped for an AR analysis of homologues C17:0, C19:0, C21:0, C23:0, and C25:0 at the Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg-Sweden. Plasma AR concentrations were measured by using normal-phase LC-MS/MS, as described elsewhere (28), with some modifications for instrumentation. Briefly, 100 μL of plasma was loaded on HybridSPEPlus phospholipid removal 96-well plates (Sigma-Aldrich) and recovered with acetone. After evaporating and resuspending the eluted samples in heptane-ethanol (95:5 volume/volume), extracts were transferred to chromatographic vials and analyzed by LC-MS/MS (QTRAP 6500+, AB SCIEX). Analytes were separated using a 50 × 2.1-mm amino column with 1.8-mm particles (Blue Orchid, Knauer) using a gradient program with solvents A and B, which were, respectively, heptane and ethanol (99.7%). The LC column was kept at 30°C. Ionization was conducted using atmospheric pressure chemical ionization in positive mode. Optimal conditions for the multiple reaction monitoring mode were set for individual AR homologues. The method has been validated against GC-MS, and results were found to be comparable (28). Quality control samples were included in each batch to assess intra- and interbatch variations, which were <15% for all homologues.

Statistical analysis

Data are presented as means and SDs for normally distributed variables or medians and 10th–90th percentiles for skewed distributions. A *t*-test for equality of means was used to compare the normally distributed variables and a Pearson's chi-squared test was used to compare rates of dichotomous variables. The Mann-Whitney U test for 2 independent samples was used to compare differences for skewed variables. Multivariate log-binomial regression [as implemented in "proc genmod" in SAS (SAS Institute)] was used to evaluate the relative risk of GDM across quartiles of dietary whole-grain consumption and plasma AR concentrations. A P for trend was evaluated using the median value in each quartile and modeling the whole grain and plasma AR variables as continuous in the regression model. Results from the regression model were presented as both crude (univariate) and multivariate adjusted data. The covariates included in our adjusted models were age, pre-pregnancy BMI kg/m^2, parity (0, 1, and ≥2), maternal smoking during pregnancy, family history of diabetes, reported intake of beans, nuts and seeds, fruit juice and coffee. In cases when missing values for covariates were low (<5%), missing values were imputed using the median or the most probable value, which was the case for pre-pregnancy BMI. For family history of diabetes (missing = 15%), missing values were accounted for using a missing category for a covariate adjustment. The covariates included in the adjusted models were selected a priori based on their potential influence on GDM diagnoses. Both IBM Statistical Package for Social Sciences (SPSS) for Windows, version 24.0, and SAS, version 9.2, were used to analyze the data. The level of significance was accepted as $P < 0.05$.

Results

The characteristics were summarized for all the participants (Table 1). The mean age of all participants was 30 years, and 44% were nulliparous. In total, 59% had a university-level or higher academic education, and 14% smoked before pregnancy. The rate of GDM was 14.9% (n = 127), and the women in the GDM group were more likely to be older

TABLE 2 Whole-grain and other carbohydrate-rich food consumption and plasma alkylresorcinol concentrations

Plasma AR nmol/L	All	Non-GDM	GDM	P^1
	$n = 853$	$n = 726$	$n = 127$	
Alkylrecorcinols total	198 (59–795)	209 (62–804)	163 (44–577)	<0.01
C17	8 (2–42)	9 (2–45)	5 (1–27)	<0.01
C19	49 (15–196)	52 (15–205)	38 (10–128)	<0.01
C21	85 (22–345)	90 (24–347)	67 (17–302)	<0.01
C23	33 (9–141)	34 (10–145)	26 (7–105)	0.01
C25	18 (5–91)	19 (5–100)	15 (4–67)	0.03
FFQ, frequency per week[2]	$n = 834$	$n = 713$	$n = 121$	
Whole grains[3]	5.8 (1.2–15.1)	6.1 (1.2–15.1)	5.1 (0.7–19.4)	0.02
Cakes, sweets, ice cream, and cookies	3.5 (1.0–7.5)	3.5 (1.0–8.0)	3.3 (0.8–7.5)	0.11
French fries and chips	0.5 (0.3–2.5)	0.5 (0.3–2.5)	0.5 (0.1–2.5)	0.53
White bread	2.5 (0.1–7.0)	2.5 (0.1–7.0)	2.5 (0.1–7.0)	0.62
Soft drinks[4]	2.0 (0.2–7.5)	1.5 (0.2–7.1)	2.6 (0.2–12.8)	0.18
Fruit juice	1.0 (0.1–7.0)	1.0 (0.1–7.0)	0.5 (0.1–7.0)	0.03
Beans, nuts, and seeds	0.5 (0.1–5.0)	0.5 (0.1–5.0)	0.5 (0.1–2.5)	0.03
Vegetables and fruits	14 (5.0–39.0)	14.1 (5.0–39.0)	14.0 (3.5–39.0)	0.52
Fish, lean and fatty	1.3 (0.4–3.0)	1.3 (0.4–3.0)	1.1 (0.2–3.4)	0.52
Processed meat	0.5 (0.1–2.5)	0.5 (0.1–2.5)	0.5 (0.1–1.9)	0.16
Dairy[5]	10.5 (1.5–25.1)	10.5 (1.6–24.7)	10.7 (140–27.4)	0.99
Coffee	0.3 (0.1–14.0)	0.5 (0.1–14.0)	0.1 (0.1–7.0)	0.03

Data are from weeks 11–14 of pregnancy in women in Pregnant Women in Iceland II that did or did not have GDM. Data are presented as medians and percentiles (10th–90th).
Abbreviations: AR, alkylresorcinol; GDM, gestational diabetes mellitus.
[1]Differences between non-GDM and GDM data were calculated using the Mann-Whitney U test for 2 independent samples.
[2]FFQ information on intake is missing for 19 participants.
[3]Including whole-grain bread such as whole-wheat and rye bread, whole-grain pasta, oatmeal, barley, and other whole-grain products.
[4]With sugar or sweetener.
[5]Not including cheese.

and have a higher pre-pregnancy BMI. Women diagnosed with GDM were more likely to be overweight or obese (74.8%) compared to non-GDM women (43.0%). Information on pre-pregnancy BMI was missing for 10 (7.9%) women with GDM. The data on plasma AR concentrations are presented along with the frequencies of consumption reported for whole-grain products and several different food sources (**Table 2**). The AR concentrations for all homologs were significantly lower among women who had developed GDM, compared with those who had not.

The proportion of pregnant women who reached the food-based dietary guideline (FBDG) of consuming whole grain twice a day was 14.9% (14.9% of non-GDM women and 15.0% of GDM women). The frequency of whole-grain consumption in weeks 11–14 of pregnancy was lower among women who were diagnosed with GDM later in pregnancy than among non-GDM women. The difference was mainly due to a lower consumption of whole-grain breads among the GDM women (median 1.5 times/week vs. 2.6 times/week among non-GDM women).

The frequency of consumption of fruit juice, as well as coffee. was significantly lower among women who had developed GDM, while consumption of beans, nuts, and seeds was skewed towards a higher consumption frequency among the non-GDM women, though the medians were the same.

The associations between the frequencies of both whole-grain consumption and AR concentrations with GDM, respectively, were assessed in a multivariate model, adjusting for age, pre-pregnancy BMI kg/m^2, parity, education, smoking during pregnancy, family history of diabetes, reported intake of beans, nuts and seeds, fruit juice and coffee. Results are presented for unadjusted and adjusted models (**Table 3**). The median for the frequency of whole-grain consumption was 7.8 times per week in the highest quartile of AR concentrations and 3.6 times per week in the lowest quartile. The RR of being diagnosed with GDM was 0.50 (95% CI: 0.27–0.90) lower among individuals in the highest quartile compared with those in the lowest quartile of plasma ARs (P-trend = 0.01).

For whole-grain consumption, similar risk estimates were observed for both quartiles 3 and 4, where the 2 quartiles with the highest frequency of whole-grain consumption were significantly associated with a lower risk of being diagnosed with GDM compared with the lowest quartile [RR = 0.47 (95% CI: 0.26–0.83) and RR = 0.48 (95% CI: 0.27–0.86) respectively], and a test for dose response was significant (P-trend > 0.01).

Associations between quartiles of individual plasma alkylresorcinol homologs and GDM were additionally explored (**Supplemental Table 2**). We also attempted to stratify the GDM associations of quartiles of plasma alkylresorcinols and FFQ reported weekly intake of whole grains with both age (**Supplemental Table 3**) and BMI (**Supplemental Table 4**).We further analyzed our model with the BMI from the first maternal visit (clinically measured) as an adjustment factor instead of the pre-pregnancy BMI (self-reported), and the results remained unchanged (data not shown). We additionally explored associations of weight gain to GDM diagnoses (**Supplemental Table 5**), finding no significant associations between weight gain in pregnancy and GDM diagnoses. In addition, the rates of GDM diagnoses were similar for women who had blood drawn the morning and afternoon (**Supplemental Table 6**).

TABLE 3 Associations of quartiles of plasma alkylresorcinols and FFQ reported weekly consumption of whole grains with gestational diabetes mellitus

		Crude	Adjusted[1]
Total plasma AR quartile,[2] (median, nmol/L), $n = 853$	Cases, *n (%)/total n*	RR (95% CI)	RR (95% CI)
AR, Quartile 1 (66)	40 (19.0)/210	1.00	1.00
AR, Quartile 2 (140)	36 (17.0)/212	0.87 (0.53, 1.43)	1.00 (0.59, 1.70)
AR, Quartile 3 (279)	30 (13.8)/217	0.68 (0.41, 1.14)	0.71 (0.41, 1.23)
AR, Quartile 4 (706)	21 (9.8)/214	0.46 (0.26, 0.82)	0.50 (0.27, 0.90)
P-trend		0.006	0.01
FFQ weekly whole grain consumption,[3] (median, times/week), $n = 834$	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
FFQ, Quartile 1 (1.2)	40 (19.9)/201	1.00	1.00
FFQ, Quartile 2 (3.8)	31 (14.5)/214	0.68 (0.41, 1.14)	0.71 (0.41, 1.22)
FFQ, Quartile 3 (7.6)	25 (12.3)/204	0.56 (0.33, 0.97)	0.47 (0.26, 0.83)
FFQ, Quartile 4 (14.5)	25 (11.6)/215	0.53 (0.31, 0.91)	0.48 (0.27, 0.86)
P-trend		0.03	0.01

Abbreviation: GDM, gestational diabetes mellitus.
[1]Adjusted for age, pre-pregnancy BMI kg/m^2, parity, smoking during pregnancy, family history of diabetes, and reported intakes of beans, nuts and seeds, fruit juice, and coffee.
[2]The medians of FFQ weekly whole-grain consumption for each AR quartile are: Q1 = 3.6, Q2 = 5.3, Q3 = 6.5, and Q4 = 7.8.
[3]FFQ data on whole-grain intake are missing for 19 women.

Discussion

In our study, we found that women who reported greater consumption of whole grains in early pregnancy, reflected in higher plasma concentrations of AR, had a decreased risk of developing GDM. Furthermore, we observed a significant decrease in the GDM risk with higher AR levels, emphasizing the importance of further investigation into the impact of diet on the GDM risk.

Several genetic and environmental factors, such as age, ethnicity, and family history of diabetes (29), are thought to affect a person's risk of being diagnosed with GDM, but it is clear that obesity in pregnancy and suboptimal nutrition are strong indicators (3). Whole-grain foods contain all parts of the grain, and therefore provide more nutrients, fiber, and phytochemicals than refined grains (30). The positive effects of a whole grain–rich diet include increased satiety, a slower digestion transit time, increased gut health, and a slower glycemic response (31). Dietary patterns that contain whole grains have repeatedly been presented as possible means of GDM prevention (12–14). Consumption of refined grains, in contrast, has been linked to a greater risk for metabolic syndrome and increased adiposity in adults (2, 32), as well as to an increased risk of GDM (13). The literature regarding the effects of refined-grain consumption during pregnancy on offspring is limited; nevertheless, results from an animal study suggest that refined carbohydrate exposure in utero may predispose offspring to an obese phenotype (33). This was supported by a study demonstrating that higher consumption of refined carbohydrates during GDM pregnancies increased the offspring's risk of being overweight or obese at 7 years old (2). Therefore, increasing whole-grain consumption during pregnancy is a modifiable factor that could benefit both the mother and her offspring.

In another recent pregnancy cohort (2013/2014) in Iceland, only 20% of the women reportedly reached the recommended minimum of 25 g/day of fiber (34). In our study, only about 15% of the women adhered to the FBDG for whole grains, which recommends 2 portions daily, while 29% consumed at least 1 portion of whole grains daily. Use of the same FFQ as that utilized in a previous PREWICE study found the rate of women reaching whole-grain consumption at least twice daily to be 9%. This indicates that whole-grain consumption might have increased somewhat among pregnant women in Iceland since 2016 (26). This is a positive change that may stem from the increased availability of whole-grain breads and other whole-grain food sources in Iceland. However, this is still a low rate, especially when considering the vast nutritional and health benefits that consumption of these foods provides in pregnancy (18, 35).

It is widely known that using a subjective method, such as an FFQ, to acquire information on diet can result in errors, since FFQs are usually meant to gather information regarding diet over a long period, and answering them relies on both memory and correct estimates of average intakes. In addition, people may underreport consumption of foods thought to be "undesirable," and portion sizes are difficult to estimate correctly (36). AR measurements have been presented as a valid, objective method of measuring whole-grain consumption (22, 23) and as a means by which to assess consumption in populations that regularly eat whole grains (37).

The apparent elimination half-life of AR homologues is about 5 hours (38). However, because the absorption half-life of AR is about 3–5 hours, the plasma concentration remains more stable than would be expected when solely considering the apparent elimination half-life. Under intervention conditions in which whole grains rich in AR were consumed regularly during the day, the fasting plasma AR concentration showed small variation (<30% within and between individuals) (39). Under free-living conditions, the intra-class correlation of AR, measured 1 month up to 3 years apart, was in the range 0.4–0.6 (37, 40, 41); this suggests that an AR measurement in a single plasma sample provides a reasonable estimate of an individual's AR concentrations over time, which are related to average long-term whole-grain consumption. Moreover, several studies have shown good correlations between estimated whole-grain wheat and rye consumption and plasma AR concentrations in controlled whole-grain intervention studies and under free-living conditions (range = 0.3–0.6) in adults (42), suggesting AR measurements are valid as concentration biomarkers of whole-grain wheat and rye consumption in populations with stable and frequent whole-grain consumption.

In our study, the participants were not fasting; as such, the median total plasma AR concentration in our cohort was fairly high compared to that of a similar study in pregnant women in Singapore, in which the fasting median was extremely low (9 nmol/L). This is, to our knowledge, the only other study that measured AR concentrations in pregnant women, although it did not investigate an association with GDM (17). For comparison, when diets are free of whole grains under controlled circumstances, the fasting median is usually below 60 nmol (43). In studies of nonpregnant participants, the fasting medians for total AR concentrations have been presented as 20 nmol/L in an elderly US cohort (44), 43 nmol/L in a Scandinavian cohort (18), and 87.7 nmol/L at baseline in the WHOLE heart study (a wholegrain intervention study) (45). In our study, the women were not fasting because that is not a requirement during the routine checkup in pregnancy through which we recruited participants for our study.

The few previous studies of nonpregnant participants that used AR concentrations to investigate the associations between whole-grain consumption and type 2 diabetes (T2D) have shown conflicting results. One study found no associations between total AR concentrations and diabetes risks in a Scandinavian cohort of men and women, but suggested that the increased ratio of rye to wheat, measured by the plasma C17:0/C21:0 homologue ratio, was associated with a lowered risk of developing T2D (18). A second study, in contrast, demonstrated results similar to ours, with an inverse association between a metabolite of AR (DHPPA) and T2D risk, as well as an association between the AR metabolite and impaired glucose regulation (46). To the best of our knowledge, ours is the first study to investigate the association between AR concentrations and GDM.

In our study, we observed similar results from both the FFQ reported consumption and the AR concentration, suggesting that the short FFQ may present reliable results. Additionally, the AR concentrations mostly represent whole grains in the form of wheat, rye, and barley, while the FFQ contains information regarding consumption frequencies of other whole-grain products as well, such as oatmeal, which may explain some differences.

In our cohort, 127 women (14.9%) in total were diagnosed with GDM, which is high in comparison to numbers seen in most other Nordic countries, such as Sweden (1.4–2.6%) (47, 48), Denmark (2.3–2.9%) (49, 50), and Norway (5.2–7.4%) (49, 51). However, in Finland, the rates seem higher (10.5–11.3%) (51, 52), and are closer to the rates seen in Iceland (11.8–16%) (53, 54). The diagnostic criteria do vary among the Nordic countries and, according to a recent meta-analysis, the worldwide GDM prevalence was estimated at 4.4% when not discerning based upon population, diagnostic criteria, or recruitment and was 10.6% when only using the IADPSG criteria (utilized in Iceland) (55).

The strengths of our study include our use of a novel, objective method to measure whole-grain consumption in the pregnant female population, in addition to the usual subjective methods of acquiring information on diet. Our study also features a large sample size (853 pregnant women), a high participation rate (75%), and data that were collected prospectively. Another strength is that we explored associations of both BMI (data not shown) and weight gain with GDM (Supplemental Table 5), which are known risk factors for a GDM diagnosis (56, 57). The limitations may stem from the fact that even though AR measurements have been demonstrated as valid for measuring whole-grain consumption (22, 23), AR does have a short to medium half-life (58). We realize that the time of day during which the subjects were assessed, as well as individual absorption, may affect results; the women were not fasting when the blood sample was taken. However, AR measurements have been presented as a reliable method of gaining information on mean whole-grain consumption in groups (22, 59), and a person eating whole-grain wheat or rye regularly is likely to display high levels of AR (21). In addition, the rates of GDM diagnoses were similar for women who had blood drawn in the morning and afternoon (**Supplemental Figure 2**).

Other limitations are that we did not have information on physical activity and that AR measurements only represent whole grains found in wheat and rye, meaning that the results do not take into account consumption of whole-grain rice and oats, for example. Despite this, we observed similar results both for reported consumption of whole grains and for AR concentrations, suggesting that this limitation did not affect our results.

In conclusion, a higher reported consumption of whole grains, also reflected by AR biomarkers, was associated with a decreased risk of a GDM diagnosis.

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The authors' responsibilities were as follows—IG, ITH, and HH: designed the research; EAT and IG: conducted the research; RL, LH, OKM, ITH, and HH: provided essential materials; EAT, TIH, and IG: analyzed the data, performed the statistical analysis, and had primary responsibility for the final content; EAT, TIH, IG, and BEB: wrote the paper; and all authors: edited the paper and read and approved thefinal manuscript.

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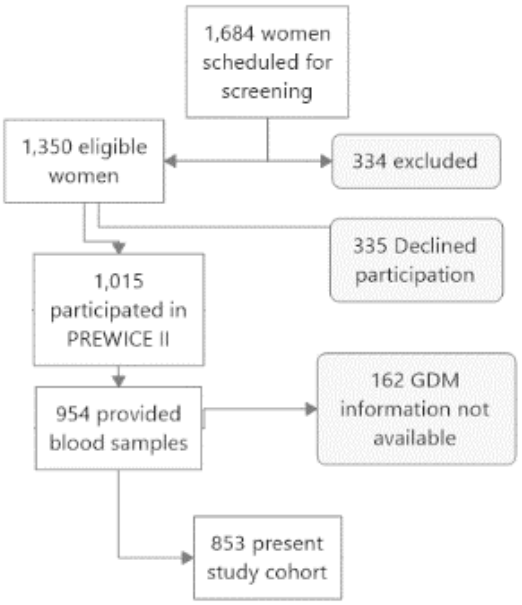
Higher alkylresorcinol concentrations, a consequence of whole-grain intake, are inversely associated with gestational diabetes mellitus in Iceland. Tryggvadottir, Ellen A. - Online Supplementary Material

Supplemental Figure 1. Participation flowchart.



Supplemental Table 1. Characteristics of the women in PREWICEII compared those not included due to unavailable data[1].

		Our cohort	missing	*p*
		(*n* = 853)	(*n* = 162)	
Age, years		30.3 ± 4.9	29.3 ± 5.4	<0.27
BMI[2], kg/m^2		26.3 ± 5.5	28.9 ± 7.1	<0.0[3]
Parity, %				
	0	44	39	
	1	36	34	
	≥ 2	20	24	0.24
Education, %				
	Elementary school	11	13	
	Technical/High school	30	27	
	University education	35	28	
	Higher academic	24	28	0.79
Marital status, %				
	Married	24	22	
	Living together	71	69	
	Single	5	4	0.90
Smoking, %				
before pregnancy	Yes	14	14	0.91
during pregnancy	Yes	5	4	0.95

[1] Data is presented as means ± std. Deviation or ratios (%).
[2] Information for BMI is missing for 32 participants in our cohort and 110 in the missing cohort
[3] Comparing BMI for only 52 of the missing cohort to 821 in our cohort
BMI: Body mass index

Supplemental Table 2. Associations between quartiles of plasma alkylresorcinol homologs and GDM, respectively.

n =853		**Crude**	**Adjusted**[1]
C17 AR tertile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
Q1 (1.9)	41 (19.2)/213	1.00 -	1.00 -
Q2 (4.9)	41 (19.2)/213	1.00 (0.62, 1.62)	1.00 (0.59, 1.67)
Q3 (11.5)	27 (12.6)/214	0.61 (0.36, 1.03)	0.56 (0.32, 0.98)
Q4 (33.2)	18 (8.5)/213	0.39 (0.21, 70)	0.35 (0.19, 0.65)
P-trend		0.0006	0.0003
C19 AR Tertile (median, nmol/L))	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
Q1 (15.9)	44 (20.7)/213	1.00 -	1.00 -
Q2 (33.0)	36 (16.9)/213	0.78 (0.48, 1.27)	0.82 (0.49, 1.38)
Q3 (69.6)	26 (12.1)/214	0.53 (0.31, 0.90)	0.52 (0.30, 0.91)
Q4 (171.2)	21 (9.9)/213	0.42 (0.24, 0.74)	0.43 (0.24, 0.77)
P-trend		0.003	0.001
C21 AR Tertile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
Q1 (25.7)	43 (20.2)/213	1.00 -	1.00 -
Q2 (60.1)	30 (14.1)/213	0.65 (0.39, 1.08)	0.75 (0.43, 1.28)
Q3 (120.6)	33 (15.4)/214	0.72 (0.44, 1.19)	0.73 (0.43, 1.23)
Q4 (311.7)	21 (9.9)/213	0.42 (0.25, 0.76)	0.46 (0.26, 0.84)
P-trend		0.009	0.01
C23 AR Tertile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
Q1 (10.5)	41 (19.2)/213	1.00 -	1.00 -
Q2 (24.2)	32 (15.0)/213	0.74 (0.45, 1.23)	0.80 (0.47, 1.38)
Q3 (44.7)	35 (16.4)/214	0.82 (0.50, 1.35)	0.81 (0.48, 1.37)
Q4 (123.5)	19 (8.9)/213	0.41 (0.23, 0.73)	0.42 (0.23, 0.77)
P-trend		0.004	0.009
C25 AR Tertile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
Q1 (5.4)	40 (18.8)/213	1.00 -	1.00 -
Q2 (13.1)	31 (14.6)/213	0.74 (0.44, 1.23)	0.71 (0.41, 1.23)
Q3 (25.4)	32 (15.0)/214	0.76 (0.46, 1.27)	0.76 (0.45, 1.31)
Q4 (77.6)	24 (11.3)/213	0.55 (0.32, 0.95)	0.53 (0.30, 0.95)
P-trend		0.06	0.05

[1] Adjusted for age, pre-pregnancy BMI kg/m2, parity, smoking during pregnancy, family history of diabetes, reported intake of beans, nuts and seeds, fruit juice and coffee.
BMI: Body mass index. CI: Confidence Interval. GDM: Gestational diabetes mellitus.
RR: Relative Risk

Supplemental Table 3. Associations of quartiles of plasma alkylresorcinols and FFQ reported weekly intake of whole grains with GDM, stratified by age.

Age <30 years (*n*=401)		**crude**	**adjusted**[1]
Total plasma AR quartile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
AR-Quartile 1 (63)	14 (13.7)/102	1.00 -	1.00 -
AR-Quartile 2 (137)	13 (12.0)/108	0.86 (0.38, 1.93)	0.72 (0.31, 1.68)
AR-Quartile 3 (281)	11 (10.6)/104	0.74 (0.32, 1.73)	0.70 (0.32, 1.69)
AR-Quartile 4 (682)	8 (9.2)/87	0.64 (0.25, 1.60)	0.62 (0.23, 1.63)
P-trend		0.35	0.43
FFQ weekly wholegrain consumption (median, times/week)[2]	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
FFQ Quartile 1 (1.2)	16 (15.5)/103	1.00 -	1.00 -
FFQ Quartile 2 (3.6)	15 (12.8)/117	0.80 (0.37, 1.71)	0.84 (0.37, 1.87)
FFQ Quartile 3 (7.6)	8 (10.5)/76	0.64 (0.26, 1.58)	0.49 (0.18, 1.31)
FFQ Quartile 4 (14.1)	7 (7.3)/96	0.43 (0.17, 1.09)	0.34 (0.12, 0.95)
P-trend		0.06	0.03
Age ≥30 years (*n*=452)			
Total plasma AR quartile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
AR-Quartile 1 (68)	26 (24.1)/108	1.00 -	1.00 -
AR-Quartile 2 (142)	23 (22.1)/104	0.90 (0.47, 1.70)	1.02 (0.53, 1.98)
AR-Quartile 3 (278)	19 (16.8)/113	0.64 (0.33, 1.24)	0.67 (0.34, 1.33)
AR-Quartile 4 (713)	13 (10.2)/127	0.36 (0.17, 0.74)	0.43 (0.20, 0.91)
P-trend		0.004	0.01
FFQ weekly wholegrain consumption (median, times/week)[2]	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
FFQ Quartile 1 (1.4)	24 (24.5)/98	1.00 -	1.00 -
FFQ Quartile 2 (3.8)	16 (16.5)/97	0.61 (0.30, 1.24)	0.56 (0.27, 1.18)
FFQ Quartile 3 (7.6)	17 (13.3)/128	0.47 (0.24, 0.94)	0.47 (0.23, 0.96)
FFQ Quartile 4 (15.0)	18 (15.1)/119	0.55 (0.28, 1.09)	0.56 (0.27, 1.16)
P-trend		0.13	0.18

[1] Adjusted for pre-pregnancy BMI kg/m2, parity, smoking during pregnancy, family history of diabetes, reported intake of beans, nuts and seeds, fruit juice and coffee.

[2] FFQ data on wholegrain intake is missing for 19 women.

BMI: Body mass index. CI: Confidence Interval. GDM: Gestational diabetes mellitus.
RR: Relative Risk.

Supplemental Table 4. Associations of quartiles of plasma alkylresorcinols and FFQ reported weekly intake of whole grains with GDM, stratified by BMI.

BMI <25 kg/m^2 *n*= 435		**crude**	**adjusted**[1]
Total plasma AR quartile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
AR-Quartile 1 (64)	15 (15.0)/100	1.00 -	1.00 -
AR-Quartile 2 (140)	16 (13.9)/115	0.92 (0.43, 1.96)	0.96 (0.43, 2.11)
AR-Quartile 3 (265)	14 (12.2)/115	0.79 (0.36, 1.72)	0.75 (0.33, 1.63)
AR-Quartile 4 (686)	6 (4.9)/123	0.29 (0.11, 0.78)	0.25 (0.09, 0.69)
P-trend		0.009	0.004
FFQ weekly wholegrain consumption (median, times/week)[2]	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
FFQ Quartile 1 (1.2)	16 (15.5)/103	1.00 -	1.00 -
FFQ Quartile 2 (3.8)	14 (12.8)/109	0.80 (0.37, 1.74)	0.79 (0.36, 1.75)
FFQ Quartile 3 (7.6)	8 (6.9)/116	0.40 (0.16, 0.99)	0.33 (0.13, 0.83)
FFQ-Quartile 4 (14.5)	10 (8.7)/115	0.52 (0.22, 1.20)	0.42 (0.17, 1.02)
P-trend		0.09	0.04
BMI ≥25 kg/m^2 *n*=396			
Total plasma AR quartile (median, nmol/L)	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
AR-Quartile 1 (67)	25 (22.7)/110	1.00 -	1.00 -
AR-Quartile 2 (140)	20 (20.6)/97	0.88 (0.46, 1.72)	0.97 (0.48, 1.96)
AR-Quartile 3 (283)	16 (15.7)/102	0.63 (0.32, 1.27)	0.66 (0.32, 1.39)
AR-Quartile 4 (721)	15 (16.5)/91	0.67 (0.33, 1.37)	0.86 (0.40, 1.79)
P-trend		0.27	0.64
FFQ weekly wholegrain consumption (median, times/week)[2]	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
FFQ Quartile 1 (1.4)	24 (24.5)/98	1.00 -	1.00 -
FFQ Quartile 2 (3.8)	17 (16.2)/105	0.60 (0.30, 1.19)	0.60 (0.29, 1.26)
FFQ Quartile 3 (7.8)	17 (19.3)/88	0.74 (0.37, 1.49)	0.61 (0.29, 1.29)
FFQ-Quartile 4 (14.5)	15 (15.0)/100	0.54 (0.27, 1.11)	0.51 (0.24, 1.11)
P-trend		0.18	0.14

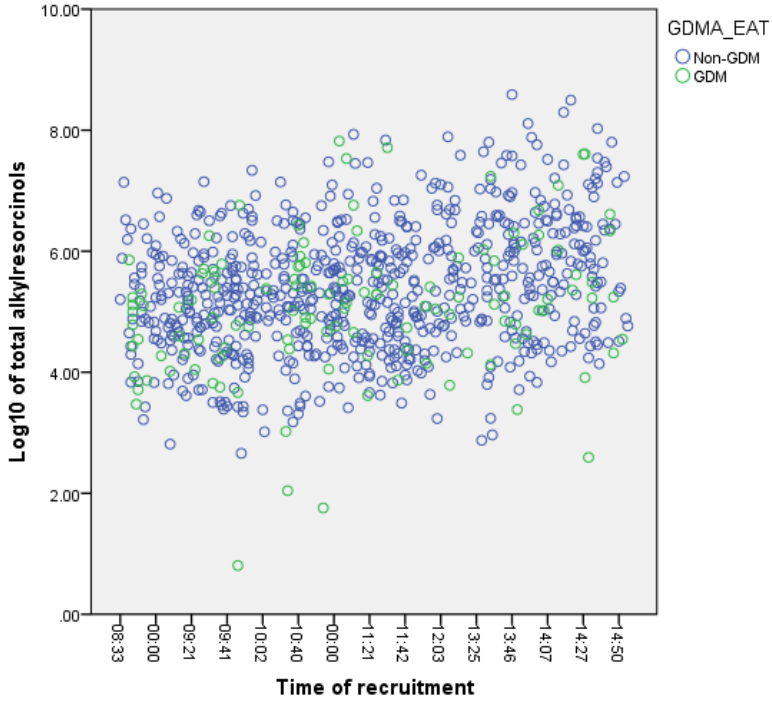
[1]Adjusted for parity, smoking during pregnancy, family history of diabetes, reported intake of beans, nuts, and seeds, fruit juice and coffee.
[2] FFQ data on wholegrain intake is missing for 19 women.
CI: Confidence Interval. GDM: Gestational diabetes mellitus. RR: Relative Risk.

Supplemental Table 5. Associations of weight gain in pregnancy, according to IOM guidelines, and GDM.

		crude	**adjusted**[1]
N=795[2]	Cases, *n* (%)/total *n*	RR (95% CI)	RR (95% CI)
ALL			
GWG continuous (kg)	110 (13.8)/795	0.90 (0.86, 0.93)	0.91 (0.87, 0.95)
Recommended	32 (12.4)/259	1.61 (0.97, 2.67)	1.76 (1.03, 3.02)
Below recommendations	40 (18.5)/216	1.00 -	1.00 -
Above recommendations	38 (11.9)/320	0.96 (0.58, 1.58)	0.63 (0.36, 1.09)
P-trend[3]		0.03	0.0006
BMI≥25 kg/m^2			
GWG continuous (kg)	67 (17.9)/374	0.88 (0.83, 0.93)	0.89 (0.83, 0.95)
Recommended	21 (20.8)/101	2.40 (1.11, 5.20)	2.22 (0.94, 5.24)
Below recommendations	17 (38.6)/44	1.00 -	1.00 -
Above recommendations	29 (12.7)/229	0.55 (0.30, 1.03)	0.48 (0.25, 0.95)
P-trend[3]		<0.0001	0.0003
BMI<25 kg/m^2			
GWG continuous (kg)	43 (10.2)/421	0.92 (0.87, 0.98)	0.93 (0.87, 0.99)
Recommended	11 (7.0)/158	2.06 (0.97, 4.38)	1.98 (0.92, 4.29)
Below recommendations	23 (13.4)/172	1.00 -	1.00 -
Above recommendations	9 (9.9)/91	1.47 (0.58, 3.69)	1.64 (0.63, 4.28)
P-trend[3]		0.29	0.47

[1]Adjusted for pre-pregnancy BMI kg/m2, parity, smoking during pregnancy, family history of diabetes, reported intake of beans, nuts and seeds, reported intake of fruit juice and reported intake of coffee.
[2] Total of women with information on both pre-pregnancy BMI and total weight gain.
[3] Chi-square test
CI: Confidence Interval. GDM: Gestational diabetes mellitus. GWG: Gestational weight gain. IOM: Institute of medicine. RR: Relative Risk

Higher alkylresorcinol concentrations, a consequence of whole-grain intake, are inversely associated with gestational diabetes mellitus in Iceland. Tryggvadottir, Ellen A. - Online Supplementary Material



Supplemental Figure 2. Plasma total alkylresorcinol and time of participation. Simple scatterplot of Log total alkylresorcinols and time of recruitment.

All women *n:*853	
Spearman correlation between Total AR and time of participation	0.24
P-value	<0.01
Non-GDM women *n:*726	
Spearman correlation between Total AR and time of participation	0.241
P-value	<0.01
GDM women *n:*127	
Spearman correlation between Total AR and time of participation	0.242
P-value	<0.01

Supplemental Table 6. Time of participation and GDM diagnoses

	Before noon	After noon	Missing time info
	n (%)	*n* (%)	*n* (%)
No GDM diagnoses	486 (85)	237 (86)	3 (43)
GDM diagnoses	85 (15)	38 (14)	4 (57)

Supplemental Table 7. Correlations of total plasma alkylresorcinol and FFQ reported whole grain intake[1].

	n	Spearman correlation	*P*-value
All women	853/834	0.26	<0.01
Non GDM women	726/713	0.25	<0.01
GDM women	127/121	0.33	<0.01

[1] FFQ reported whole grain intake is missing for 19 women.

Paper III

BMJ Open Diabetes Research & Care

Early pregnancy plasma fatty acid profiles of women later diagnosed with gestational diabetes

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ABSTRACT

Introduction Fatty acid (FA) concentrations have previously been associated with gestational diabetes mellitus (GDM). However, few studies on GDM have examined FA profiles in early pregnancy or before diagnosis. This study aimed to compare early pregnancy plasma FA profiles of women with and without GDM diagnoses as well as their reported dietary consumption.

Research design and methods The subjects comprised 853 women from the prospective study: Pregnant Women in Iceland II (PREWICE II), attending their 11–14 weeks ultrasound appointment in 2017–2018. During the visit, blood samples were collected for plasma FA analysis, and dietary habits were assessed using a short food frequency questionnaire. Information on GDM diagnoses was then later extracted from medical records. Differences in FA profile between GDM cases and non-cases were evaluated using the Mann-Whitney U test.

Results GDM was diagnosed in 127 women (14.9%). Concentrations of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids (PUFA) n-6, PUFA n-3 and total FA were higher in the women who later developed GDM compared with those who did not ($p \leq 0.05$). The medians for total FA were 2898 µg/mL for the women with GDM and 2681 µg/mL for those without GDM. Mean adjusted difference for total FA between the groups was 133 µg/mL (95% CI 33 to 233). Similar results were observed in prepregnancy normal-weight women and overweight women/women with obesity. Overall diet quality in early pregnancy appeared to be lower among the women later diagnosed with GDM.

Conclusion We found that plasma FA profiles in early pregnancy were different for women later diagnosed with GDM compared with those who were not, independent of the women's body mass index.

Significance of this study

What is already known about this subject?

- Fatty acid (FA) profiles have been associated with gestational diabetes; however, most studies were performed during or after gestational diabetes mellitus (GDM) diagnoses and few have additionally investigated dietary intake.

What are the new findings?

- Plasma FA profiles in early pregnancy were different for women with and without GDM diagnoses later in pregnancy.
- Differences in plasma FA profiles were independent of the women's body mass index.
- Women that were later diagnosed with GDM appeared to have lower overall diet quality in early pregnancy.

How might these results change the focus of research or clinical practice?

- Further research is needed on early pregnancy FA profiles as a predicter for GDM.
- Increased dietary quality during pregnancy should be a clinical focus in GDM prevention.

INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications and a strong risk factor for later development of type 2 diabetes (T2D).[1] Potential risk factors for GDM include prepregnancy overweight and obesity, age and unhealthy dietary habits.[2] With respect to dietary fat intake, high maternal intake of total fat,[3] saturated fatty acids (SFA) as well as cholesterol[4] have previously been associated with an increased risk of GDM,[3 5] whereas intake of polyunsaturated fatty acid (PUFA) and alpha-linolenic acid (ALA) has been associated with reduced risk.[6] Circulating FA can derive from both dietary intake[7] and catabolism or endogenous synthesis, both of which being partly influenced by glucose homeostasis.[8] As an example, free fatty acid (FFA) levels have been associated with altered secretion of insulin as well as insulin resistance (IR), which may progress to diabetes.[9–12]

In most previous studies on FA profiles in pregnant cohorts, FA analysis was performed during or after GDM diagnoses,[12] whereas few studies have investigated the FA profile during the first trimester, before GDM diagnoses.[13–17] Their results indicated that women

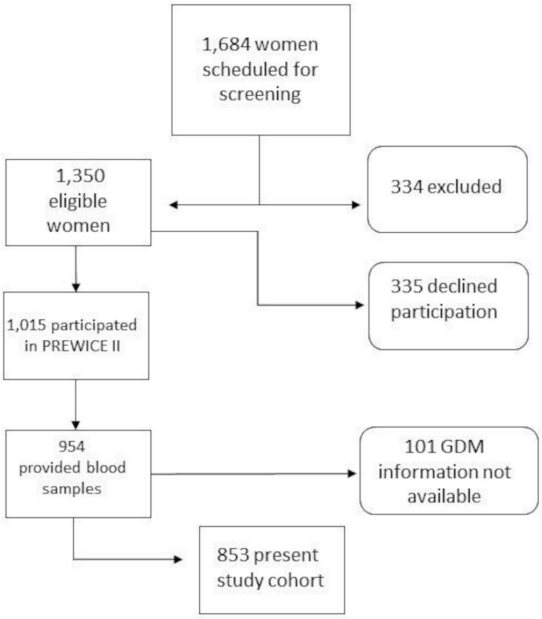

Figure 1 Participant flow chart. GDM, gestational diabetes mellitus; PREWICE II, Pregnant Women in Iceland II.

diagnosed later with GDM had higher total concentrations of SFA,[13–15] MUFA,[13 15] PUFA n-6[16] and PUFA n-3[13] and sometimes lower concentrations of PUFA n-6[13 15] and PUFA n-3.[15–17]

Our aim was to investigate whether the total plasma FA profile during the first trimester was associated with increased odds of GDM diagnoses. Because overweight and obesity can increase the risk of IR and GDM and is associated with altered FA profiles,[12] we also stratified our analysis according to pre-pregnancy body mass index (BMI). Furthermore, self-reported dietary consumption was also compared between the two groups of women.

SUBJECTS AND METHODS

Subjects

Between October 2017 and March 2018, all pregnant women attending routine screening at gestational weeks 11–14 at the Prenatal Diagnostic Unit at The National University Hospital (Reykjavik, Iceland) were invited to become participants in the study. Of the 1684 women scheduled for an appointment, 1350 were eligible by being able to answer an Icelandic Food Frequency Questionnaire (FFQ) in addition to their gestational age being between 11 and 14 weeks. Participant flow chart is shown in figure 1. A total of 1015 women agreed to participate (75%). Most of the women provided a blood sample during this routine visit, at which time additional tubes were drawn for the purpose of FA analysis. A total of 954 participants provided a non-fasting blood sample. We were able to acquire information on GDM diagnoses from medical data for 853 of these women; hence, the entire analysis consisted of 853 women or 84% of the enrolled study participants. The source population and cohort has been described in detail in previous publications.[18 19]

Dietary intake and characteristics

During recruitment, the participants answered a short FFQ in electronic format on dietary intake, which also contained questions on age, education, smoking, parity, prepregnancy weight and height. The FFQ assessed the frequency of consumption of 40 food items and beverages and dietary supplement intake, requiring participants to refer to their intake during the previous 3 months. The women chose between 10 potential frequency responses ranging from 'less than once a month' to 'more than 5 times a day'. The FFQ development has previously been described in detail.[18 20–22]

In brief, the FFQ was pilot-tested and compared with results from a 4-day weighed food record with acceptable correlation for most food groups/items (Spearman's correlation >0.3).[20] Recent publications from our PREWICE II study have described a dose-dependent association between the consumption of dairy and urine iodine concentration,[18] wholegrain consumption and alkylresorcinol concentrations (a biomarker for wholegrains)[19] and reported intake of vitamin D supplements and 25-OH-D in plasma.[22]

Measurement of plasma fatty acids in plasma

The voluntary blood samples provided at Landspitali University Hospital in weeks 11–14 by consenting participants were processed within 1 hour after collection to separate plasma from red blood cells and buffy coat via centrifugation at 3000 rpm for 10 min. Plasma was aliquoted into cryotubes and stored in a freezer at −80°C until shipped for FA analysis at the Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden. The total plasma fatty acid (FA) composition was determined according to the method described by Stråvik *et al*,[23] which is a modification of the method described by Masood *et al*.[24] In total, 24 FA were quantified. An internal standard solution (100 µL of 0.1 mg C23:0 methyl ester/mL toluene) was added to 50 µL of thawed plasma samples, and 1.8 mL of acetyl chloride-MeOH solution (10% (v/v) fortified with butylated hydroxytoluene (2.78 µg/mL) was added. Samples were incubated at 70°C for 60 min in a water bath with shaking. Single extraction of fatty acid methyl esters (FAMEs) was carried out by adding 1.5 mL of hexane. The extraction solvent was evaporated using a vacuum concentrator (125 mbar, 30°C, 30 min). FAME was dissolved in 200 µL of hexane before injection into the GC-FID for analysis. Two water blanks (Millipore-purified water) and six quality controls (pooled plasma) were prepared and run together with the study samples for each batch of a maximum of 50 study samples.

FAMEs were separated using the GC-FID system (Thermo Scientific Focus GC, FID detector, Pal GC-xt

autosampler, AD-100 H_2 generator and a MicroClip XT hydrogen gas alert and a ZA 1500 zero air generator) equipped with a Zebron ZB-FAME column (20 m×0.18 µm ID×0.15 µm). The oven program was as follows: initial 80°C with a 1.5 min hold; ramp: 40°C/min to 160°C, 5°C/min to 185°C with a 0 min hold and then 30°C/min to 260°C with a 0 min hold. The instrumental condition was as follows: nitrogen as the carrier gas, constant flow, carrier flow 1.25 mL/min, Inlet temperature 260°C, split flow 12.5 mL/min, split ratio: 15. Detector temperature 260°C. Gas flow: air 450 mL/min; hydrogen 35 mL/min; makeup gas 10 mL/min. The injection volume was 1 µL. The concentration of FAs in samples was quantified against external standard calibrations made from GLC-462 mixed FAMEs (Nu-Check Prep, Elysian, Minnesota, USA) dissolved in toluene. The external standard included 24 FAMEs ranging from C12:0 to C24:1. An equal amount of internal standard (C23:0 methyl ester) was added to the external standards as added to the study samples, and all analyte peaks were normalized with the peak of the internal standard before calibration.

Gestational diabetes mellitus diagnoses

The GDM diagnoses are based on The International Association of the Diabetes and Pregnancy Study Groups (IADPSG).[25] During the first routine maternal care visit in Iceland (around 10 weeks of pregnancy), women at risk of developing GDM, based on age (≥40 years), BMI (≥30 kg/m^2), ethnicity and history of diabetes or macrosomia (≥4500 g), are invited to provide a fasting blood sample to measure fasting blood glucose. If results are ≥5.1 mmol/L, the women are diagnosed with GDM or in some cases T2D, if fasting blood sugar is >7 mmol/L or hemoglobin A1c is ≥48 mmol/mol. The rate of women diagnosed with GDM during this early selective screening is unknown in Iceland. However, a study in France also using selective screening and the IADPSG criteria reported an early hyperglycemia rate of 2.3% in a cohort of almost 800 000 women, corresponding to 26.9% of the women diagnosed with hyperglycemia overall.[26]

Later in pregnancy, usually at weeks 24–28, the women considered at risk of GDM undergo a 2-hour oral glucose tolerance test (OGTT). During the OGTT blood sugar levels are measured during fasting, after 1 hour and after the second hour.[27] Previously reported rates of GDM diagnoses in Iceland have been in the range of 11.8%–16%.[28 29] Information on GDM diagnoses for this study was gathered from medical records at the National University Hospital. The records did not always differentiate between early detected GDM, GDMA1 (controlled with diet) or GDMA2 (medication needed); therefore, the GDM diagnoses cover all categories.

Statistical analysis

Data are presented as means and SD for normally distributed variables or median and 10th–90th percentiles for skewed distributions. A t-test for equality of means was used to compare the normally distributed variables, and Pearson's χ^2 test was used to compare dichotomous variables. The Mann-Whitney U test for two independent samples was used to compare differences for skewed variables.

For classification of FA groups, the concentrations of different types of FA were combined to determine the total for SFA, MUFA, PUFA n-6, PUFA n-3 as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) especially. Ratios of individual and groups of FA were calculated by dividing their concentration by the total FA concentration.

The value for six of the FA was below the limit of quantification in a few cases. For four of the FA 0.1%–0.2% of the participants had levels below quantification, but for myristoleate (14:1) the rate was 3% and for docosatetraenoic (22:4) it was 10%. Values below limit of quantification were set to half of the lowest value quantified for an FA in our data.

A linear regression model to compare means was used to evaluate the adjusted difference in FA types, between the women who were diagnosed later with GDM and those who were not.

Multivariate binary logistic regression was used to evaluate the OR of GDM across quartiles of FA types as well as relative FA. P for trend was evaluated by using the median value in each quartile and modeling the plasma FA and relative FA variables as continuous in the regression model. Results from the regression model are presented as multivariate-adjusted. A Spearman's correlation was used to investigate correlations between dietary intake and FA. IBM Statistical Package for Social Sciences for Windows, V.24.0 (Armonk, New York, USA), was used to analyze the data. The level of significance was accepted as $p<0.05$.

Covariates

The covariates included in the adjusted models were selected a priori based on their potential influence on GDM diagnoses.[30–34] In the adjusted linear regression model, covariates were age, prepregnancy BMI (kg/m^2), weekly weight gain (kg/week) and maternal smoking during pregnancy.

The covariates included in our multivariate adjusted models were age; prepregnancy BMI (kg/m^2); weekly weight gain (kg/week); parity (0, 1 and ≥2); maternal smoking during pregnancy (yes/no) and family history of diabetes (yes/no/unknown). In cases when missing values for covariates were low (<5%), they were imputed using the median or the most probable value, which was the case for prepregnancy BMI. When prepregnancy BMI values were missing (2.6% overall, 7.6% of the women diagnosed with GDM), values were imputed using the median or the most probable value based on BMI at the first maternal care visit. For family history of diabetes (missing=15%), missing values were accounted for by use of the missing category for covariate adjustment.

Medical records provided information on gestational age and maternal weight at maternal care visits. From

Table 1 Characteristics of the women in PREWICE II

	All (n=853)	Non-GDM (n=726)	GDM (n=127)	P value*
Age, years	30.3±4.9	29.9±4.8	32.4±5.5	**<0.01**
Prepregnancy BMI†, kg/m^2	25.8±5.7	25.4±5.4	28.4±6.8	**<0.01**
BMI ≥25 kg/m*, %	47	45	60	
Total weight gain‡, kg	12.3±5.5	12.8±5.2	9.6±6.1	**<0.01**
Weight gain, kg/week§	0.49±0.2	0.50±0.2	0.39±0.2	**<0.01**
Parity¶, %				
0	44	45	41	
1	36	35	40	
≥2	20	20	19	0.60
Education**, %				
Elementary school	11	11	13	
Technical/High school	30	29	31	
University education	35	36	28	
Higher academic	24	24	28	0.36
Marital status††, %				
Married	24	23	26	
Living together	71	72	69	
Single	5	5	5	0.77
Smoking‡‡, %				
Before pregnancy: yes	14	14	17	0.39
During pregnancy: yes	5	4	6	0.55
Family history of diabetes§§, %	7	6	14	**<0.01**

Data are presented as means±SD or ratios.
*Differences between non-GDM and GDM using t-test for equality of means, Pearson's χ^2 test and Mann-Whitney U test for two independent samples. Bold text indicates a statistically significant difference with a p-value ≤ 0.05.
†Information on prepregnancy BMI is missing for 22 women.
‡Information on weight gain is missing for 45 women. Total weight gain is the difference between measured weight at first and last maternal care visit.
§Weekly weight gain is the total weight gain divided by number of weeks between first and last maternal care visit.
¶Information on parity is missing for 6 women.
**Information on education is missing for 5 women.
††Information on marital status is missing for 21 women.
‡‡Information on smoking is missing for 6 women.
§§Information on family history of diabetes is missing for 128 women.
BMI, body mass index; GDM, gestational diabetes mellitus; PREWICEII, Pregnant women in Iceland II.

these data, we were able to calculate total weight gain and weight gain per week by dividing total weight gain with the number of weeks between visits.

Self-reported prepregnancy weight and height from the FFQs was used to calculate prepregnancy BMI (kg/m^2). A BMI <18.5 kg/m^2 was defined as underweight, 18.5–24.9 kg/m^2 as normal weight, 25–29.9 kg/m^2 as overweight and ≥30.0 kg/m^2 as obese.

RESULTS

Characteristics of the participants are presented in table 1.

The mean age of all participants was 30 years, and 44% were nullipara. In total, 59% had a university-level or higher academic education, and 14% smoked before pregnancy. The prevalence of GDM diagnoses was 14.9% (n=127). The women diagnosed with GDM were more likely to be older and have a higher prepregnancy BMI. They were, therefore, also more likely to be overweight or obese prior to pregnancy (59.8%) compared with women without GDM (44.6%).

The concentrations of total and relative SFA, MUFA, PUFA n-6 and n-3 at gestational weeks 11–14 are provided in table 2, whereas the concentrations of individual FAs for women with and without later GDM diagnoses are presented in online supplemental table 1.

The total concentration of FA was significantly higher in women diagnosed with GDM, as were the concentrations

Table 2 Fatty acid concentrations at gestational weeks 11–14 in women with and without GDM diagnosis later in pregnancy, also stratified by BMI*

	Non-GDM	GDM		Mean adjusted difference	Non-GDM	GDM	
	Median (10th–90th percentile)				Median (10th–90th percentile)		
	Total µg/mL		P value	µg/mL (95% CI)†	Ratio %‡		P value
All	*(n=726)*	*(n=127)*			*(n=726)*	*(n=127)*	
SFA	906 (712–1166)	966 (747–1230)	**<0.01**	37 (1 to 75)	34 (32–36)	33 (31–36)	0.39
MUFA	688 (521–928)	784 (562–1041)	**<0.01**	75 (40 to 110)	26 (23–29)	27 (24–31)	**<0.01**
PUFA n-6	955 (793–1178)	989 (813–1188)	**0.02**	21 (–11 to 52)	36 (32–40)	35 (31–38)	**<0.01**
PUFA n-3	128 (94–177)	134 (99–180)	**0.05**	–0,3 (–8 to 7)	4.7 (3.7–6.2)	4.6 (3.8–5.9)	0.38
EPA+DHA	93 (66–135)	97 (73–133)	0.11	–2 (–8 to 5)	3.4 (2.5–4.8)	3.4 (2.6–4.5)	0.40
Total fatty acids	2681 (2174–3392)	2898 (2287–3632)	**<0.01**	133 (33 to 233)	–	–	
BMI <25 kg/m²	*(n=396)*	*(n=44)*			*(n=396)*	*(n=44)*	
SFA	887 (705–1153)	941 (730–1282)	0.17	45 (–11 to 100)	34 (32–36)	34 (31–36)	0.54
MUFA	660 (516–906)	753 (522–1034)	**<0.01**	88 (38 to 138)	25 (22–29)	27 (23–30)	**<0.01**
PUFA n-6	947 (789–1178)	969 (769–1203)	0.18	24 (–23 to 72)	36 (32–40)	35 (32–38)	**0.02**
PUFA n-3	126 (93–178)	133 (99–176)	0.14	3 (–8 to 15)	4.7 (3.8–6.2)	4.7 (3.8–6.0)	0.99
EPA+DHA	92 (64–133)	98 (73–136)	0.14	2 (–7 to 12)	3.4 (2.5–4.8)	3.5 (2.6–5.0)	0.87
Total fatty acids	2615 (2127–3340)	2773 (2178–3701)	**0.04**	161 (14 to 307)	–	–	
BMI >25 kg/m²	*(n=324)*	*(n=77)*			*(n=324)*	*(n=77)*	
SFA	918 (727–1186)	973 (766–1231)	**0.01**	30 (–23 to 83)	34 (31–36)	33 (31–36)	0.68
MUFA	717 (535–962)	822 (570–1092)	**<0.01**	60 (12 to 109)	26 (23–30)	27 (24–32)	**<0.01**
PUFA n-6	963 (796–1180)	990 (814–1191)	0.07	16 (–27 to 59)	36 (32–39)	35 (30–38)	**0.01**
PUFA n-3	131 (95–181)	134 (104–182)	0.34	–3 (–13 to 6)	4.7 (3.7–6.2)	4.6 (3.8–5.9)	0.30
EPA+DHA	96 (67–136)	95 (74–132)	0.52	–5 (–12 to 3)	3.5 (2.6–4.7)	3.3 (2.5–4.5)	0.27
Total fatty acids	2769 (2210–3412)	2944 (2323–3632)	**<0.01**	103 (–35 to 240)	–	–	

Bold text indicates a statistically significant difference with a p-value ≤ 0.05.
*BMI information is missing for 12 women.
†Adjusted for age, prepregnancy BMI, weekly weight gain and smoking during pregnancy when all women are included. No adjustment for prepregnancy BMI when stratifying for BMI.
‡Relative FA concentrations as a ratio of total FA.
BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; GDM, gestational diabetes mellitus; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

of all types of FA, except for long n-3 FAs EPA+DHA. When stratified by prepregnancy BMI (<25 kg/m^2 vs ≥25 kg/m^2), the same tendency toward a higher concentration of total FA and MUFA in women who later were diagnosed with GDM was observed in both groups. When comparing relative FA concentrations of women later diagnosed with GDM with those who were not, MUFA was significantly higher, and PUFA n-6 was significantly lower for the women who later developed GDM. This difference remained after stratifying by prepregnancy BMI (<25 kg/m^2 vs ≥25 kg/m^2).

Table 3 shows the OR for developing GDM according to quartiles of FA concentrations (total plasma concentration and ratio of total plasma concentration).

When examining the total concentration, a significant trend toward increased odds of GDM was noted with higher concentrations of MUFA, PUFA n-6 and total FA. However, when FA was expressed as a ratio of total plasma concentration, increased odds for GDM were seen with a higher ratio of MUFA. The odds of GDM were lower in the highest quartile of PUFA n-6 ratio compared with the lowest quartile, although p for trend did not reach significance. No association was observed between PUFA n-3 and GDM; neither for total concentration nor the ratio of total plasma PUFA n-3.

Table 4 shows a comparison of dietary intake in early pregnancy between the women who were later diagnosed with GDM and those who were not.

Women who were later diagnosed with GDM had a significantly lower intake of fatty fish and skimmed milk compared with women who did not develop GDM. They also tended to use a higher proportion of saturated fat but a lower proportion of vegetable oil when cooking (50% vs 29% p=0.06) versus (50% vs 71% p=0.06), respectively. Reported intake of omega-3 supplements also tended to be lower for women who were later diagnosed with GDM, with differences close to significance. Other differences observed in dietary intake between the two groups included a lower frequency intake of whole grains, fruit juice, beans, nuts, seeds and coffee during

Table 3 The associations between quartiles of fatty acid concentrations and ratios with GDM diagnoses

	Total plasma*			Ratio† of total plasma*		
N=853	Median (µg/mL)	No. cases (%)/*n*	OR (95% CI)	Median %	No. cases (%)/*n*	OR (95% CI)
SFA quartile						
SFA-Q1	736	22 (10.3)/213	1.00	31.7	36 (16.8)/214	1.00
SFA-Q2	855	28 (13.1)/214	1.05 (0.55 to 1.99)	33.0	29 (13.5)/215	0.67 (0.37 to 1.22)
SFA-Q3	970	33 (15.5)/213	1.11 (0.58 to 2.13)	34.0	34 (16.1)/211	1.14 (0.64 to 2.03)
SFA-Q4	1145	44 (20.7)/213	1.69 (0.92 to 3.11)	35.8	28 (13.1)/213	0.76 (0.41 to 1.40)
P-trend			0.059			0.65
MUFA quartile						
MUFA-Q1	541	21 (9.9)/213	1.00	23.1	16 (7.5)/213	1.00
MUFA-Q2	646	21 (9.8)/214	0.77 (0.39 to 1.53)	25.0	19 (8.9)/214	0.89 (0.42 to 1.87)
MUFA-Q3	750	29 (13.6)/213	0.87 (0.45 to 1.70)	26.6	37 (17.4)/213	1.82 (0.94 to 3.52)
MUFA-Q4	925	56 (26.3)/213	**2.21 (1.22 to 3.99)**	29.0	55 (25.8)/213	**3.01 (1.16 to 5.67)**
P-trend			**0.001**			**<0.001**
PUFA n-6 quartile						
PUFA n-6-Q1	812	26 (12.2)/213	1.00	32.2	41 (19.2)/213	1.00
PUFA n-6-Q2	916	26 (12.1)/214	0.94 (0.49 to 1.81)	34.6	36 (16.7)/215	1.03 (0.58 to 1.81)
PUFA n-6-Q3	1009	28 (13.1)/213	1.02 (0.54 to 1.92)	36.5	34 (16.0)/212	1.08 (0.61 to 1.91)
PUFA n-6-Q4	1161	47 (22.1)/213	1.67 (0.94 to 2.99)	38.9	16 (7.5)/213	**0.47 (0.24 to 0.94)**
P-trend			**0.046**			0.065
PUFA n-3 quartile						
PUFA n-3-Q1	97	22 (10.3)/213	1.00	3.8	31 (14.6)/213	1.00
PUFA n-3-Q2	120	34 (16.0)/213	1.62 (0.85 to 3.07)	4.4	37 (17.3)/214	1.13 (0.63 to 2.02)
PUFA n-3-Q3	140	35 (16.4)/214	1.22 (0.64 to 2.34)	5.0	33 (15.5)/213	0.97 (0.53 to 1.79)
PUFA n-3-Q4	173	36 (16.9)/213	1.37 (0.72 to 2.59)	5.9	26 (12.2)/213	0.67 (0.35 to 1.25)
P-trend			0.59			0.14
Total plasma concentration						
Total FA-Q1	2237	23 (10.8)/213	1.00	–	–	–
Total FA-Q2	2570	22 (10.3)/214	0.74 (0.38 to 1.47)	–	–	–
Total FA-Q3	2886	32 (15.0)/213	1.10 (0.59 to 2.07)	–	–	–
Total FA-Q4	3323	50 (23.5)/213	1.76 (0.98 to 3.16)	–	–	–
P-trend			**0.014**			

Bold text indicates a statistically significant difference with a p-value ≤ 0.05.
*Adjusted for age, prepregnancy BMI, parity, weekly weight gain, smoking during pregnancy, family history of diabetes.
†Relative FA concentrations as a ratio of total FA.
BMI, body mass index; FA, fatty acids; GDM, gestational diabetes mellitus; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

early pregnancy in women who later were diagnosed with GDM.

There were no clear correlations between dietary intake and FA concentrations except in the case of fatty fish and omega-3 supplements, both of which correlated positively with concentrations of PUFA n-3 and EPA+DHA (online supplemental tables 2 and 3) pregnancy (59.8%) compared with women without GDM (44.6%).

One limitation of our study is that we did not have exact data at the time of diagnoses for our GDM cases. As subjects with known risk factors are more likely to be diagnosed early (online supplemental table 4), we examined the concentrations of total and relative SFA, MUFA, PUFA n-6 and n-3, excluding all women with known risk factors that prompt early GDM screening (BMI ≥30 kg/m^2, age ≥40 years and parity ≥1, to exclude all women with previous GDM/macrosomia).

DISCUSSION

We found that the total concentrations of plasma FAs, as well as total MUFA and MUFA ratio measured at 11th–14th week of pregnancy, were significantly higher and PUFA n-6 ratios lower in women who were later diagnosed with GDM, independent of the women's BMI. The fact that stratifying by BMI did not alter our results is

Table 4 FFQ reported weekly intake of foods at 11th–14th week of pregnancy*

FFQ, frequency per week†	Non-GDM (n=742) Median (10th–90th percentile)	GDM (n=123)	P value‡
Fish, fatty	0.5 (0.1–1.0)	0.1 (0.1–1.0)	**<0.01**
Fish, lean	1.0 (0.1–2.5)	1.0 (0.1–2.5)	0.39
Omega-3 supplements	1.0 (0.3–14.1)	0.3 (0.3–10.9)	0.09
Red meat	1.0 (0.1–2.5)	1.0 (0.1–2.5)	0.94
Poultry	1.0 (0.1–2.5)	1.0 (0.1–2.5)	0.41
Processed meat	0.5 (0.1–2.5)	0.5 (0.1–1.3)	0.39
Whole milk	0.1 (0.1–5.0)	0.1 (0.1–7.0)	0.68
Low fat milk	1.0 (0.2–7.1)	0.6 (0.2–14.1)	0.55
Skimmed milk	0.2 (0.2–1.7)	0.2 (0.2–1.1)	**0.02**
Sour dairy	2.5 (0.1–7.0)	2.5 (0.1–7.0)	0.18
Cheese	5.0 (1.0–14.0)	5.0 (1.0–14.0)	0.11
Butter on bread	5.0 (0.5–14.0)	5.0 (0.3–7.0)	0.42
Butter for cooking	1.0 (0.1–5.0)	2.5 (0.1–5.4)	0.76
Vegetable oil for cooking	5.0 (1.0–7.0)	2.5 (0.3–7.0)	0.07
French fries and chips	0.5 (0.3–2.5)	0.5 (0.1–2.5)	0.53
Cakes, sweets, ice cream and cookies	3.5 (1.0–8.0)	3.0 (0.6–7.5)	0.11
Soft drinks	1.5 (0.2–7.1)	2.6 (0.2–10.8)	0.44
Fruit juice	1.0 (0.1–7.0)	0.5 (0.1–7.0)	**0.01**
Fruits and vegetables	14.0 (5.0–39.0)	14.0 (3.4–39.0)	0.12
Beans, nuts and seeds	0.5 (0.1–5.0)	0.5 (0.1–2.5)	**0.01**
Wholegrains	6.0 (1.2–15.0)	4.0 (0.5–19.2)	**<0.01**
White bread	2.5 (0.1–7.0)	2.5 (0.1–7.0)	0.97
Coffee	0.5 (0.10–14.0)	0.1 (0.1–7.0)	**0.01**

*Data are presented as medians and percentiles (10th–90th).
†FFQ information on intake is missing for six participants.
‡Differences between non-GDM and GDM using the Mann-Whitney U test for two independent samples. Bold text indicates a statistically significant difference with a p-value ≤ 0.05.
FFQ, Food Frequency Questionnaire; GDM, gestational diabetes mellitus.

important to note because obesity, IR and FA profiles in GDM are strongly inter-related.[12]

In most previous studies on FA profiles in pregnancy cohorts, FA analysis was performed during or after GDM diagnoses.[12] These studies found that SFA concentrations appeared to be higher in women diagnosed with GDM when compared with a control group and that PUFA n-6 and PUFA n-3 concentrations were both lower in women with GDM.[12] A recent meta-analysis similarly reported that women with GDM had higher total concentrations of FFAs in the second and third trimester compared with women without GDM, with concentrations decreasing as pregnancy progressed.[35]

The results of the few studies that have investigated FA concentrations in early pregnancy, prior to GDM diagnosis, agree with the present study's findings, showing higher total concentrations of SFA[13–15] and MUFA[13 15] as well as PUFA n-6[16] and PUFA n-3[13] in women who were later diagnosed with GDM compared with those who were not. On the other hand, some of these studies reported lower PUFA n-6[13 15] and PUFA n-3[15–17] concentrations in women who later received a GDM diagnosis.

FA profiles have been proposed as a means of predicting later T2D diagnosis in non-pregnant populations, where higher relative concentrations of FFA,[36] PUFA n-6,[37] MUFA[36 37] and SFA[36 37] have been associated with an increased risk of impaired glucose tolerance and T2D risk. These studies have reported similar results as observed for circulating FA in the pregnant population in our study. Associations between higher PUFA n-6 relative to total FA and increased insulin sensitivity have also been found in previous studies in non-pregnant populations,[36] which is in accordance with the results seen in the present pregnancy cohort.

The FA profiles may be a result of differences in intake or absorption of both carbohydrates and fat.[12] Plasma MUFA and SFA concentrations do not only represent dietary intake as FAs can be synthesized endogenously, mainly from carbohydrates, which is then referred to as de novo lipogenesis.[38 39] Other factors that could influence FA profile are FA synthesis

and incorporation of FA into cell membranes. It remains unclear how the plasma total FA profile is physiologically associated with diabetes. Lipogenesis is stimulated by insulin and suppressed by the hormones glucagon and epinephrine. Some studies have suggested that higher FFA may alter insulin signalling, secretion and glucose production.[40 41] It is, therefore, possible that an abnormal increase of insulin in the blood may lead to higher FA concentrations and vice versa.

Some differences were found in food consumption in the first trimester between women with and without GDM diagnoses, and it is not clear how this difference might be reflected in the plasma FA profile. It could have been expected to see a difference in EPA and DHA concentrations between the two groups because women who later were diagnosed with GDM had a less frequent intake of both fatty fish and omega-3 supplements. However, because FA can be synthesized endogenously from excess carbohydrates, the overall quality of the diet, including carbohydrate quality and amount consumed, might explain some of the difference observed in FA concentration between the two groups, overall carbohydrate quality being one.[19 21 42]

As we have previously reported in this cohort, the women who were later diagnosed with GDM had lower quality of carbohydrate intake as they had a lower intake of wholegrains (estimated by biomarkers).[19] Intake of soft drinks also tended to be greater for women diagnosed with GDM as well as use of saturated fat for cooking, even though the difference was not statistically significant. This could suggest that the overall diet quality in early pregnancy was lower among the women later diagnosed with GDM.

In summary, a stronger association has been observed between plasma FA and diabetes risk compared with dietary intake estimates.[37] It is important to note that different sources of FA measurements can represent varying dietary intake periods, such as adipose tissue (long-term FA intake 1–1.5 years), skeletal muscle cells, erythrocytes (120 days), serum, total plasma, phospholipids (1–2 weeks[12]), cholesteryl esters and FFAs.[37] Erythrocytes have been claimed as a preferable option to evaluate differences in recent FA intake, at least regarding PUFA n-3.[39] However, FA from erythrocytes and plasma have been found to correlate.[37] In our study, we analyzed total plasma FA, which includes the FAs from cholesteryl esters, phospholipids, triacyclglycerols and FFA thought to represent very recent intake (1–2 weeks), whereas the answers in the FFQ covered intake during the previous 3 months.

It might be considered a limitation that our participants were not fasting. This might have resulted in lower concentrations of FFA because they are reduced in response to higher insulin levels postmeals[43] and makes a comparison with other studies challenging. However, comparisons of our results with other studies may be affected by the non-fasting state of our participants and by the difference in methodology used when analysing FA profiles. Another limitation is that we did not have exact information on date of diagnoses, thereby part of our GDM cases may have received their diagnosis at or close to drawing of the blood sample in which FA profiles were quantified. However, we did see a similar trend in the results (online supplemental 4) when we ran our analysis excluding all women with known risk factors that prompt early GDM screening (BMI, age and previous GDM/macrosomia). We therefore believe that this limitation did not majorly affect our main results.

The strength of our study is that we analyzed plasma FA early in pregnancy, in addition to acquiring subjective data on dietary intake. Our study also features prospectively collected data from a large sample size of 853 pregnant women with high participation rate (75%). We report both total concentrations of all FA subgroups as well as their ratios. We adjusted for BMI, and stratified our FA results by BMI, a process that, to our knowledge, has not been previously performed in a pregnancy cohort.

In conclusion, we found that women who were later diagnosed with GDM had a higher concentration of total plasma FA, total MUFA and MUFA ratios as well as lower PUFA n-6 ratios in early pregnancy, independent of the women's BMI, compared with women who remained free of GDM. The women who were not diagnosed with GDM also tended to have better diet quality in early pregnancy. These results suggest that FA biomarkers in early pregnancy may predict GDM. However, further studies are required to confirm this hypothesis.

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Supplement - Early pregnancy plasma fatty acid profiles of women later diagnosed with gestational diabetes

Supplemental Table 1. Early pregnancy oncentrations of individual fatty acids in women with and without later GDM diagnosis, in Iceland.

	Non-GDM (n=726)	**GDM (n=127)**	
All in µg/ml	*median (10th - 90th percentile)*		***P***
SAFA			
12:0 Lauric	3 (1 - 10)	3 (1 - 9)	*0.08*
14:0 Myristic	33 (20 - 59)	34 (19 - 63)	0.93
16:0 Palmiate	649 (506 - 859)	703 (528 - 934)	**0.00**
18:0 Stearic	186 (148 - 233)	191 (153 - 234)	0.16
20:0 Arachidic	7 (6 - 10)	8 (6 -11)	**<0.01**
22:0 Behenic	10 (8 - 13)	10 (8 - 13)	0.06
24:0 Lignoceric	9 (7 - 12)	9 (7 - 11)	0.09
MUFA			
14:1 Myristoleate	2 (1 - 3)	1.6 (1 - 4)	0.22
16:1 Palmitoleic	51 (29 - 87)	62 (34 -104)	**<0.01**
18:1n9 Oleic	561 (427 - 760)	635 (456 - 849)	**<0.01**
18:1n7 Vaccenic	46 (34 - 62)	53 (37 - 70)	**<0.01**
20:1 11-eicosenoate	5 (4 - 8)	6 (4 - 9)	**0.05**
24:1 Nervonic	20 (16 - 26)	22 (18 - 27)	**<0.01**
PUFA n-6			
18:2 LA	712 (577 - 894)	728 (581 - 920)	0.31
18:3n6 GLA	6 (3 - 11)	6 (4 - 11)	**0.02**
20:2 11-14-eicosenoate	6 (4 - 9)	6 (4 - 8)	0.88
20:3n6 DGLA	49 (33 - 69)	55 (35 - 71)	**<0.01**
20:4 ARA	177 (133 - 229)	194 (146 - 252)	**<0.01**
22:2 Docosadientoate	1 (0.4 - 1)	1 (0.5 - 1)	**<0.01**
22:4 Docosatetraenoic	4 (0.1 - 7)	5 (0.1 -7)	0.17
PUFA n-3			
18:3n3 ALA	22 (14 - 35)	23 (14 -38)	0.07
20:5 EPA	19 (10.5 - 37.3)	21 (13 -36)	0.14
22:5n3 DPA	11 (7.7 - 16.3)	11 (8 -16)	0.97
22:6 DHA	73 (52 - 102)	75 (56 - 100)	0.09

Supplemental Table 2. Spearman correlations between frequency of dietary intake and total concetrations of fatty acid types (µg/ml), in pregnant women in Iceland.

	SFA	**MUFA**	**PUFA n-6**	**Total PUFA n-3**	**PUFA n-3 EPA+DHA**

	Spearman Correlation	P	Spearman Correlation	P	Spearman Correlation	P	Spearman Correlation	P	Spearman Correlation	P
Fatty fish	-0.01	0.77	-0.09	0.01	0.00	0.92	**0.20**	<0.01	**0.24**	<0.01
Lean fish	-0.02	0.57	-0.06	0.08	-0.04	0.31	**0.14**	<0.01	**0.18**	<0.01
Omega 3 supplements	**-0.09**	0.01	**-0.15**	0.00	**-0.07**	0.03	**0.25**	<0.01	**0.31**	<0.01
Red meat	0.02	0.55	0.01	0.81	0.03	0.38	**0.08**	0.02	**0.10**	<0.01
Poultry	-0.03	0.38	-0.04	0.23	0.01	0.87	-0.03	0.39	-0.03	0.32
Processed meat	0.01	0.81	0.03	0.43	-0.02	0.62	0.00	0.89	-0.03	0.43
Whole milk	0.02	0.59	-0.02	0.51	-0.05	0.13	0.07	0.06	**0.08**	0.02
Low fat milk	0.02	0.55	0.01	0.79	-0.04	0.25	0.05	0.18	0.04	0.21
skimmed milk	0.03	0.38	0.04	0.28	0.03	0.40	0.05	0.11	0.04	0.21
Soured dairy products	-0.04	0.25	**-0.10**	<0.01	**-0.08**	0.02	0.04	0.27	0.06	0.10
Cheese	-0.01	0.67	**-0.10**	<0.01	-0.02	0.54	-0.03	0.44	-0.02	0.52
Butter on bread	0.00	0.89	-0.06	0.10	-0.01	0.79	0.04	0.30	0.04	0.23
Butter	0.01	0.88	-0.03	0.46	0.01	0.71	0.04	0.29	0.03	0.41
Vegetable oil	-0.02	0.50	-0.04	0.24	0.01	0.74	**0.09**	0.01	**0.09**	0.01
French fries	**-0.08**	0.02	-0.01	0.83	-0.05	0.15	**-0.15**	<0.01	**-0.19**	<0.01
Cakes and condiments	-0.03	0.42	-0.05	0.13	-0.04	0.21	-0.05	0.17	-0.04	0.23
Soft drinks	0.06	0.07	**0.11**	<0.01	0.01	0.67	-0.03	0.34	**-0.08**	0.02
Fruit juice	-0.02	0.63	-0.01	0.71	-0.06	0.07	0.00	0.89	0.00	0.90
Fruits and vegetables	**-0.10**	<0.01	**-0.14**	<0.01	-0.04	0.25	0.02	0.48	0.06	0.06
Bens nuts and seeds	**-0.11**	<0.01	**-0.12**	<0.01	-0.04	0.22	-0.01	0.69	0.01	0.82
Wholegrains	0.00	0.94	-0.02	0.58	-0.01	0.83	0.03	0.40	0.03	0.35
Coffee	-0.06	0.07	**-0.12**	<0.01	-0.02	0.56	-0.02	0.48	-0.02	0.61

Supplemental Table 3. Spearman correlations between frequency of dietary intake and ratios[1] of fatty acid types, in pregnant women in Iceland.

	SFA ratio[1]	**MUFA ratio[1]**	**PUFA n-6 ratio[1]**	**PUFA n-3 ratio[1]**	**PUFA n-3 EPA+DHA ratio[1]**

	Spearman Correlation	P	Spearman Correlation	P	Spearman Correlation	P	Spearman Correlation	P	Spearman Correlation	P
Fatty fish	0.01	0.69	**-0.17**	0.00	0.05	0.17	**0.26**	<0.01	**0.28**	<0.01
Lean fish	0.04	0.30	**-0.09**	0.01	-0.01	0.67	**0.21**	<0.01	**0.23**	<0.01
Omega 3 supplements	-0.05	0.16	**-0.18**	<0.01	0.05	0.19	**0.38**	<0.01	**0.39**	<0.01
Red meat	0.00	0.98	-0.04	0.29	0.02	0.56	**0.09**	0.01	**0.10**	<0.01
Poultry	-0.02	0.63	-0.06	0.07	**0.08**	0.02	-0.02	0.61	-0.02	0.58
Processed meat	0.01	0.75	0.05	0.12	-0.05	0.17	-0.01	0.71	-0.04	0.28
Whole milk	**0.11**	<0.01	-0.02	0.50	**-0.08**	0.01	**0.10**	<0.01	**0.10**	<0.01
Low fat milk	0.06	0.08	0.01	0.75	**-0.07**	0.05	0.04	0.21	0.04	0.30
skimmed milk	0.00	0.93	-0.01	0.88	-0.03	0.33	0.04	0.23	0.03	0.43
Sour dairy	**0.10**	0.00	**-0.11**	<0.01	0.00	0.98	**0.11**	<0.01	**0.11**	<0.01
Cheese	**0.11**	0.00	**-0.15**	<0.01	**0.07**	0.04	0.01	0.70	0.01	0.78
Butter on bread	**0.07**	0.04	**-0.10**	<0.01	0.03	0.44	0.06	0.10	**0.06**	0.10
Butter	0.02	0.49	-0.06	0.08	0.02	0.49	0.05	0.15	0.03	0.33
Vegetable oil	-0.02	0.53	**-0.08**	0.03	0.04	0.20	**0.11**	<0.01	**0.10**	<0.01
French fries	**-0.10**	<0.01	**0.08**	0.02	0.04	0.27	**-0.14**	<0.01	**-0.18**	<0.01
Cakes and condiments	**0.07**	0.03	-0.04	0.23	0.00	0.99	-0.01	0.70	-0.01	0.72
Soft drinks	0.02	0.52	**0.14**	0.00	**-0.11**	<0.01	**-0.10**	<0.01	**-0.15**	0.00
Fruit juice	0.05	0.19	0.02	0.62	-0.05	0.19	0.03	0.44	0.01	0.83
Fruits and vegetables	-0.04	0.22	**-0.13**	<0.01	**0.12**	<0.01	**0.10**	<0.01	**0.12**	<0.01
Bens nuts and seeds	**-0.07**	0.05	**-0.12**	<0.01	**0.12**	<0.01	**0.07**	0.04	**0.08**	0.02
wholegrains	0.03	0.33	-0.03	0.35	-0.01	0.76	0.05	0.15	0.04	0.23
Coffee	0.02	0.61	**-0.15**	<0.01	**0.12**	<0.01	0.05	0.17	0.04	0.22

[1] Relative FA concentrations as a ratio of total FA.

Supplement Table 4. Comparison of early pregnancy fatty acid concentrations and ratios between women with and without GDM diagnosis later in pregnancy, only including women without known GDM risk factors[1].

	Non-GDM median (10th - 90th percentile)	**GDM**		**Mean adjusted difference**	**Non-GDM** median (10th - 90th percentile)	**GDM**	
	Total µg/ml		**P**	**µg/ml (95%CI)[2]**	**Ratio %[3]**		**P**
All	*(n=278)*	*(n=34)*			*(n=278)*	*(n=34)*	
SFA	896 (715 - 1129)	930 (737 – 1146)	0.36	15 (-48, 78)	33.4 (32 - 36)	33.2 (31 - 35)	0.31
MUFA	679 (531 - 895)	754 (550 - 970)	**<0.01**	57 (0.02, 114)	25.5 (23 - 29)	26.4 (24 - 31)	**<0.01**
PUFA n-6	947 (818 - 1168)	996 (806 - 1163)	0.14	6 (-50, 63)	36.0 (33 - 40)	35.2 (31 - 38)	0.13
PUFA n-3	124 (92 - 175)	128 (97 - 165)	0.63	-0,06 (-14, 12)	4.6 (3.6 - 6.2)	4.7 (3.8 - 5.7)	0.71
EPA + DHA	90 (92 - 175)	94 (72 - 125)	0.49	-0.08 (-11, 11)	3.4 (2.4 - 4.7)	3.4 (2.6 - 4.3)	1.00
Total Fatty acids	2647 (2174 - 3269)	2765 (2259 - 3336)	*0.07*	78 (-89, 246)	-	-	

[1] Excluding women with BMI ≥ 30 kg/m^2, age ≥ 40 years and parity ≥ 1

[2] Adjusted for age, pre-pregnancy BMI, weekly weight gain and smoking during pregnancy when all women are included. No adjustment for pre-pregnancy BMI when stratifying for BMI.

[3] Relative FA concentrations as a ratio of total FA.

BMI: Body mass index. FA: Fatty acids GDM: Gestational diabetes mellitus.

Paper IV

Neyslutíðni matvæla eða bætiefna og fylgni við styrk langra ómega-3 fitusýra í blóðvökva barnshafandi kvenna

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ÁGRIP

TILGANGUR

Fyrri rannsóknir benda til að hluti barnshafandi kvenna á Íslandi uppfylli ekki ráðlögð viðmið fyrir neyslu langra ómega-3 fitusýra, sem eru taldar mikilvægar fyrir fósturþroska. Markmið rannsóknarinnar var að meta neyslutíðni barnshafandi kvenna á fæðutegundum og bætiefnum sem innihalda langar fjölómettaðar ómega-3 fitusýrur og kanna fylgni við styrk þeirra í blóðvökva.

AÐFERÐIR

Þátttakendur voru 853 barnshafandi konur sem mættu í fósturgreiningu við 11.-14. viku meðgöngu. Upplýsingar um fæðuval, notkun ómega-3 bætiefna sem innihalda eikósapentaensýru (EPA) og dókósahexaensýru (DHA) og bakgrunn þátttakenda var aflað með fæðutíðnispurningalista. Blóðsýni voru tekin til mælinga á styrk fitusýra í blóðvökva. Fylgni var metin með Spearman-fylgnistuðli.

NIÐURSTÖÐUR

Miðgildi neyslu á mögrum fiski var 1,3 skipti í viku og á feitum fiski eitt skipti í mánuði. Um 50% tóku ómega-3bætiefni daglega eða oftar. Hærri heildartíðni fiskneyslu og notkun bætiefna með ómega-3 fitusýrum endurspeglaðist í hærri heildarstyrk þeirra í blóðvökva (r=0,37 p<0,001). Jákvæð fylgni var á milli tíðni lýsisneyslu (r=0,23, p=0,001) sem og neyslutíðni ómega-3 hylkja/olíu (r=0,20, p=0,001) við styrk ómega-3 fitusýra í blóðvökva. Hins vegar sást engin fylgni á milli neyslutíðni íslensks fjölvítamíns fyrir þungaðar konur (sem inniheldur ómega-3) við styrk ómega-3 í blóðvökva (r=0,03, p=0,98).

ÁLYKTANIR

Neysla matvæla og bætiefna sem innihalda ómega-3 fitusýrur endurspeglaðist í styrk þeirra í blóðvökva, að undanskildu íslensku meðgöngu fjölvítamíni. Helstu niðurstöður okkar eru að rétt rúmlega þriðjungur barnshafandi kvenna borðaði fisk að minnsta kosti tvisvar sinnum í viku í samræmi við ráðleggingar. Um það bil helmingur kvennanna notaði einhver bætiefni með ómega-3 fitusýrum daglega.

Inngangur

Langar ómega-3 fjölómettaðar fitusýrur líkt og eikósapentaensýra (EPA) og dókósahexaensýra (DHA) eru taldar mikilvægar fyrir heilbrigði móður og barns þar sem þær gegna meðal annars mikilvægu hlutverki í þroska miðtaugakerfis.[1,2] Algengasta fitusýran í heilavef er til að mynda DHA og eykst því þörf hennar á meðgöngu vegna uppbyggingar á heilavef fósturs.[1] Þegar hana skortir getur það valdið sjóntruflunum, hegðunarbreytingum og breytingu á efnaskiptum ýmissa boðefna.[3] Samantekt sem gerð var á niðurstöðum íhlutandi rannsókna, sem könnuðu tengsl inntöku á DHA á meðgöngu við útkomubreytur á meðgöngu og fæðingu, svo sem vaxtarskerðingu fósturs, háþrýsting og fyrirburafæðingar, gaf til kynna að til að fullnægja þörfum bæði móður og vaxandi fósturs á meðgöngu þurfi meðalinntaka DHA að vera að lágmarki um 200 mg á dag.[4,5] Þá virðist vera óhætt að neyta allt að eins gramms á dag án neikvæðra afleiðinga.[4] Stærri skammtar af DHA hafa

hins vegar verið tengdir við auknar líkur á ógleði, brjóstsviða og aukinni blæðingu í fæðingu vegna blóðþynnandi áhrifa.[6] Langar ómega-3 fitusýrur er helst að finna í sjávarafurðum eins og fiski og fiskiolíu. Barnshafandi konum er ráðlagt að borða fisk tvisvar til þrisvar í viku, þar af feitan fisk í að minnsta kosti eitt skipti, meðal annars til að ná æskilegum markmiðum um neyslu á EPA og DHA.[4,7] Sem dæmi er í meðalskammti (150 g) af þorski um 105 mg EPA og 228 mg DHA, í ýsu um 143 mg EPA og 195 mg DHA og í laxi 621 mg EPA og 987 mg DHA. Þessar þrjár máltíðir á einni viku myndu því gefa um það bil 200 mg DHA og 124 mg EPA á dag að jafnaði.[8] Undanfarin ár hafa niðurstöður rannsókna á mataræði íslenskra kvenna á meðgöngu, bent til þess að hluti barnshafandi kvenna fullnægi ekki ráðlögðum viðmiðum fyrir neyslu á löngum ómega-3 fitusýrum. Þær aðferðir sem eru almennt notaðar til að kanna mataræði geta þó haft vissa galla[9] og því getur verið gagnlegt að sannreyna niðurstöður með lífmerkjamælingum.[10] Markmið rannsóknarinnar var að meta neyslutíðni barnshafandi kvenna á fæðutegundum og bætiefnum sem innihalda langar ómega-3 fitusýrur, með fæðutíðnispurningalista, ásamt því að mæla styrk fitusýranna í blóðvökva.

Efniviður og aðferðir

Þátttakendur voru 853 barnshafandi konur úr rannsókninni PREgnant Women in ICEland II (PREWICE II), sem fór fram á 6 mánaða tímabili frá október 2017 til mars 2018. Konunum var boðin þátttaka við fósturgreiningu í 11.-14. viku á fósturgreiningardeild Landspítala. Á þessu tímabili voru 1684 konur bókaðar í fósturgreiningu sem samsvarar um 77% af heildarfjölda þungaðra kvenna á Íslandi á rannsóknatímabilinu. Af þessum hópi voru 1350 konur sem mættu í skoðun ásamt því að uppfylla önnur skilyrði fyrir þátttöku í rannsókninni sem voru: staðfest þungun með fósturskimun, að vera í 11.-14. viku meðgöngu við þátttöku og að geta svarað íslenskum spurningalista. Af þeim 1350 konum sem boðin var þátttaka í rannsókninni, vildu 335 konur ekki taka þátt, en 1015 konur samþykktu (76%). Af þeim voru 853 konur sem veittu blóðsýni ásamt því að hafa aðgengilegar upplýsingar um meðgönguna í rafrænni sjúkraskrá og eru niðurstöður birtar eingöngu fyrir þennan hóp.

Fæðuval var kannað með rafrænum fæðutíðnispurningalista sem innihélt einnig spurningar um notkun bætiefna, aldur, menntun, fjölda barna, ógleði á meðgöngu, þyngd fyrir meðgöngu og hæð. Við svörun á spurningalistanum er miðað við fæðuval undanfarinna þriggja mánaða. Svarmöguleikar varðandi tíðni fæðuvals eru 10 talsins og eru allt frá: „sjaldnar en einu sinni í mánuði“ upp í „oftar en 5 sinnum á dag“. Fjallað hefur verið nánar um spurningalistann í fyrri vísindagreinum.[11-14]

Blóðsýni voru fengin til fitusýrumælinga hjá þeim konum sem fóru í blóðsýnatöku sem hluta af fósturskimun við 11.-14.viku meðgöngu á Landspítalanum. Sýnin voru unnin innan klukkustundar þar sem blóðvökvi var aðgreindur í skilvindu við 3000 snúninga á mínútu í 10 mínútur. Í kjölfarið var blóðvökvinn frystur við -80°C þar til hann var sendur til fitusýrugreininga við Chalmers-Tækniháskólann í Gautaborg. Styrkur 24 tegunda fitusýra var mældur með aðferð sem áður hefur verið lýst.[15] Niðurstöðum fyrir styrk DHA og EPA í blóðvökva er bæði lýst sem heildarstyrk og sem hlutfalli af heildarstyrk allra fitusýra í blóðvökva. Niðurstöður eru settar fram sem meðaltöl og staðalfrávik fyrir normaldreifðar breytur, hlutföll eða miðgildi og 10.-90. hundraðshlutar. Mann-Whitney U-próf var notað til að kanna marktækan mun milli breyta sem voru ekki normaldreifðar og fylgni var metin með Spearman-fylgnistuðli. Marktækni var skilgreind sem <0,05.

Tafla I. *Lýsandi einkenni þátttakenda í PREWICE II rannsókninni. Niðurstöður eru settar fram sem meðaltöl ± staðalfrávik eða hlutföll. Niðurstöður fitusýrumælinga eru settar fram sem miðgildi og hundraðshlutar (10-90).*

		(*n* = 853)
Aldur, ár		30,3 ± 4,9
LÞS fyrir meðgöngu[1], kg/m²		25,8 ± 5,7
LÞS ≥25 kg/m², %		47
Heildarþyngdaraukning[2], kg		12,3 ± 5,5
Þyngdaraukning á meðgöngu, kg/viku[3]		0,49 ± 0,2
Fyrri fæðingar[4], %	0	44
	1	36
	≥ 2	20
Menntun[5], %	Grunnskóli	11
	Menntaskóli	30
	Grunnnám háskóla	35
	Framhaldsnám háskóla	24
Hjúskaparstaða[6] %		
	Gift	24
	Sambúð	71
	Einstæð	5
Reykingar[7], %		
fyrir meðgöngu	Já	14
á meðgöngu	Já	5
Heildarstyrkur, µg/ml		
EPA		19 (11-37)
DHA		74 (52-102)
EPA+DHA		94 (66-135)
Hlutfallslegur styrkur, %		
EPA		0,7 (0,4-1,3)
DHA		2,7 (2,0-3,5)
EPA+DHA		3,4 (2,5-4,7)

[1]Upplýsingar um LÞS fyrir meðgöngu vantar fyrir 22 konur.
[2]Upplýsingar um þyngdaraukningu vantar fyrir 45 konur. Heildarþyngdaraukning er mismunur á milli fyrstu og síðustu mældri þyngd í mæðraeftirliti.
[3]Vikuleg þyngdaraukning er heildarþyngdaraukning deilt með fjölda vikna á milli mælinga.
[4]Upplýsingar um fyrri fæðingar vantar fyrir 6 konur.
[5]Upplýsingar um menntun vantar fyrir 5 konur.
[6]Upplýsingar um hjúskaparstöðu vantar fyrir 21 konu.
[7]Upplýsingar um reykingar vantar fyrir 6 konur.
EPA: eikósapentaensýra. DHA: dókósahexaensýra. .LÞS: Líkamsþyngdarstuðull. PREWICEII: Pregnant women in Iceland II.

Niðurstöður

Í töflu I má sjá upplýsingar um aldur, líkamsþyngdarstuðul (LÞS) fyrir meðgöngu, þyngdaraukningu á meðgöngu, fjölda fyrri

Tafla II. *Niðurstöður fæðutíðnispurningarlista varðandi vikulega fæðutíðni matvæla við 11.-14. viku meðgöngu og fylgni[1] við heildar- og hlutfallslegan styrk EPA og DHA. Sett fram sem hlutföll eða miðgildi og hundraðshlutar (10-90).*

	(n=853)				
Vikuleg fæðutíðni	miðgildi (10-90 hundraðshluti)	EPA+DHA, µg/ml	P	EPA+DHA, %[2]	P
Allur fiskur og öll ómega-3 bætiefni	7,5 (1,0-16,3)	0,34	<0,001	0,41	<0,001
Allur fiskur, lýsi og ómega-3 bætiefni[3]	3,3 (0,9-14,7)	0,37	<0,001	0,46	<0,001
Allur fiskur	1,3 (0,4-3,0)	0,24	<0,001	0,28	<0,001
Fiskur, magur	1,0 (0,1-2,5)	0,18	<0,001	0,23	<0,001
Fiskur, feitur	0,3 (0,1-1,0)	0,24	<0,001	0,28	<0,001
Öll ómega-3 bætiefni	7,0 (0,4-14,2)	0,28	<0,001	0,35	<0,001
Lýsi og Ómega-3 olía/hylki	0,7 (0,3-14,0)	0,31	<0,001	0,40	<0,001
Lýsi	0,1 (0,1-7,0)	0,21	<0,001	0,27	<0,001
Ómega-3 olía/hylki	0,2 (0,2-7,1)	0,19	<0,001	0,25	<0,001
Meðgöngu fjölvítamín	0,1 (0,1-7,0)	0,01	0,835	0,001	0,977

[1]Spearman fylgnistuðull. [2]Hlutfall fitusýra af heildarstyrk fitusýra í blóðvökva. [3]Inniheldur ekki meðgöngu fjölvítamín með ómega-3.
EPA: eikósapentaensýra. DHA: dókósahexaensýra.

Tafla III. *Heildar- og hlutfallslegur styrkur EPA og DHA í blóðvökva skipt upp eftir neyslutíðni fisks, sett fram sem hlutföll eða miðgildi og hundraðshlutar (10-90).*

		N%	EPA+DHA, µg/ml	EPA+DHA, %
Allur fiskur	≥2 vikulega	35,1	100 (71-142)	3,7 (2,7-5,1)
	1x mánaðarlega - 1x vikulega	55,9	90 (66-131)	3,3 (2,5-4,6)
	Aldrei	9,0	87 (60-118)	3,0 (2,2-4,1)
	P[1]		**<0,01**	**<0,01**
Fiskur, magur	≥1 vikulega	59,0	98 (70-139)	3,6 (2,6-4,9)
	1x mánaðarlega - <1x vikulega	29,7	87 (64-128)	3,3 (2,5-4,6)
	Aldrei	11,3	89 (62-132)	3,1 (2,3-4,5)
	P[1]		**<0,01**	**<0,01**
Fiskur, feitur	≥1 vikulega	22,5	103 (68-144)	3,7 (2,8-5,2)
	1x mánaðarlega - <1x vikulega	42,0	97 (70-137)	3,5 (2,7-4,9)
	Aldrei	35,5	86 (61-122)	3,1 (2,4-4,3)
	P[1]		**<0,01**	**<0,01**

[1]Kruskal Wallis próf notað til kanna mun á milli neyslutíðnihópa.
EPA: eikósapentaensýra. DHA: dókósahexaensýra.

barna, menntunarstig, hjúskaparstöðu og reykingar, bæði fyrir og á meðgöngu, ásamt heildar- og hlutfallslegum styrk EPA og DHA í blóðvökva þátttakenda. Upplýsingar um neyslutíðni fæðutegunda og bætiefna sem innihalda langar ómega-3 fitusýrur má sjá í töflu II. Þátttakendur borðuðu magran fisk að jafnaði 1,3 sinnum í viku og feitan fisk um einu sinni í mánuði. Jákvæð fylgni var á milli neyslu magurs fisks og feits fisks hjá konunum (r=0,39 p<0,001) (ekki birt í töflu). Hærri heildartíðni fiskneyslu og notkun bætiefna sem innihéldu langar ómega-3 fitusýrur endurspeglaðist í hærri heildarstyrk ómega-3 í blóðvökva (plasma) (r=0,34 p<0,001) og einnig hærri hlutfallslegum styrk EPA + DHA í blóðvökva (r=0,41 p<0,001). Einnig sást jákvæð fylgni á neyslutíðni fisks (r=0,24 p<0,001 og r=0,28 p<0,001) og ómega-3 bætiefna (r=0,28 p<0,001 og r=0,35 p<0,001) við bæði heildar- og hlutfallslegan styrk EPA og DHA í blóðvökva. Þegar skoðuð var sérstaklega fylgni stakra tegunda bætiefna sem innihalda langar ómega-3 fitusýrur við styrk og hlutfall EPA og DHA í blóðvökva sást að bæði neysla á lýsi og ómega-3 olíu eða hylkjum endurspeglaðist í hærri styrk, en ekki neysla á íslensku meðgöngu fjölvítamíni sem inniheldur EPA og DHA (r=0,01 og r=0,001). Þegar íslenska meðgöngu fjölvítamínið var undanskilið og fylgni könnuð á ný, sást hærri fylgni á milli neyslu fisks og bætiefna (r=0,37 p<0,001 og r=0,46 p<0,001) við heildar- og hlutfallslegan styrk EPA og DHA. Í töflu III er samanburður á heildar- og hlutfallslegum styrk EPA og DHA í blóðvökva út frá neyslutíðni kvennanna á fisk. Um 35% borðuðu einhvern fisk tvisvar í viku eða oftar og voru þær með hærri heildar- og hlutfallsstyrk EPA og DHA í blóði, borið saman við þær sem borðuðu fisk sjaldnar eða aldrei.

Í töflu IV sést samanburður á heildar og hlutfallslegum styrk EPA og DHA í blóðvökva, milli kvennanna sem tóku bætiefni sem innihalda langar ómega-3 fitusýrur að minnsta kosti daglega og þeirra sem tóku þau sjaldnar eða aldrei. Í heild tóku um 50% kvennanna einhver bætiefni sem innihalda ómega-3 og voru um 40% að taka lýsi og/eða ómega-3 olíu/hylki daglega. Af þeim sem

Tafla IV. *Heildar- og hlutfallslegur styrkur EPA og DHA í blóðvökva skipt upp eftir neyslutíðni ómega-3 bætiefna. Sett fram sem hlutföll eða miðgildi og hundraðshlutar (10-90).*

		N%	EPA+DHA, µg/ml	EPA+DHA, %1
Öll bætiefni með ómega-3	≥Daglega	50,4	102 (70-148)	3,7 (2,7-5,3)
	<Daglega	12,5	89 (64-120)	3,3 (2,4-4,2)
	Aldrei	37,0	86 (62-118)	3,1 (2,4-4,0)
	***P*[1]**		**<0,01**	**<0,01**
Lýsi og ómega-3 bætiefni	≥Daglega	39,7	105 (72-151)	3,9 (2,8-5,5)
	<Daglega	12,1	91 (66-128)	3,4 (2,5-4,5)
	Aldrei	48,2	87 (62-118)	3,1 (2,4-4,0)
	***P*[1]**		**<0,01**	**<0,01**
Lýsi	≥Daglega	18,8	108 (76-157)	4,0 (3,0-5,6)
	<Daglega	10,4	93 (66-128)	3,4 (2,6-4,7)
	Aldrei	70,8	91 (64-129)	3,3 (2,5-4,5)
	***P*[1]**		**<0,01**	**<0,01**
Ómega-3 olía/hylki	≥Daglega	27,5	103 (70-145)	3,8 (2,8-5,2)
	<Daglega	6,8	92 (71-144)	3,5 (2,5-4,7)
	Aldrei	65,7	90 (64-130)	3,3 (2,5-4,4)
	***P*[1]**		**<0,01**	**<0,01**
Meðgöngu fjölvítamín	≥Daglega	17,1	98 (65-134)	3,4 (2,6-0,5)
	<Daglega	5,3	88 (65-125)	3,2 (2,4-4,3)
	Aldrei	77,6	93 (66-136)	3,4 (2,5-4,8)
	***P*[1]**		0,25	0,41

[1]Kruskal Wallis próf notað til kanna mun á milli tíðnihópa.
EPA: eikósapentaensýra. DHA: dókósahexaensýra.

Tafla V. *Magn EPA og DHA sem bætiefni veita samkvæmt innihaldslýsingu framleiðanda.*[1]

	Meðgöngu fjölvítamín	Lýsi	Ómega-3 olía
(mg)			
EPA	150	114	160
DHA	100	150	100

[1]Byggt á ráðlagðri inntöku viðkomandi bætiefnis.
EPA: eikósapentaensýra. DHA: dókósahexaensýra

tóku daglega bætiefni tóku 27,5% ómega-3 olíu/hylki, 18,8% tóku lýsi og 17,1% tóku íslenskt meðgöngu fjölvítamín sem inniheldur ómega-3 fitusýrur. Marktækur munur var á heildar- og hlutfallsstyrk EPA og DHA í blóðvökva þeirra kvenna sem tóku lýsi og ómega-3 bætiefni í formi olíu eða hylkja daglega borið saman við styrk þeirra sem tóku þau sjaldnar. Hins vegar var enginn marktækur munur á heildar- og hlutfallsstyrk EPA og DHA hjá þeim konum sem tóku meðgöngu fjölvítamínið daglega, borið saman við þær sem tóku það sjaldnar. Í töflu V eru upplýsingar um þann styrk EPA og DHA sem helstu bætiefnin eru sögð veita samkvæmt upplýsingum frá framleiðenda.[16-18]

Umræða

Niðurstöður okkar sýna að einungis um 35% barnshafandi kvenna borða fisk samkvæmt ráðleggingum frá Embætti landlæknis, eða að minnsta kosti tvisvar sinnum í viku.[7,19] Algengara er að konurnar velji magran fisk frekar en feitan fisk, en þar sem jákvæð fylgni var á milli neyslu magurs og feits fisks hjá konunum eru það líklega sömu konurnar sem borða magran og feitan fisk. Miðgildin á neyslutíðni fisks voru þau sömu og sáust í fyrri niðurstöðum PREWICE frá 2015/2016, eða 1,3 skipti í viku.[20] Þess ber þó að geta að tíðni neyslu var könnuð í 11.-14.viku meðgöngu bæði í fyrri og núverandi PREWICE-rannsókn, þar sem konur voru beðnar um að meta tíðni neyslu valinna fæðutegunda síðastliðna þrjá mánuði. Það er því mögulegt að ógleði sem er algengur kvilli á fyrsta þriðjungi meðgöngu gæti hafa haft áhrif á tíðni neyslu á fiski. Hins vegar sást mjög sambærileg fiskneyslutíðni (að meðaltali einu sinni í viku) í annarri íslenskri rannsókn meðal barnshafandi kvenna frá 2012-2013.[21] Þar var fiskneysla könnuð á öðrum þriðjungi meðgöngu, með fjögurra daga matardagbókum, þegar ógleði er yfirleitt liðin hjá. Því virðist allt benda til að fiskneysla barnshafandi kvenna hafi nokkurn veginn staðið í stað síðastliðinn áratug.

Samkvæmt opinberum ráðleggingum er barnshafandi konum ráðlagt að borða fisk tvisvar til þrisvar í viku og að velja feitan fisk helst einu sinni í viku.[7] Fiskur er ein helsta fæðuuppspretta langra ómega-3 fitusýra og afar fáar aðrar fæðutegundir innihalda langar ómega-3 fitusýrur. Myndun EPA og DHA getur þó átt sér stað í líkamanum úr lífsnauðsynlegu ómega-3 fitusýrunni alfa-línólensýra (ALA).[22] Þá eru ensím notuð til að lengja ALA fitusýruna úr 18:3 yfir í 20:5 (EPA) og í kjölfarið er hægt að lengja EPA yfir í 22:6

(DHA).[23] Þessi umbreytingarhæfni er þó misgóð milli einstaklinga og geta ýmsir þættir haft þar áhrif, líkt og heildar fitusýrusamsetning fæðunnar, erfðir, aldur og heilsufar.[24] Þannig virðist mikil neysla á fæði sem er ríkt af ómega-6 fitusýrunni linoleic (LA) eða mjög mikil neysla á ALA ríku fæði geta takmarkað myndun á löngum ómega-3 fitusýrum.[22,23] Staða þekkingar í dag bendir hins vegar til þess að það magn ALA sem er umbreytt í DHA í líkamanum sé afar takmarkað.[4,25]

Styrkur DHA og EPA hefur mælst lægri hjá grænmetisætum, sem borða ekki kjöt eða fisk, borið saman við alætur og enn lægri hjá þeim sem teljast grænkerar.[24,26,27] Grænmetisætur og grænkerar geta þó tekið bætiefni, sem eru unnin úr þörungum og innihalda EPA og DHA fitusýrur.[24,27] Mikilvægt er þó að taka fram að neysla á þörungum eða þara er talin óæskileg fyrir barnshafandi konur, þar sem hætta er á að joð-innihald geti verið umfram hættulaust viðmið.[28] Í rannsókninni frá 2012-2013 kom fram að dagleg meðalneysla barnshafandi kvenna á lýsi og öðrum fiskiolíum var mjög lág, eða um eitt gramm og að aðeins 35% kvennanna fullnægði ráðlögðum viðmiðum um inntöku DHA (≥200 mg/dag að jafnaði).[21] Í PREWICE I frá 2015/2016 voru birt miðgildi á sameinaðri inntökutíðni D-vítamíns og fiskiolíu, sem var 7,1 skipti í viku.[20] Þegar við sameinum inntökutíðni D-vítamíns og fiskiolíu í okkar rannsóknarhóp eru niðurstöðurnar svipaðar, eða 7,3 skipti í viku.

Í okkar rannsóknarhópi notaði 50% barnshafandi kvenna bætiefni sem innihalda ómega-3 daglega. Neysla langra fitusýra endurspeglaðist í styrk þeirra í blóðvökva. Um 17,1% kvennanna tók fjölvítamín ætlað barnshafandi konum, sem inniheldur ómega-3, daglega en neysla þess endurspeglaðist ekki í styrk ómega-3 í blóðvökva. Mögulega er það vegna áhrifa lífaðgengis bætiefnisins. Lífaðgengi ómega-3 fitusýra er mismikið og spila margir þættir þar inn í.[29] Það form sem fitusýrur eru á getur haft áhrif, sýruþol hylkis getur hamlað upptöku, fita sem neytt er samhliða getur aukið upptöku og önnur efni geta truflað ferlið.[29] Algengustu form ómega-3 bætiefna eru: fríar fitusýrur, þríglýceríð, monoacylglycerol, etýlester og fosfólípíð.[29] Samkvæmt rannsóknum sem gerðar hafa verið á upptöku fitusýra virðist besta lífaðgengið vera þegar þær eru á formi frírra fitusýra eða þríglýceríða og það sísta þegar þær eru á formi etýlestera.[30] Þar sem fríar fitusýrur eru óstöðugri og líklegri til að skemmast eru bætiefnin oftast á formi þríglýceríða eða etýlestera.[30]

Samkvæmt upplýsingum um innihaldsefni bætiefnanna frá framleiðendum kom í ljós að íslenska fjölvítamínið fyrir barnshafandi konur inniheldur fiskiolíu á forminu etýlester á meðan ómega-3 töflur og lýsi eru á þríglýseríð formi.[16-18] Líkleg ástæða fyrir því að styrkur ómega-3 fitusýra þeirra kvenna sem tóku inn fjölvítamínið, endurspeglaðist ekki í í blóðvökva þeirra er að frásog fitusýrunnar er ófullnægjandi á forminu etýlester. Einnig skiptir þó máli að fitu sé neytt með notkun á ómega-3 bætiefnum til að auka á frásog þeirra.[31] Þá er frásog einnig háð meltingarkerfinu, þar sem þörf er á meltingarlípösum frá brisi til að vinna úr þríglýceríðum og etýlesterum, og er það niðurbrot talið mun hægar þegar um etýlestera er að ræða.[30,32] Önnur möguleg skýring á þessum mismun er sú að þar sem bætiefnið inniheldur fjölda annarra næringarefna, geta önnur innihaldsefni mögulega hindrað upptöku fitusýranna. Sem dæmi má nefna að kalkjónir geta bundist ómega-3 fitusýrum í meltingarkerfinu og þannig hindrað upptöku þess.[29] Þetta gæti því verið raunin þegar um fjölvítamínið fyrir barnshafandi konur er að ræða, þar sem ráðlagt magn inniheldur 300 mg af kalki, ásamt öðrum næringarefnum.[18]

Það gæti talist takmarkandi að ekki var gerð krafa um að þátttakendur væru fastandi þegar blóðprufan var tekin. Það sama átti þó við um allar konurnar og ættu niðurstöðurnar því að vera samanburðarhæfar. Eins erum við ekki með upplýsingar um nákvæmar skammtastærðir, er kemur að fiskneyslu og töku bætiefna, heldur aðeins fæðutíðni. Aftur á móti sást jákvæð fylgni á milli fæðutíðni matvæla og bætiefna við styrk ómega-3 í blóði. Það að myndun EPA og DHA getur átt sér stað í líkamanum, í mismiklu magni milli einstaklinga og að hluti þessara fitusýra flyst yfir til fósturs getur haft áhrif á niðurstöður mælinga og fylgni útreikninga. Sterkasta fylgnin sem sást í gögnunum, var á milli neyslu alls fisks og allra bætiefna með ómega-3, að undanskildu meðgöngu fjölvítamíninu (r=0,46). Við gildismat matvæla og lífmerkja er fylgni um 0,3-0,4 talin meðalgóð en ákjósanlegust er fylgni á bilinu 0,4-0,7 eða hærra.[9,33]

Fyrri niðurstöður PREWICE II styðja einnig að svör barnshafandi kvenna við spurningum fæðutíðnispurningalistans endurspegla raunverulega neyslu þeirra á heilkornum[13] og tíðni mjólkur- og fiskneyslu tengist styrk joðs í þvagi.[12] Niðurstöður okkar sýna að æskilegt er að hvetja enn frekar til þess að fiskur sé á borðum barnshafandi kvenna, en það gæti einnig dregið úr hættu á joðskorti sem greint hefur verið frá í þýðinu á öðrum vettvangi.[12]

Styrkleikar rannsóknarinnar eru meðal annars að við erum með stóran rannsóknarhóp með háu þátttökuhlutfalli (75%) þar sem við höfum bæði upplýsingar úr fæðutíðnispurningalista ásamt niðurstöðum lífmerkja sem voru mæld í blóði.

Helstu niðurstöður okkar eru að rétt rúmlega þriðjungur barnshafandi kvenna borðaði fisk að minnsta kosti tvisvar sinnum í viku í samræmi við ráðleggingar. Um það bil helmingur kvennanna notaði einhver bætiefni með ómega-3 fitusýrum daglega. Af þeim 554 konum (65%) sem borðuðu ekki fisk tvisvar í viku eða oftar, var um helmingur sem tók einhver bætiefni með ómega-3 (ekki birt í töflu). Neysla matvæla og bætiefna sem innihalda ómega-3 fitusýrur endurspeglaðist í styrk þeirra í blóðvökva, að undanskildu íslensku fjölvítamíni fyrir barnshafandi konur. Líkleg ástæða er ófullnægjandi frásog fitusýrunnar á því formi sem hún er í bætiefninu (etýlester). Niðurstöðurnar benda til þess að stór hluti barnshafandi kvenna fullnægi ekki þörf sinni fyrir langar ómega-3 fitusýrur á meðgöngu. Því er mikilvægt að afla upplýsinga um fiskneyslu í upphafi meðgöngu og út frá því ákvarða hugsanlega þörf fyrir bætiefni, ef konan getur ekki aukið fiskneyslu sína. Einnig er mikilvægt að hafa í huga á hvaða formi ráðlagða bætiefnið er til þess að upptaka þess sé nægileg.

Þakkir

Sérstakar þakkir fær starfsfólk fósturgreiningardeildar Landspítalans fyrir aðstoð við öflun þátttakenda. Einnig þakka höfundar öllum þeim sem unnu við framkvæmd rannsóknarinnar. Rannsóknin fékk styrk frá bæði Rannsóknasjóði Háskóla Íslands og Vísindasjóði Landspítala.

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ENGLISH SUMMARY

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Correlation between intake of fish or supplements containing omega-3 fatty acids and early pregnancy plasma concentrations.

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INTRODUCTION: Long-chain polyunsaturated omega-3 fatty acids are considered important for fetal development, but previous studies suggest suboptimal intake in part of pregnant women in Iceland. The study aim was to evaluate intake of food and supplements containing omega-3 fatty acids, among pregnant women in Iceland and correlations to fatty acid composition in plasma.

MATERIALS AND METHODS: Subjects were 853 pregnant women attending their 11-14 weeks ultrasound appointment. Information on intake of food and supplements containing long-chain omega-3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) as well as background was obtained by a questionnaire. Blood samples were collected for analysis of plasma fatty acids. Correlation was evaluated using the Spearman correlation.

RESULTS: Median intake of lean fish was 1.3 times per week, while fatty fish was consumed once monthly. About 50% of the women took omega-3 containing supplements daily. Higher intake of both fish and omega-3 containing supplements was reflected in higher omega-3 plasma levels ($r=0.37$ $p<0.001$). A positive correlation was seen between intake of cod liver oil/capsules ($r=0.23$, $p=0.001$), omega-3 oil/capsules ($r=0.20$, $p=0.001$) and plasma concentration of omega-3. However, no correlation was seen between intake of a maternal multivitamin containing omega-3 and corresponding plasma concentration ($r=0.03$, $p=0.98$).

CONCLUSION: Intake of food and supplements containing omega-3 fatty acids was reflected in plasma concentration, except for an Icelandic maternal multivitamin. One third of the women followed the recommendation of eating fish at least twice weekly. About 50% had a daily intake of supplements containing omega-3 fatty acids.

Appendix

Participant Number:

It is not necessary to answer all questions in the list if they make you feel uncomfortable or if you are unsure regarding the answer. It is however recommended that as many questions as possible are answered, for the purpose of the study.

FOOD SELECTION

Do you avoid (or never eat) certain types of food? Yes ___ No ____

If yes, check the types of food that you avoid or do not eat.

Cereal products	
Vegetables	
Fruits	
Fish	
Meat	
Eggs	
High-fat foods	
Dairy products	

CONSUMPTION OF VARIOUS FOODS

Keeping in mind the last 4 weeks, put one cross in each row.

How many times per month or week or day do you eat the following types of food?
Questions on fish and meat (including processed meat products) apply to main meals, not sandwich meats or spreads.

	Per month			**Per week**				**Per day**		
	<1	**1**	**2-3**	**1**	**2-3**	**4-6**	**1**	**2**	**3-4**	**≥5**
Vegetables										
Fruits										
Lean fish (e.g., haddock or cod)										
Fatty fish (e.g., salmon, trout or large halibut)										
Red meat (beef, lamb or pork)										
Poultry										
Processed meat products, meat dough products or sausages										
Soured dairy products (sour milk, skyr or yogurt)										
Cheese on bread										
Whole-grain products, other than bread*										

Bean dishes										
Nuts and seeds (not in breads)										
French fries and/or packaged snacks										
Cakes and/or cookies										
Candy										
Ice cream										

*E.g., brown rice, barley or whole-wheat pasta, as accompaniment or part of main meals. Oatmeal or other whole grain porridges as a meal.

FATTY FOODS

Keeping in mind the last 4 weeks, put one cross on each row.

How often do you use the following high-fat foods?

	Per month			**Per week**				**Per day**		
	<1	**1**	**2-3**	**1**	**2-3**	**4-6**	**1**	**2**	**3-4**	**≥5**
Oil or other soft fat for food preparation										
Butter or other hard fat for food preparation										
Butter or oil-blended butter as bread spread										

BEVERAGES

Keeping in mind the last 4 weeks, put one cross on each row.

How many portions per month or per week or per day do you drink of the following beverages?
Assume that one portion is about 250 ml.
Remember to include milk used on morning cereal or porridges and in coffee. Carbonated and non-carbonated beverages with added sugar or sweeteners include all types of carbonated beverages, sports drinks, fruit drinks (other than pure fruit juices) and energy drinks.

	Per month			**Per week**				**Per day**		
	<1	**1**	**2-3**	**1**	**2-3**	**4-6**	**1**	**2**	**3-4**	**≥5**
Pure fruit juice										
Whole milk										
Low-fat milk										
Low-fat milk fortified with vitamin D										
Fortified nonfat milk (vitamin D- and protein fortified)										
Nonfat milk										
Carbonated and non-carbonated drinks with added sugar										
Carbonated and non-carbonated drinks with sweeteners										
Coffee										
Alcohol										

BREAD

Keeping in mind the last 4 weeks, put one cross on each row.

How many slices per month or per week or per day do you eat of the following kinds of bread?

	Per month			Per week			Per day			
	<1	1	2-3	1	2-3	4-6	1	2	3-4	≥5
Whole-grain bread. This means breads marked with the Keyhole or specified as fiber rich or whole grained with at least 25% wholegrains or a fiber content ≥6g per 100g.										
Other breads. This means "common" wheat breads (Heimilisbrauð, Bónus/Krónu-brauð, ciabatta, soft-cheese bows, etc.)										
Rye bread with added sugar. Here, this pertains only to Icelandic rye bread. Please record other rye bread, e.g., Danish rye bread, as whole-grained bread.										

SUPPLEMENTS

Do you use the following supplements?

	Yes	No
Cod liver oil		
Maternal multivitamin with child		
Vitamin-D		
Omega 3 with vitamin D		
Omega 3 without vitamin D		
Folate		
Iron		
Multivitamin with vitamin A		
Multivitamin without vitamin A		
Another supplement _____		

If yes, how often per month or per week or per day do you use the following supplements?

	Per month			Per week			Per day				
	<1	1	2-3	1	2-3	4-6	1	2	3-4	≥5	<1
Cod liver oil											
Maternal multivitamin with child											
Vitamin-D											

Omega 3 with vitamin D											
Omega 3 without vitamin D											
Folate											
Iron											
Multivitamin with vitamin A											
Multivitamin without vitamin A											
Another supplement _____											

Additional questions

Did you have a fishmeal yesterday? Yes/No
Did you use dairy products yesterday? Yes/No - if yes: How much_____
Did you eat eggs yesterday? Yes/No - if yes: How much_____
Do you take a supplement containing iodine? Yes/No/Not sure. IF yes, what is it called_____

Thank you for participating!