Thesis

First trimester biomarkers for gestational diabetes
Ersttrimester-Biomarker für Schwangerschaftsdiabetes

submitted by

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Declaration of Academic Integrity

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Graz, 02.07.2025 Victoria Laura Mogg m.p.

Zusammenfassung in Deutsch

Ziel dieser Diplomarbeit ist es, potenzielle frühe Biomarker für Schwangerschaftsdiabetes (GDM) zu identifizieren. Eine umfassende Literaturrecherche wurde in PubMed unter Verwendung der folgenden Schlüsselwörter: GDM/gestational diabetes – biomarker/screening – early pregnancy/first trimester durchgeführt. Diese Suche ergab eine Vielzahl an Studien, die gründlich analysiert wurden. Nach Ausschluss doppelter Studien und solcher, die die Einschlusskriterien nicht erfüllten oder nicht kategorisiert werden konnten, wurden insgesamt 182 Studien in die endgültige Analyse einbezogen. Diese Studien untersuchten den Zusammenhang zwischen verschiedenen Biomarkern und Screening-Methoden mit GDM. Die Ergebnisse wiesen auf nachweisbare Unterschiede im ersten Trimester zwischen Frauen hin, die später einen GDM entwickelten, und solchen, bei denen dies nicht der Fall war. Zu den vielversprechenden identifizierten Biomarkern, zählen verschiedene Adipokine, Ferritin, Ultraschallmessungen und mikrobielle Merkmale. In Anbetracht der Vielfalt potenzieller Risikoprädiktoren lässt sich schließen, dass der effektivste Ansatz zur Früherkennung von GDM in einer Kombination der vielversprechendsten Biomarker bestehen könnte.

Abstract in English

The aim of this review is to identify potential early biomarkers for gestational diabetes mellitus (GDM). A comprehensive literature search was conducted in PubMed using the key words: GDM/gestational diabetes – biomarker/screening – early pregnancy/first trimester. This search yielded a wide range of studies, which were thoroughly analysed. After excluding duplicate studies and those that did not meet the inclusion criteria or could not be categorized, a total of 182 studies from around the world were included in the final analysis. These studies explored the association between various biomarkers and screening methods with GDM. The results indicated detectable differences in the first trimester between women who later developed GDM and those who did not. Among the promising biomarkers identified are various adipokines, ferritin, ultrasound measurements, and microbial features. Given the diversity of potential early risk predictors, it can be concluded that the most effective approach for the early detection of GDM may involve a combination of the most promising biomarkers.

Contents

1. Introduction

Gestational diabetes (GDM) is characterized by high blood sugar levels, which occur for the first time in pregnancy. It is one of the most common complications in pregnancy and can have severe acute and long-term health consequences for both mother and child. According to the International Diabetes Federation (IDF), the global prevalence of hyperglycemia in pregnancy was estimated at 16.7% in the year 2021, with 80.3% of these cases attributed to GDM. In Europe, the prevalence was slightly lower at 15.0%, meaning that one in seven live births in Europe is affected by hyperglycemia during pregnancy. The IDF´s atlas also shows that the prevalence of GDM increases rapidly with maternal age. Additional risk factors include obesity, excessive weight gain, and a family history of diabetes. As more pregnant women present with one or more of these risk factors, the overall risk of developing GDM continues to rise (Magliano & Boyko, 2021).

Currently, GDM is diagnosed according to the IADPSG criteria (Metzger et al., 2010) between the 24th and 28th week of gestation using an oral glucose tolerance test (OGTT). This test involves measuring fasting blood glucose levels, followed by the ingestion of 75 grams of glucose, after which blood samples are taken one and two hours later. The glucose concentrations are measured in venous blood.

Diagnostic thresholds for GDM are as follows:

- Fasting blood glucose: 92mg/dl or 5.1 mmol/l
- One hour after glucose ingestion: 180mg/dl or 10.0 mmol/l
- Two hours after glucose ingestion: 153mg/dl or 8.5 mmol/l

Due to this late diagnosis, the fetus is exposed to a hyperglycemic environment for an extended period. This leads to excessive glucose transfer to the fetus, resulting in increased insulin production and subsequent beta-cell hyperplasia and hypertrophy. One acute outcome is fetal macrosomia. Other associated complications include shoulder dystocia, neonatal hypoglycemia, hypocalcemia, polycythemia, hyperbilirubinemia and respiratory distress syndrome. If left undiagnosed or untreated, GDM can lead to intrauterine fetal death - 28% of prenatal deaths are attributed to GDM. Furthermore, damage to the fetal beta cells increases the risk of diabetes later in life. Children born to mothers with poorly controlled GDM are also at higher risk for obesity and diabetes. Compared to normoglycemic pregnancies, those affected by GDM show higher incidences of urinary tract infections,

gestational hypertension and preeclampsia, as well as increased rates of cesarean sections and operative vaginal deliveries. Moreover, women with GDM have a 30-84% likelihood of developing glucose tolerance in subsequent pregnancies and an elevated risk of type 2 diabetes mellitus (Claudi-Böhm & Böhm, 2011).

To improve maternal and fetal outcomes, early diagnosis and treatment of GDM are essential. This requires the development of biomarkers and screenings that can predict GDM as early as possible. The following chapters summarize the results of my literature review conducted via PubMed.

2. Methods

I searched the PubMed database for studies dealing with early biomarkers indicating the determination of gestational diabetes later in pregnancy, I conducted my investigation over the period from December 2021 to March 2022. I used the following key words:

GDM/gestational diabetes – biomarker/screening – early pregnancy/first trimester.

Six different keyword combinations were applied. After removing duplicates, 644 studies remained. Applying the exclusion criteria reduced the total to 257 studies. These included prospective and retrospective studies, meta-analyses and systemic reviews conducted worldwide, mainly in Asia and Europe.

To improve clarity, I categorized the selected studies into 16 groups based on the type of biomarker or screening method investigated. This selection process left me with 182 studies for the final analysis.

Inclusion criteria:

- Data collection before the 16th week of gestation
- Biomarker and/or screening tool for early (risk) prediction of GDM
- Investigated biomarker and/or screening tool had to belong in at least one of the compiled categories

Exclusion criteria:

- Data collection/time of screening after the 16th week of gestation
- Studies focused solely on consequences of GDM, or general risk factors for GDM (e.g., depression, sleep behavior/snoring, weight, BMI, lifestyle)
- No results reported
- Full text was not available in English or German

3. Lipid markers

3.1 Introduction

This chapter covers the category of lipid markers. These are the following biomarkers in particular: triglyceride, LDL, HDL, VLDL and fatty acids. From the pool of collected studies eighteen fell into this group. All studies collected their data before the 16th week of gestation.

3.1.1 Lipid marker during pregnancy

Lipid markers have drawn significant attention from various research groups, as elevated levels are known to contribute to insulin resistance and promote beta cell apoptosis – both of which are established risk factors for the development of diabetes. Starting around the 12th week of gestation, levels of triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol and phospholipid begin to rise due to influence of estrogen and increasing insulin resistance. This physiological elevation is a crucial adaptation that enables the maternal body to transition into a catabolic state. At this stage, maternal fat stores become the primary energy source for the mother, allowing glucose and amino acids to be preserved for fetal use. Moreover, the rise in available cholesterol supports fetal development by contributing to the formation of cell membranes, bile acids, and steroid hormones as well as playing a part in cell proliferation and overall growth. These changes indicate that a certain degree of lipid elevation is both beneficial and necessary for a healthy fetal development. However, abnormally elevated lipid levels – particularly in the first trimester – have been associated with metabolic complications such as gestational diabetes and preeclampsia as well as fetal outcomes like macrosomia (Ghio et al., 2011). As a result, the aim of several research groups has been to determine whether early pregnancy lipid profiles could serve as effective biomarkers for predicting the risk of GDM.

3.1.2 Triglyceride, cholesterol, fatty acids, sphingolipids

Triglycerides, or more precisely triacylglycerols, consist of a glycerol backbone bonded to three fatty acid chains. These lipids are the primary form of energy storage in the human body and serve as a crucial energy source, especially during periods of increased energy demand (Horn, 2020i).

Cholesterol, composed of 27 carbon atoms, belongs to the sterol subgroup of lipids. It plays essential roles in numerous physiological processes, including incorporation into cell membranes, the synthesis of steroid hormones, cardioactive glycosides, vitamin D and the production of bile acid (Horn, 2020b). To be transported throughout the body, lipids require

carrier particles known as lipoproteins. Lipoproteins are complexes made up of proteins and various lipids, such as triglycerides, free cholesterol, cholesteryl ester and phospholipids. Functionally, they can be divided into two main groups: triglyceride-rich lipoproteins (chylomicrons, very-low-density lipoproteins (VLDL)) and cholesterol-rich lipoproteins (low-density lipoproteins (LDL) and high-density lipoproteins (HDL)). Once VLDL particles deliver their triglycerides content, they are transformed into intermediate-density lipoproteins (IDL) and eventually into LDL. LDL particles can remain in circulation for several days, acting as mobile cholesterol reservoirs for peripheral tissues. In contrast, HDL particles are responsible for collecting excess cholesterol esters from the peripheral tissues and transporting them back to the liver, a process known as reverse cholesterol transport (Horn, 2020f).

Fatty acids are carboxylic acids with a hydrocarbon chain containing at least four carbon atoms. They exist either freely or as components of more complex lipids. Through the oxidation of fatty acids – primarily via beta-oxidation in the mitochondria - organs, such as liver, heart and actively working skeletal muscles obtain approximately 50% of their energy needs. Depending on the body´s metabolic state, fatty acids may be oxidized for energy or incorporated into triglycerides or phospholipids. In an anabolic state, when glucose is sufficiently available, fatty acids are more likely to be stored rather than broken down (Horn, 2020d).

Sphingosines form the backbone of sphingolipids, another important class of lipids. In cellular membranes, sphingosine is typically bonded to a fatty acid via an amide linkage, forming a molecule known as ceramide. Depending on additional components, ceramides can give rise to various complex lipids such as sphingomyelin (a phospholipid), or the glycolipids cerebroside and ganglioside. Sphingolipids are vital components of the cell membranes and are involved in numerous biological functions, including signaling and structural integrity (Horn, 2020h).

3.2 Collected Studies

3.2.1 Triglyceride and Cholesterol marker

Fifteen studies investigated the correlation between abnormal triglyceride blood levels and the development of GDM. And a total of nine studies dealt with cholesterol, its transport-lipoproteins (LDL, HDL) and cholesterol synthesis marker. The majority of reviewed studies were conducted in Asia and the remaining ones in Australia, Europe and North America. All

blood samples were taken before the gestational week 16, however, the exact time frames were different. A difference between the studies, that also should be noted, is the number of participants they looked on and the different approaches to diagnose GDM.

Conducted by the Obstetrics Department of Fujian Maternity and Child Health Hospital (affiliated to Fujian Medical University) a retrospective study (X. Wang et al., 2021) to determine the clinical values of afamin, triglyceride and PLR as risk predictor for GDM was performed. After going through their medical record from May 2018 to December 2018 they compared the data of 607 with GDM and 833 without GDM. The blood samples were taken between the 10^{th} and the 12^{th} week of gestation. In the direct comparison the triglyceride level of the GDM group were significantly higher.

"TG (mmol/L): 1.56 ± 0.73 (GDM) 1.31 ± 0.47 (non-GDM) p value <0.001" (The Clinical Values of Afamin, Triglyceride and PLR in Predicting Risk of Gestational Diabetes During Early Pregnancy, p. 4)

Other significant differences between the groups were detected in the afamin and PLR levels and in the age of the participants. They then used a multivariate logistic regression analysis to determined independent risk factors for GDM and created a risk prediction model. The risk prediction model combined the independent risk factors: age, afamin levels, triglyceride levels and PLR levels. To evaluate the performance of the risk factors alone and the model they used the ROC and AUC metrices. It turned out, that the model had an even better prediction rate. With the Hosmer-Lemeshow test they showed that the model has also a good discrimination ability. The Hosmer-Lemeshow test is a tool used in statistic to evaluate the goodness of fit of logistic regressions (Paul et al., 2013). In conclusion this study showed that abnormal high blood levels of triglycerides early in the pregnancy are significant risk predictors for GDM later on in pregnancy. But to get an even more accurate prediction, it is necessary to combine different risk predictors (X. Wang et al., 2021).

A second study (Ren et al., 2020), accomplished by the Peking University International Hospital showed similar findings. The triglyceride blood levels showed also a significant difference between the GDM and the non-GDM group. In this case the difference was even more drastic. Although it should be mentioned that the number of participants in this study was slightly smaller. They also not only looked on the importance of the triglyceride and recommend using multiple biomarkers for the risk prediction of GDM.

A few years before that, another study group from Beijing dealt with the topic of risk prediction of GDM (Chen Wang et al., 2016). The collected data were part of a greater retrospective study: "Systemic Random Sampling Survey On The Prevalence Of Gestational Diabetes Mellitus In Beijing (GDM prevalence survey, GPS." In this study they collected the data of 15194 women from 15 different hospitals. For the retrospective analysis, published in the year 2016, they looked on the data of 5265 from the 15194 pregnant women. The measurement of the triglyceride levels had to be done before the 14th week of gestation. Women with diagnosed gestational diabetes had significantly higher fasting glucose, triglyceride (TG) and cholesterol levels then the women without GDM. Additionally, TG/HDL-C and LDL-C/HDL-C ratios were significantly increased in the case group. In a multivariate binary logistic regression analysis, after adjusting for maternal age and family history of diabetes mellitus, they were able to show that early pregnancy cholesterol concentrations a significantly related to GDM in normal-weight and obese women and triglyceride levels in normal-weight and overweight women. Nevertheless, the promising results, fasting glucose showed to be an even better predictor for GDM in each prepregnancy-BMI category. In general, the predictive quality of every studied marker increased along with the pre-pregnancy-BMI. Therefore, pre-pregnancy BMI levels should always be taken into consideration when determining GDM based on early markers.

In the same year another retrospective analysis was done (C. Wang et al., 2017). They also took the collected data from the same greater retrospective study and examined the connection between early lipid profiles (total cholesterol (TC), HDL-C, LDL-C, triglyceride (TG)) and pregnancy outcome. For this analysis the looked on the lipid profiles of 5218 women. 1053 women were diagnosed with GDM. Higher TG and LDL-C as well as lower HDL-C levels showed an association with a higher risk of developing GDM. A multivariate logistic regression analysis determined that early TC, LDL-C and TG levels were independently associated with GDM even after adjusting for cofounders.

In the year 2013 another study took place in a hospital in Beijing (G. Li et al., 2015). In this case it was a prospective cohort study which collected the data of 2488 women between January 2013 – April 2013. Blood samples were drawn between 6 – 15 weeks of gestation. This study also showed that women with GDM had significantly elevated TG, cholesterol, LDL-C levels and LDL/HDL ratio, as well as decreased HDL levels compared to women without GDM. Furthermore, they did subgroup analysis (lean (BMI < 24) vs. obese (≥ 24)), where they found strong associations between high triglyceride levels and increased risk for

gestational diabetes in both subgroups. Whereas women with BMI levels under 24 and high HDL seemed to be protected from developing GDM.

Between July 2016 and July 2017 a prospective cohort study took place in the Fu Xing Hospital, Capital Medical University (H. Zhu et al., 2020). 581 women with gestational diabetes and 2368 women without were included in the study. The study group measured the triglyceride levels between six to eight weeks of gestation (TG0) and between sixteen to eighteen weeks of gestation (TG1) and determined the triglyceride elevation between first and second trimester. Because of that, they divided the women in three groups according to their TG0 levels and their TG elevation. Both comparisons showed that the incidence of GDM increased with the increase of TG0 levels and the TG elevation. These statements remained the same even after adjustment for age, pre-BMI, fasting blood glucose and family history of diabetes mellitus. However, there was no association seen between low TG0 levels and high TG elevations with the development of GDM. They assumed that women with low triglyceride levels in the first trimester require high triglyceride elevation to fulfil the need of the growing fetus. This study had some limitations, there was no known diet records, the pre-BMI was self-reported and the study population only included Chinese women.

In the year 2021 a study accomplished by the Tsinghua University First Hospital was published (Jian-Wei Liang et al., 2021). In their retrospective study they looked on the data of 326 women with GDM and 1790 women without GDM between January 2018 – December 2019. All women had their first visit and blood work done before the twelfth weeks of gestation and an OGGT between 24 and 28 weeks of gestation. They also showed a significant difference by the triglyceride levels between the GDM and the non-GDM group.

“The cutoff value for TG was 1.83 mmol/L based on the Youden index, with sensitivity and specificity values of 69.8% and 76.5%, respectively.” (Potential Biomarkers in Early Pregnancy for Predicting Gestational Diabetes Mellitus and Adverse Pregnancy Outcomes, 2021, p. 3)

In their conclusion they summarized that the triglyceride levels could be a validate predictor of GDM. Measurements above 1.83 should be monitored closely.

A study group from Shanghai took a different approach on the topic (Xue et al., 2021). They investigated the effects of high triglyceride levels in early and late pregnancy. Therefore, they looked in the data from 12 715 pregnant women. Blood samples were taken between 9-

13 weeks of gestation and between 28-42 weeks of gestation. For their analysis the divided in two groups: low and high (> 90th percentile = > 2 mM = 177mg/dl) triglyceride levels. In the low triglyceride level group 12.44% of the women were diagnosed with gestational diabetes and in the high triglyceride group 27.93% of the women. That led to the conclusion, that higher triglyceride levels early in pregnancy increase the risk of getting GDM.

Published in the year 2021 a nested case-control-study based on the Tongji-Shuangliu Birth Cohort (TSBC), a prospective cohort study accomplished in Shuangliu Maternal and Child Health Hospital, Chengdu, China, investigated 328 lipids from 21 classes/subclasses and the risk of developing GDM (Yi Wang et al., 2021). From the data of 6143, collected between March 2017 and June 2019, 336 cases and 672 matched controls were included in this case-control study. Blood samples were taken until the fifteenth week of gestation. Women in the case group had significantly higher total cholesterol, triglyceride and LDL-C levels as well as lower HDL levels. From the lipid biomarker panel 161 of the 328 lipids showed a significant difference between the groups. After using penalized conditional logistic regression and generalized LASSO regression ten lipids remained significant. The addition of these different lipids improved the risk prediction quality of GDM.

As one of the two Australian studies, a study group (Harrison et al., 2015) from the Medical University of Melbourne detected significantly higher triglyceride levels in women with GDM. However, after they adjusted the data for age, ethnicity, baseline BMI, family history, previous GDM, the difference between cases and controls was no longer significant. Which could lead to the conclusion that triglycerides are a more sophisticated biomarker in the Asian ethnicity and not so much in the Australian/Caucasian. However, the study had some limitations. On one hand they only included women, who were overweight (BMI $\geqslant$ 25 kg/m2 or BMI $\geqslant$ 23 kg/m2 with high-risk ethnicity), on the other hand the number of participants were limited. Because of that it could be possible, that triglyceride levels from women with a lower BMI could be predictive for GDM, also in Australian/Caucasian ethnicity. It should be noted, that they applied the ADIPS and IADPSG criteria on the participants. For the IADPSG group low HDL belonged to the risk predictors for GDM development, However, this association did not remain significant after adjustment with the risk factors mentioned above.

Moreover, a study group from Sydney (Sweeting et al., 2019) detected a higher elevation of the triglycerides in the GDM group compared with the control group. Cholesterol levels on

the other hand did not differ significantly between cases and controls. Triglyceride was one of the significant biomarkers for the risk prediction. When they evaluated the performance of the biomarkers as single predictive parameters, triglyceride showed the best results in South Asian women, followed by Caucasian women. In their conclusion the recommend a multivariant risk prediction model for GDM in early pregnancy, which includes triglyceride levels among other biomarkers.

Between January 2011 and January 2013, a prospective cohort study(Kumru et al., 2016), to observe the association among SHBG, HbA1c, FBG, insulin, TSH, fT4, total cholesterol (TC), triglycerides (TG), LDL-C, HDL-C levels and the risk of gestational diabetes, took place in the obstetric department of a hospital in Istanbul, Turkey. Blood samples were taken between 6 to 13+6 weeks of gestation. They analysed the data from 333 women with low-risk pregnancies. 38 women with GDM and 295 without it. All participants of the GDM group had an abnormal 50g GCT plus two abnormal levels at the 100g OGTT. Due to a logistic regression analysis triglyceride (TG) and LDL-C (and SBHG and HOMA) were found independent significant risk predictors for GDM in low-risk pregnancies. According to their findings they determined cut-off levels for each factor and calculated the sensitivity, specificity, positive and negative predictive value. Both (LDL-C and TG) cut-off levels showed a high percentage for specificity and negative predictive value and low percentage for sensitivity and positive predictive value. These discoveries show that first trimester levels of LDL-C and TG in low-risk pregnancies can predict GDM, however the optimal cut-off levels are to be found.

An American study group accomplished a case-control study within a greater cohort study, located in twelve different hospitals across the US (Rahman et al., 2021). In the timeframe from 2009 to 2013 2802 pregnant women were included. For the case-control study 107 women with GDM and 214 matched controls were determined. Gestational diabetes was diagnosed following the Carpenter and Coustan criteria. The determined 420 non-targeted lipid-metabolites divided in four groups (glycerol-lipids, glycerophospholipids, sphingolipids, sterol lipids) and analysed the links between lipid networks, individual lipid-metabolites and GDM. At visit 0 (8-13 weeks of gestation) they identified eight networks, which they colour-coded. The “yellow” and the “brown”, both primarily consisting of short saturated/ low unsaturated triglyceride showed positive association and “turquoise (comprised cholesteryl ester, phosphatidylcholins and long, polyunsaturated triglyceride)” and “blue (sphingomyelins and ceramides)” showed negative associations with GDM.

Moreover, higher levels of 41 (30 triglycerides) and lower levels of six (all cholesteryl esters) metabolites were significantly linked with the risk of gestational diabetes.

The study group from Finland (Miettinen et al., 2014) looked not only on the main lipid markers, such as triglycerides, total cholesterol, LDL-C, HDL-C, but also on the cholesterol synthesis marker. In their study they included 52 obese pregnant women (BMI > 30kg/m^2), at risk for GDM. In twenty-two women gestational diabetes was diagnosed with a 75g OGTT. Blood samples were taken between ten to fourteen weeks of gestation. The GDM and the control group showed no significant differences in total cholesterol, LDL-C, HDL-C, triglycerides or phospholipids levels. Participants with later diagnosed gestational diabetes had elevated serum delta8-cholestenol concentrations and a higher ratio to cholesterol. Moreover, the ratio of lathosterol to that of sitosterol was increased in comparison to the control group. This ratio reflects the cholesterol synthesis.

F. Ianniello et al. (2013) studied additionally to adiponectin levels also lipid markers in overweight/obese women and their connection to GDM. 32 women with BMI levels ≥ 25 kg/m^2, including 16 women, who developed GDM. GDM was diagnosed if two glucose levels from the three-hour 100g OGTT were abnormal. Normal glucose levels were by definition: fasting: 95 mg/dl, one-hour: 180 mg/dl, two-hour: 155 mg/dl and three-hour: 140 mg/dl. Adiponectin and lipid markers were measured at three points during the pregnancy. Firstly, between 8 to 11 gestational weeks. Secondly, between 23 to 25 gestational weeks and thirdly, between 33 to 36 gestational weeks. Women with GDM had lower adiponectin levels throughout the pregnancy. On the contrary, triglyceride, cholesterol level and fatty acids did not differ significantly between cases and controls.

3.2.2 Fatty acids

In total three studies investigated fatty acids as early risk predictors for gestational diabetes. One each is from Italy, Iceland and China. The Italian study group (F. Ianniello et al., 2013) couldn´t find any significant differences in fatty acids levels between cases and controls. Further details are mentioned above.

The study from Iceland (Tryggvadottir et al., 2021) included 853 pregnant women between October 2017 and March 2018. 127 of those women were later diagnosed with GDM. Blood samples were taken between eleven to fourteen weeks of gestation. The study group investigated the absolute and relative (ratio to total fatty acids) concentrations of different fatty acids. Total and monounsaturated fatty acids (MUFA) were significantly higher in

women with gestational diabetes. The relative concentrations of MUFA were higher and the relative concentrations of polyunsaturated fatty acids n-6 (PUFA n-6) were lower in the GDM group. These discoveries stayed significant even after they were stratified for BMI.

The Asian study group (X.-F. Pan et al., 2021) analysed two prospective nested case-control studies from western and central China. The first study was taken between March 2017 and June 2019 and the second between October 2013 and October 2016. In total they analysed the results from 1618 pregnant women (336 cases vs. 672 controls and 305 cases vs. 305 controls). The analysed studies measured the plasma phospholipid fatty acids and the plasma total fatty acids at approximately 10.4 and 13.2 weeks of gestation. For the analyse twenty-one major individual fatty acids, which were investigated in both studies, were taken into consideration. The fatty acids were divided in six groups. Higher levels of even-chain saturated fatty acids (SFAs) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) were associated with gestational diabetes in both studies. Whereas higher levels of n-6 PUFAs showed correlations with the absence of GDM.

3.2.3 Sphingolipids

A Polish study group (Juchnicka et al., 2022) analysed the sphingolipids in pregnant women with and without GDM and in healthy non-pregnant women. In total they analysed the results of 172 women (53 GDM vs. 82 normal glucose tolerance vs. 37 healthy, non-pregnant). Blood samples for determination of the sphingolipid levels were taken in the first (tenth to twelfth week of gestation) and second trimester. GDM was diagnosed with a 75g OGTT between twenty-four and twenty-eight weeks of gestation. First trimester serum C18:1-Cer was significantly higher in pregnant women with GDM, then in pregnant women without it.

3.3 Conclusion

Overall, the studies in Asia consistently reported significantly elevated levels of triglyceride, total cholesterol and LDL-C along with decreased HDL-C levels in women diagnosed with GDM. In contrast, findings from Australia and Europe were more varied. Both Australian studies identified significantly higher triglyceride levels in women with GDM, however, in the study from Harrison et al. (2015) these results lost statistical significance after adjusting for other known risk factors for GDM. Similarly, studies from Italy (F. Ianniello et al., 2013) and Finland (Miettinen et al., 2014) did not find significant differences in lipid markers between cases and controls. It is important to note that both these European studies included only women with elevated BMI, which may have had an influence on the results. In contrast,

a study from Turkey (Kumru et al., 2016) focused on women with low-risk pregnancies and found significantly increased levels of triglyceride and LDL-C in women with GDM compared to normoglycemic women. These findings suggest that lipid markers might be more effective predictors of GDM in women with lower BMI levels. To further explore this hypothesis, future studies in Caucasian populations should include women with a broad range of BMI values.

Regarding fatty acids, studies from Iceland (Tryggvadottir et al., 2021) and China (X.-F. Pan et al., 2021), both indicated that higher levels of n-6 polyunsaturated fatty acids (PUFAs) may have a protective effect against the development of GDM. Additionally, both studies found elevated total fatty acid levels in women with GDM. However, the Italian study (F. Ianniello et al., 2013) did not confirm this findings. To strengthen the evidence presented by these more recent studies, further research involving larger study populations is needed. Lastly, Juchnicka et al. (2022), the only study in this review focusing on sphingolipids, reported higher serum levels of C18:1 ceramide in women with GDM compared to normoglycemic controls. Given the novelty of this finding, additional studies are required to confirm the potential of sphingolipids as early biomarkers for GDM.

4. Inflammatory markers

4.1 Introduction

Tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) are cytokines that act as key mediators in the acute inflammatory response. C-reactive protein (CRP), an acute-phase reactant produced by the liver, is synthesized in response to inflammation. Specifically, IL-1 and IL-6 stimulate hepatocytes to increase CRP synthesis. CRP can recognize molecular ligands on bacterial membranes, bind to them and mark them for clearance by apoptetic cells. Due to this role, measuring CRP levels is commonly used as an indicator of systemic inflammation. inflammation. Chronic low-grade inflammation is recognized as an independent risk factor for the development of GDM. Furthermore, high-sensitive CRP (hs-CRP) has been associated with increased insulin resistance. Given these associations, inflammatory markers such as TNF-α, IL-6 and hs-CRP, are currently under investigation as potential early risk predictors for gestational diabetes (Abbas et al., 2017; Horn, 2020c).

4.2 Collected Studies

Twelve studies are part of this category. Most of them focused on high-sensitivity C-reactive protein (hs-CRP), with two also examining tumor-necrosis factor-alpha (TNF-α) and one including Interleukin-6 (IL-6). These studies were conducted across various countries, including two in Iran, and one each in Turkey, Ireland, Sudan, China, Bangladesh, the United Kingdom, the United States and Greece.

Overall, the majority of the investigated studies showed no significant difference of early (hs-)CRP levels between women with and without gestational diabetes. However, three studies conducted in Turkey (Kansu-Celik et al., 2019), Bangladesh (Fatema Nargis et al., 2016) and Iran (Alamolhoda et al., 2020) presented CRP as a significantly predictive factor for GDM. A Chinese study group (W. Liu et al., 2021) was also able to measure higher CRP levels in the GDM group through the whole pregnancy, but they could only confirm the predictive value of the increased CRP levels for the second trimester.

The study from the UK (Syngelaki et al., 2016) also examined TNF-α as an early biomarker. They measured the biomarker levels from 1000 pregnant women. 200 of them were later diagnosed with GDM. Although there was a significant increase of the TNF-α levels in the women with gestational diabetes, they study authors could not see, that its consideration would make a great difference to screening with maternal characteristics. The US study

group (Nicholson et al., 2013) could also not confirm TNF-α as a useful early predictor for GDM.

Meanwhile the study from Greek (Hassiakos et al., 2016) chose Interleukin-6 as their inflammation marker. The case-control study was part of a prospective observational study for the first trimester prediction of GDM. They compared the IL-6 levels between eleven to fourteen weeks of gestation. Participants divided in 40 cases and 94 controls. All pregnancies were considered low-risk pregnancies. IL-6 was significantly higher in the women with GDM.

4.3 Conclusion

In conclusion some studies reported significantly higher levels of (hs-)CRP in pregnant women, who were later diagnosed with gestational diabetes. However, the majority of studies did not observe a significant increase in CRP levels. To determine whether (hs-)CRP can serve as a reliable early biomarker, larger and long-term studies are needed. Similarly, TNF-α a key cytokine involved in insulin resistance and inflammatory pathways, has been investigated as a potential early indicator for GDM. To date, these findings have been inconclusive, failing to establish a strong predictive value for TNF-α in this context. Interleukin-6, another pro-inflammatory cytokine, showed more promising results in a small study cohort, where elevated levels were associated with later GDM diagnosis. While these findings are encouraging, they must be interpreted with caution due to the limited sample size. Further research involving larger and more diverse study populations is required to assess the reliability and generalizability of IL-6 as a predictive marker.

Overall, although inflammation is widely recognized as a contributing factor to GDM development, the current evidence on specific inflammatory biomarkers remains mixed. Further research, with larger, multi-center cohorts, is needed to clarify the potential of these markers, particularly hs-CRP and IL-6, in early GDM risk prediction.

5. Sonographic markers

5.1 Introduction

The sonographic markers are divided into four groups, based on six different measurements. Two of these measurements are taken from the mother, three from the unborn fetus and one from the placenta. The first group includes studies that investigate adipose tissue thickness and neck circumference as early predictors. Traditionally seen as a passive energy storage, the adipose tissue is now recognized as an active endocrine organ, involved in expressing adipocytes and enzymes linked to steroid hormone metabolism. Excess adipose tissue is associated with metabolic complications, such as insulin resistance and hyperglycaemia. Due to these associations, several research groups have examined adipose tissue thickness, measured via ultrasound, as a potential early predictor for gestational diabetes (Kershaw & Flier, 2004). The second method in this group involves measuring neck circumference (NC), which serves as a marker for upper body subcutaneous fat (Ping Li et al., 2018).

The second and third marker group involve ultrasonographic measurements of the fetus. The second group focuses on nuchal translucency (NT), a fluid-filled area between the fetal neck and skin that is visible in the first trimester ultrasounds. Increased NT measurements are connected with a range of chromosomal and nonchromosomal abnormalities, including heart defects, genetic syndromes and adverse perinatal outcomes (Sharifzadeh et al., 2015).

The third group includes of the fetal heartrate and fetal growth during the first trimester. Fetal heartrate is already part of standard first trimester screening used to assess the risk of chromosomal abnormalities, such as trisomy 21 (Kagan et al., 2008). Sirico, Sarno, et al. (2019) found that fetal heartrate in the first trimester was significantly higher in women with preexisting diabetes mellitus compared to those without it. As a next step, Sirico, Lanzone, et al. (2019) investigated a possible connection between the fetal heartrate and GDM. The well-established association between GDM and increased birth weight has also raised interest in fetal growth during the first trimester as a potential early indicator (Brand et al., 2018).

The fourth group focuses on ultrasonographic measurements of placental vascularisation. Previous studies have shown links between placental vascularisation and various pregnancy outcomes, including preeclampsia and fetal growth restriction (Leijnse et al., 2018; I. Y. Park et al., 2021). The aim of the studies in this marker group was to explore potential associations between early placental vascular indices and GDM.

5.2 Collected Studies

This chapter includes 16 studies, of which seven belong in the first group, four in the second, two in the third and three in the fourth. The ultrasound examinations were conducted between 4 and 14 weeks of gestation.

5.2.1 Adipose tissue thickness and neck circumference

Group one includes studies from Canada, Turkey, China, Italy, Egypt and Brazil. The studies got mixed results. Ping Li et al. (2018) were the only one in this group, who investigated the neck circumference as an early predictor for GDM. GDM was diagnosed using a 75g OGTT between 24 to 28 gestational weeks. A total of 371 pregnant women, including 97 with GDM formed the study population. Neck circumference was measured between 11 to 13+6 gestational weeks. The mean NC was significantly higher in the GDM group; however, its diagnostic accuracy was similar to that of pre-pregnancy BMI.

Bourdages et al. (2018) investigated subcutaneous and visceral tissue thickness as potential predictors for GDM. GDM screening included at first and 50g GCT, if the glucose level met or exceeded 11.1 mmol/L, the test was seen as positive and GDM was diagnosed. If the glucose level was between 7.8 and 11.1 mmol/L, the women had a 75g OGTT as follow-up test. GDM then was diagnosed if at least one glucose level was abnormal. The final study cohort included 977 women without GDM and 61 with GDM. Adipose tissue thickness was rather predictive for GDM. However, its discriminative value was similar to that of BMI.

Aydın et al. (2021) evaluated the predictive value of early adipose tissue measurements for GDM. GDM was diagnosed using a 75g OGTT between 24 to 28 gestational weeks. The study cohort included 142 pregnant women, 19 of them developed GDM. Subcutaneous and visceral adipose tissue was measured between 11 to 14 gestational weeks. Adipose tissue thickness was significantly increased in the GDM group. Nevertheless, BMI showed to be a more accurate predictor of GDM.

The study group from Egypt (Saif Elnasr & Ammar, 2021) observed in a prospective cohort study the potential of visceral and subcutaneous adipose tissue (VAT and SAT) measurements for predicting GDM. VAT was measured between 11 to 14 weeks of gestation. Screening for GDM took place between 16 to 22 gestational weeks with a 75g OGTT. 83 pregnant women, including 12 with GDM, formed the final study cohort. VAT measurements were significantly different cases and controls. They also detected that,

visceral adiposity was positively associated with HOMA-IR and negatively associated with insulin sensitivity.

A second Canadian study group (Souza et al., 2016) measured visceral adipose tissue, total adipose tissue and hepatic fat between 11 to 14 weeks of gestation and studied their potential as risk predictor for problems concerning the glucose homeostasis. To define the state of the glucose homeostasis mid-pregnancy, a 75g OGTT between 24 to 28 gestational weeks was carried out. They then diagnosed an impaired fasting glucose (IFG) as a fasting glucose level ≥ 5.3 mmol/L, an impaired glucose tolerance (IGT) as one-hour glucose level ≥ 10.6 mmol/L or a two-hour glucose level ≥ 8.9 mmol/L. GDM was diagnosed if at least to glucose levels met or exceeded the glucose level cut-off mentioned above. 50 out of 476 pregnant women were diagnosed as either IFG, IGT or GDM positive and formed the case group. The remaining women were the control group. The combination of hepatic fat and high visceral adipose tissue or total adipose tissue depth was a valid predictor of impaired glucose tolerance.

The Brazilian study group (Alves et al., 2020) measured and evaluated first trimester visceral adipose tissue depth in pregnant women as early predictor for GDM. GDM was diagnosed using an 75g OGTT between 24 to 28 gestational weeks. The study population included 431 normoglycemic women and 87 women with GDM. Visceral adipose tissue depth was significantly higher in women with GDM. The difference stayed significant even after adjusting for maternal age and pre-pregnancy BMI. Increased Visceral adipose tissue depth showed to be an even better predictor for GDM than pre-pregnancy BMI.

Gur et al. (2014) studied the possibility of visceral fat thickness measurements as an early predictor for GDM and metabolic syndrome. Visceral fat thickness was measured between 4 to 14 gestational weeks. Impaired glucose tolerance and GDM were diagnosed using an two-step approach. Regardless of their fasting conditions, all women were given a 50g one-hour GCT at about 24 weeks of gestation. Women with a one-hour glucose level ≥ 140 mg/dl were also given a 100g three-hour OGTT. Impaired glucose tolerance was diagnosed if one of the following glucose levels was met or exceeded and GDM was diagnosed if at least glucose levels were abnormal. The glucose cut-offs were as follow: fasting ≥ 105 mg/dl, one-hour ≥ 190 mg/dl, two-hour ≥ 165 mg/dl and three-hour ≥ 145 mg/dl. 94 pregnant women, including 6 with IGT and 10 with GDM were included in the final study population. The measured maximal visceral fat thickness was significantly higher in women with GDM.

5.2.2 Nuchal translucency thickness

Group two contains four European studies., including two from Italy (Luchi et al., 2011), (Visconti et al., 2019) and one each from Slovenia (Tul et al., 2003) and Austria (Heinz Leopold et al., 2005). Luchi et al. (2011) evaluated if first trimester nuchal translucency thickness could predict GDM. GDM screening was two-step approach. The first step was a 50g GCT between 24 to 28 weeks of gestation. If the glucose level after one-hour met or exceeded 140mg/dl, a 100g three-hour OGTT was performed. GDM was diagnosed if at least two glucose levels were abnormal. The defined glucose cut-offs were as followed: fasting; 95 mg/dl, one-hour: 180 mg/dl, two-hour: 155 mg/dl and three-hour: 140 mg/dl. The study population included 678 pregnant women with GDM and 420 pregnant women as controls. The between 11 to 13+6 gestational weeks measured nuchal translucency thickness did not differ significantly between cases and controls.

The second Italian study (Visconti et al., 2019), a retrospective population-based study, evaluated the first trimester Combined test (FTCT) as an early predictor of GDM. GDM screening took place using a 75g OGTT between 16 to 18 weeks of gestation and/or 24 to 28 weeks of gestation. GDM was diagnosed if at least one of the following glucose levels was met or exceeded: fasting ≥ 92 mg/dl, one-hour ≥ 180 mg/dl, two-hour ≥ 153 mg/dl. The study population included in total 2410 singleton pregnant women. 42 of them were diagnosed with GDM in the first screening time range and 554 were diagnosed in the second screening time range. The Combined test is calculated based on the maternal age, nuchal translucency measurement, free beta-hCG and PAPP-A levels between 11 to 13+6 gestational weeks. A logistic regression analysis with a calculated Combined test of < 1: 10000 had an increased risk for developing GDM. However, nuchal translucency thickness was not significantly different between cases and controls.

Tul et al. (2003) studied the predictive quality of a first trimester screening, consisting of nuchal translucency measurement and serum levels of beta-hCG, PAPP-A and inhibin-A, for pregnancy complications. The first trimester screening took place between 10 to 14 weeks of gestation. GDM was one of the investigated pregnancy complications. Diagnosis of GDM was made by using a 100g three-hour OGTT. 1136 pregnant women, including 27 with GDM formed the study population. Nuchal translucency measurement did not differ significantly between pregnancies affected by GDM and pregnancies without complications.

The Austrian study group (Heinz Leopold et al., 2005) performed an open study to evaluate first trimester nuchal translucency measurements as predictors for GDM. GDM was diagnosed using an 75g OGTT between 24 to 28 gestational weeks. 485 pregnant women, including 135 with GDM formed the study cohort. Nuchal translucency measurements between 11 to 14 gestational weeks did not differ significantly between cases and controls.

5.2.3 Fetal heart rate and growth

The third group only includes two studies, a retrospective analysis from Italy (Sirico, Lanzone, et al., 2019) and prospective study from the United Kingdom (Brand et al., 2018). Sirico, Lanzone, et al. (2019) investigated the possible predictive ability of the first trimester fetal heartrate (FHR). Therefore, they used the data from 199 women with GDM and 404 women without GDM (controls), who had a first trimester screening between 2016 and 2018 in three different Italian hospitals. GDM was diagnosed via 75g OGTT between the 24 and 28 weeks of gestation. The fetal heartrate was significantly higher in the case group. Using 162 bpm as cut-off value, the detection rate was 76.9%, the specificity was 67.1% and the negative predictive value (NPV) was 85.5% for GDM. A significantly higher percentage of women in the GDM group had a higher fetal heart compared to women in the control group.

Brand et al. (2018) took a deeper look on the connection between fetal growth and GDM. This study group used the data from the population-based pregnancy cohort study Born in Bradford (BIB). They included 10705 women, 4747 women with European origin and 5958 with South Asian origin. 832 of them were diagnosed with GDM with a 75g OGTT between the 26^{th} and 28^{th} week of gestation. Fetuses of women with GDM showed smaller measurements (mean head circumference at 12 weeks and mean abdominal circumference and estimated fetal weight at 16 weeks of gestation) early in the pregnancy. This early growth restriction was followed by an increased growth. These observations were independent from the ethnicity. However, South Asian fetuses were smaller than White European fetuses, which should result in different growth cut-offs.

5.2.4 Placental vascular indices

The fourth group contains three studies. One each is from the United Kingdom, Taiwan and China. The study group from UK (Savvidou et al., 2013) investigated the uterine artery pulsatility index as an early predictor for GDM. Diagnosis of GDM was made by using a 75g OGTT between 24 to 28 gestational weeks. They included 57 686 pregnant women, 1037 of them were later diagnosed with gestational diabetes. The measurements were taken

between the 11th and 14th week of gestation with a three-dimensional doppler ultrasound. Median uterine artery pulsatility index did not significantly differ between the women with and without GDM.

The study group from Taiwan (Wong et al., 2019) evaluated first and second trimester placenta vascularization and volume in pregnancies affected by GDM. Screening of GDM was performed using a two-step approach. The first step was 50g GCT between 24 to 28 weeks of gestation. If the one-hour glucose level exceeded 140 mg/dl, a 100g three-hour OGTT was performed. Diagnosis of GDM was made if at least two glucose levels met or exceeded the following levels: fasting ≥ 105 mg/dl, one-hour ≥ 190 mg/dl, two-hour ≥ 165 mg/dl and three-hour ≥ 145 mg/dl. 155 pregnant women with risk factors for GDM, including 31 with GDM formed the study cohort. Risk factors were defined as maternal age ≥ 35 years, BMI ≥ 25 kg/m2, history of a macrosomic child (birthweight ≥ 4 kg)/GDM in a previous pregnancy and history of diabetes mellitus in first-/second-degree relatives. Placenta vascularization index (VI) and vascularization flow index (VFI) were significantly lower in pregnancies complicated by GDM in the first and second trimester. The placental flow index, placental volume and uterine artery index did not differ significantly in the first trimester.

The Chinese study (Han et al., 2021) group did get similar results. They not only had significant lower VI and VFI values, but also significant lower placental volume (PV) and flow indexes (FI). GDM was diagnosed with 75g OGTT between 24 to 28 weeks of gestation. The study cohort included 106 pregnant women with GDM and 35 pregnant women without GDM.

5.2 Conclusion

Although all six studies investigating adipose tissue thickness reported significantly higher measurements in the GDM groups, two of them (Aydın et al., 2021; Bourdages et al., 2018) did not find a predictive advantage of ultrasonographic measurements compared to pre-pregnancy-BMI. In contrast, the remaining four studies (Alves et al., 2020; Gur et al., 2014; Saif Elnasr & Ammar, 2021; Souza et al., 2016), not only confirmed increased adipose tissue in women with gestational diabetes but also identified high levels of visceral adipose tissue as a better predictor for GDM. In summary, ultrasonographic measurement of adipose tissue shows promise as an early biomarker for GDM, but its predictive value relative to BMI requires further investigation. Ping Li et al. (2018) was the only study in the first group, to

evaluate neck circumference as an early predictor for GDM. Although mean neck circumference was significantly higher in the GDM group, its diagnostic accuracy was comparable to that of pre-pregnancy BMI.

A common strength among all studies in the second group (Heinz Leopold et al., 2005; Luchi et al., 2011; Tul et al., 2003; Visconti et al., 2019) was their large sample sizes. As part of the first trimester screening conducted between the 11^{th} and 14^{th} weeks of gestation, nuchal translucency (NT) was measured. Each of the four studies used slightly different diagnostic criteria for GDM. Despite these variations, all reached the same conclusion: NT thickness did not differ significantly between GDM cases and control groups, suggesting it is not a useful early predictor.

The third group consisted of two studies, each focusing on a different parameter - fetal heartrate (Sirico, Lanzone, et al., 2019) and fetal growth (Brand et al., 2018) - as early predictors for GDM. The former found that fetal heartrate was significantly higher in pregnancies affected by GDM. The latter observed that fetuses of women with GDM were smaller during the first trimester, followed by accelerated growth later in pregnancy. These findings suggest that fetal ultrasound measurements may serve as promising early indicators for GDM, though additional studies are necessary to confirm these results.

The fourth group included three studies (Han et al., 2021; Savvidou et al., 2013; Wong et al., 2019). The first two examined placental vascularization and volume indices, and both detected lower values of the vascularization index (VI) and the vascularization flow index (VFI) in pregnant women with gestational diabetes. Wong et al. (2019) and Savvidou et al. (2013) also investigated the uterine artery pulsatility index and reached the same conclusion: there was no significant difference between GDM and non-GDM pregnancies. In conclusion, early measurement of VI and VFI may be useful for the predicting of GDM, while the uterine artery pulsatility index appears to have limited predictive value.

6. Glycemic markers

6.1 Introduction

In total, 44 studies from around the world were included in this category. I divided them into four groups, based on the marker they investigated. The markers include first trimester determination of HbA1c, fasting plasma glucose, glycosylated fibronectin, glycated albumin and the effectiveness of first trimester oral glucose tolerance tests (OGTT) and glucose challenge tests (GCT). Group one contains 16 studies, group two 18, group three 4 and group four 10. Some studies examined more than one of the markers discussed here.

HbA1c, a type of glycohemoglobin, is a form of haemoglobin, that undergoes non-enzymatic glycation in response to blood glucose concentration. It serves as a reliable marker of the average blood glucose level over the previous eight to ten weeks. The physiological concentration of HbA1c is typically between 4-6% of the total haemoglobin. Therefore, it is a useful and objective tool for assessing and individual´s glucose tolerance. The hypothesis is that determining HbA1c levels early in pregnancy may help predict whether a woman will develop gestational diabetes (Arasteh et al., 2024).

Fasting plasma glucose (FPG), also known as fasting blood glucose (FBG), is a quick method for evaluating a person´s current glycemic status. FPG is measured by analysing a blood sample taken after a fasting period of eight to twelve hours. Results are reported in either in mmol/L or mg/dl, for comparability, all values in this work are converted to mmol/L. The normal physiological range for FPG is between 70 mg/dl (3.9 mmol/l) and 100 mg/dl (5.6 mmol/l) (World Health Organization).

Fibronectin is a dimeric glycoprotein that exists in various isoforms and play key roles in processes such as cell adhesion, migration and differentiation. It is present on cell surfaces, in plasma and within the extracellular matrix. Cellular fibronectin is produced by endothelial cells, fibroblasts, and smooth muscle cells. Normally, less than 1-2% of total plasma fibronectin is cellular fibronectin. Elevated levels of circulating fibronectin have been associated with metabolic disorders. In particular, elevated levels of cellular fibronectin may reflect the extent of vascular damages and extracellular matrix alterations in individuals with diabetes. The studies referenced in this thesis chapter focus especially on glycosylated plasma fibronectin as a potential marker for GDM (Alanen et al., 2020; Kanters et al., 2001).

6.2 Collected studies

6.2.1 Early detection of HbA1c

Sixteen studies dealt with the question of the efficacy of HbA1c as an early screening tool for gestational diabetes. One major difference between them was the inclusion criteria for the participants. Groups of interest were low-risk pregnancies, high-risk pregnancies, women with polycystic ovary syndrome (PCOS) and a mix of it. Common ground of fourteen out of the sixteen studies is, that the first trimester HbA1c value was significantly higher in women who developed GDM (Amylidi et al., 2016; Arbib et al., 2019; Benaiges et al., 2017; Berggren et al., 2017; Çetin et al., 2021; Hinkle et al., 2018; Jian-Wei Liang et al.; Jian-Wei Liang et al., 2021; Kansu-Celik et al., 2021; Kumru et al., 2016; Maegawa et al., 2003; Osmundson et al., 2016; Pezeshki et al., 2019; Punnose et al., 2020; Sun et al., 2021). However, the calculated cut-off-levels (for diagnosing GDM) are in a range between 5.33 to 6.0%, with different sensitivity, specificity, and predictive value. Nonetheless, HbA1c values above 5.7%, which is considered prediabetic, seem to have significant association with the development of GDM. The two exceptions are a PCOS (polycystic ovary syndrome) study from Norway (Odsæter et al., 2015) and a case-controlled study from China (Y. Pan et al., 2020). The first one showed no significant associations between early HbA1c levels and GDM in women with PCOS. The second one considered 2119 pregnant women in their investigation. 386 women were diagnosed with gestational diabetes. In this study population no significant difference between the HbA1c values of the case and the control group could be found.

6.2.2 Fasting plasma glucose

A total of eighteen studies investigated first trimester fasting plasma/blood glucose levels as early biomarker for gestational diabetes. The study results showed that early FPG values are significantly different between women, who developed GDM and those who did not. Twelve out of the eighteen studies (Harrison et al., 2015; Jian-Wei Liang et al., 2021; Kansu-Celik et al., 2019; Maegawa et al., 2003; Meek et al., 2016; Min Hao & Li Lin, 2017; Ozgu-Erdinc et al., 2015; Y. Pan et al., 2020; Riskin-Mashiah et al., 2010; Sesmilo et al., 2020; Tong et al., 2022; Chen Wang et al., 2016) compared the early fasting blood sugar value and then calculated optimal cut-off-points. For the estimation of these, they often used the Youden index. The cut-off-points are among 4.6 mmol/l and 5.05 mmol/l. Based on these results, the studies considered the early fasting plasma/blood glucose level as an independent biomarker. Two Chinese studies (Y. Pan et al., 2020; Chen Wang et al., 2016) took additionally the BMI

into consideration. One of them (Chen Wang et al., 2016) estimated different optimal cut-off-points based on the BMI and the other one (Y. Pan et al., 2020), showed a better predictive value of the combination FPG and BMI.

The remaining seven studies (Abell et al., 2019; Bojnordi et al., 2021; Corrado et al., 2012; Del López Val et al., 2019; Kuehn et al., 2021; Ozgu-Erdinc et al., 2022; Yeral et al., 2014) looked on diagnostic accuracy of FPG as a screening tool for GDM. Chosen cut-off-point was 5.1 mmol/l. A higher prevalence of GDM cases in women with FPG values above 5.1 mmol/l could be shown. However, there was a fair amount of false positive and negative cases. Due to that circumstance, the study groups recommended using FPG as a predictive value and not as a diagnostic tool.

6.2.3 Glycosylated Fibronectin

Two case-control studies from Finland were investigating the usability of glycosylated fibronectin as a first trimester biomarker for GDM. Their investigated results were on opposite sides. The first study (Alanen et al., 2020) measured the glycosylated fibronectin levels between the nineth and eleventh week of gestation. The study population included 19 pregnant women with GDM and 59 women with unaffected pregnancies. Although they found slightly higher levels in the women with gestational diabetes, the difference between cases and controls was not statistically significant. The second study (Rasanen et al., 2013) took the blood samples between the fifth and thirteenth week of gestation. They compared the results between 90 pregnancies complicated by GDM and 92 uncomplicated pregnancies. Their findings showed significantly higher levels of glycosylated fibronectin in the GDM cases.

6.2.4 First trimester OGTT and GCT

The studies from the fourth group looked into the effectiveness of early screening tests. Oral glucose tolerance test and/or glucose challenge test were taken in the first trimester and in case of a negative test result again in the momentarily recommend timeframe of 24 to 28 weeks of gestation. They then compared the results of the first and second trimester screening and/or the results of the different tests with each other.

Two studies investigated early screening and diagnosis criteria for GDM. One of them is a study from the United Kingdom (Plasencia et al., 2011), in which they took a 50g GCT at six to fourteen weeks of gestation and confirmed a positive result with a 100g GTT. Women with negative GCT or GTT result were measured again between twenty and thirty weeks of

gestation. From 1716 participants, 416 had positive GCT results in the first trimester, but only twenty-seven cases were confirmed. 1689 were screened a second time later on, 547 of it had a positive GCT test, fifty-eight were confirmed. However, forty-seven of the fifty-eight had positive result at the glucose challenge test, but negative glucose tolerance test in the first trimester. All measured post-glucose-load levels were lower in the first compared to the second trimester. Therefore, the study group recommended lowering the cut-off values of GCT and GTT in the first trimester to get better effectiveness. A study from Finland (Jokelainen et al., 2020) came to the similar conclusions, that adapting the glycemic thresholds based on the gestational age are necessary for an early GDM diagnosis.

Singh and Kujur (2016) compared first and second trimester gestational diabetes screening with the development of complications. The study population contained 3000 pregnant women, all of them were screened for GDM with a 75g OGTT in the first trimester and if negative a second time between 24 to 28 weeks of gestation. 156 women were diagnosed with GDM, 61.54% of them were diagnosed in the first trimester. Women who were diagnosed early had significant lower rates of ceaseran deliveries, macrosomia and needed insulin therapy. Therefore, they concluded that first trimester screening is an effective way to lower maternal and perinatal outcomes in GDM.

A Hungarian study from Tamás Bitó et al. (2005) investigated possible glucose level cut-offs before the 16th gestational week as predictors for GDM in high-risk pregnancies. 163 pregnant women with at least one risk factor (family history of diabetes, obesity, age $\geq$ 35 years, glucosuria) were included. Gestational diabetes screening was taken with an OGTT. 88 women were diagnosed with GDM, including eight before the 16th gestational week, which resulted in their exclusion from the study. Remaining GDM diagnosis were made at 24-28 or 32-34 gestational weeks. Women in the case group had significantly higher BMI, fasting glucose and 2h postprandial glucose levels. The combined best cut-off levels of fasting and 2h-postprandial glucose before the sixteenth week of gestation for the prediction of GDM development were 5.3 mmol/l and 6.8 mmol/l.

A Canadian descriptive study in Sherbrooke (Allard et al., 2015) evaluated the clinical implantation of recommendations concerning GDM from a GDM Regional Committee containing primary care physicians, nurses obstetricians, fetal-maternal health specialists, dietitians and endocrinologist. The recommendations were to take a 50g GCT as early as possible, followed by self-monitoring blood glucose (SMBG) four times a day for a week.

If more than 50% of the levels were above the cut-off (fasting ≥ 5.3 mmol/l or 2h-postprandial ≥ 6.7 mmol/l) of the Canadian Diabetes Association (CDA) they were diagnosed with GDM. If the initial GCT was negative or in the SMBG less than 50% of the levels were above the cut-offs, a rescreening in the second trimester was recommended. The results showed that the recommendations were well implemented in this specific area. According to the findings, early screening followed by rescreening in the second trimester, if the first screening is negative, is important. This way a 75g OGTT can be avoided.

A study from Japan (Maegawa et al., 2003) compared the first trimester 50g GCT, HbA1c levels and FPG. 50g GCT had the highest sensitivity. Yeral et al. (2014) compared GCT, FPG and 75g GTT. From this comparison, GTT resulted in the highest sensitivity. In contrast, a study (Lekva et al., 2018)based on a normal to low-risk population could not see a benefit, based on low sensitivity, by taking an early OGTT.

6.3 Conclusion

The interpretation of the results concerning first trimester HbA1c suggest that early HbA1c measurement can be used as a screening tool to identify women at risk of developing GDM. As such it may be considered a risk factor, rather than a definitive biomarker, for gestational diabetes. However, in women with PCOS, early HbA1c levels did not differ significantly between those who later developed GDM and those who remained normoglycemic. This should into account when using first trimester HbA1c measurements as a risk factor/predictive tool for GDM. In summary, earl fasting plasma glucose (FPG) levels were significantly different between women affect by GDM and normoglycemic controls, and they demonstrated strong predictive value. Therefore, it appears reasonable to include first trimester FPG levels in early risk prediction models for GDM. Nevertheless, the optimal cut-off values remain unclear and require further investigation. The studies examining glycosylated fibronectin yielded inconclusive results. Given this, larger-scale studies are recommended to better assess the utility of glycosylated fibronectin as a first trimester biomarker for gestational diabetes. Findings related to the use of glucose screening tools in the first trimester were not unanimous. However, studies did show that earlier diagnosis of GDM was associated with a reduced rate of complications for both mother and child. To implement standard glucose screening tools (e.g. OGTT or GCT) in the first trimester for GDM diagnosis, new cut-off values – possibly in combination with established risk factors – will need to be determined.

7. Insulin-resistance markers

7.1 Introduction

Insulin resistance (IR) is a condition in which the cells of the muscles, fat and liver do not respond effectively to insulin, which leads to higher blood glucose levels. In order to reduce these glucose concentrations, the pancreas must produce even more insulin to help glucose enter the cells. Over time cells can get even more insulin resilient, which results in hyperglycemia. Therefore, IR is considered a preliminary state in the development of diabetes (Cleveland Clinic, 2024). Given its well-established role in the pathogenesis of diabetes mellitus (DM), the studies in this category explored the relationship between IR, its associated markers, and the development of gestational diabetes. A total of 14 studies were included, some of which also examined additional potential biomarkers.

One commonly studied marker for IR is Sex Hormone Binding Globulin (SHBG), a glycoprotein primarily secreted by the liver. For a long time, SHBG was known mainly for its role in the transportation of sex steroids – hence its name. However, research has shown that low SHBG levels are associated with IR and even type 2 diabetes mellitus (I. R. Wallace et al., 2013).

Another group of IR-related assessments includes the Quantose Insulin Resistance test and the Triglyceride-Glucose Index (TyG). The Quantose-IR score, introduced in 2013, is a fasting blood test based on insulin, α-hydroxybutyric acid (α-HB), linoleoylglcerophosphocholine (L-GPC) and oleate levels. Compared to HOMA-IR, BMI, fasting glucose and insulin alone, the Quantose-IR demonstrated superior performance in detecting insulin resistance in non-pregnant human individuals (Cobb et al., 2013). The TyG index, which combines fasting triglyceride and glucose levels, has also shown potential in identifying insulin resistance in non-pregnant people (Guerrero-Romero et al., 2010). Each of these tools was the focus of a single study within this review.

The Homeostasis Model Assessment (HOMA) is another widely used method for evaluating insulin resistance and beta-cell function. HOMA is a mathematical model based on fasting glucose and insulin levels (Gordon et al., 2009). The original HOMA1 model was introduced in 1985 and later updated to HOMA2 in 1996, to better account for variations in hepatic and peripheral glucose resistance (T. M. Wallace et al., 2004). Similarly, the Quantitative Insulin Sensitivity Check Index (QUICKI) is a tool used to assess insulin sensitivity in humans, calculated using the same fasting glucose and insulin parameters (Katz et al., 2000).

Another approach for assessing insulin resistance involves measuring C-Peptide and insulin levels directly. Insulin is a peptide hormone composed of 51 amino acids and is synthesized in the beta cells of the pancreas. C-Peptide, made up of 31 amino acids, is part of the proinsulin molecule. In the pancreas, proinsulin is enzymatically cleaved into C-peptide, Insulin and two pairs of dipeptides. Following cleavage, all components are secreted into the bloodstream (Halwachs-Baumann, 2006). Because C-peptide is released in equal amounts with endogenous insulin, it serves as a reliable marker for assessing endogenous insulin production (Horn, 2020e).

7.2 Collected Studies

7.2.1 Sex Hormone Binding Globulin

Seven of the studies put their focus on Sexual Hormone Binding Globulin as a possible early biomarker for gestational diabetes. The studies are located all over the world. Common ground of all was, that early SHBG levels are lower in women with GDM compared to normoglycemic women. Differences are seen in the significance and the prediction quality of the results. Four studies (Kumru et al., 2016; W. Liu et al., 2021; Nanda et al., 2011; T. Zhang et al., 2018) found significant lower levels in the GDM group. The study with the largest study population was the British study from Nanda et al. (2011), were they compared the results of 11 167 women without and 297 women with GDM.

Berggren et al. (2017) compared early HbA1c and SHBG from 259 pregnant women. Fourteen women were diagnosed with GDM. SBHG were significantly lower in women with GDM. However, the study also showed that its power to identify women at risk for glucose intolerance is not better than the BMI.

Two studies could not find statistically different levels. One of them is a matched case-control study within the Eunice Kennedy Shriver National Institute of Child Health and Human Development Fetal Growth Studies-Singleton Cohort (M.-Y. Li et al., 2020). They matched 214 women without to 107 women with gestational diabetes. The SHBG levels in the GDM group were lower, however the discrepancy was not significant. The other one was a study from Ireland (Corcoran et al., 2018), were they compared the results of 224 women. After adjusting for BMI, ethnicity, and family history the difference between cases and controls was not significant anymore.

7.2.2 Quantose Insulin Resistance test and Triglyceride and Glucose Index

Eid et al. (2022) investigated not only the quality of the Quantose IR test as a biomarker to predict GDM but also compared it to the one-hour Glucose Tolerance Test and HOMA-IR. The study included 100 women, ten of them were diagnosed with gestational diabetes. Quantose IR test showed to be a valid measurement for GDM prediction. However, the first trimester one-hour GTT showed to be an even better predictor for GDM.

Triglyceride and Glucose index (TyG) was the object of investigation of a Mexican study group (Sánchez-García et al., 2020). They compared the TyG results of 164 Latin American women, including 29 of those diagnosed with gestational diabetes. The first trimester TyG results showed no significant difference between women with and without GDM.

7.2.3 HOMA-IR and QUICKI

Both instruments, HOMA-IR and QUICKI were the focus in the following three studies.

Ozgu-Erdinc et al. (2015) measured FPG, FPI, hs-CRP and calculated HOMA-IR, HOMA-beta and QUICKI. This study group could not determinate any significant differences between the case and control group, therefore they concluded that there is no predictive value of the insulin sensitivity indices for GDM. However, the other two studies (Grewal et al., 2012; Ozcimen et al., 2008) were able to find correlations between higher HOMA-IR levels and GDM. Grewal et al. (2012), took a three-hour OGTT in the first trimester and calculated with the Matsuda index the insulin sensitivity and resistance indices. Women with a GDM diagnosis at twenty-four to twenty-eight weeks had a higher insulin resistance then women with a normal glucose tolerance. Optimal calculated cut-off value for the HOMA-IR was 1.77 with a sensitivity of 73.3% and specificity of 61.6% and for QUICKI a cut-off level lower than 0.35 with a sensitivity of 62.3% and specificity of 68.7%. The comparison of the fasting plasma insulin levels showed that women with a FPI value lower then 7.45μU/mL were unlikely to develop GDM.

The second study (Ozcimen et al., 2008) compared the results of 253 women. They divided the participants into two groups, depending on their HOMA-IR values. Group one had levels lower than 2.38 and the second group equal and higher levels of 2.38. Eighteen of the twenty women with gestational diabetes were in the second group. The optimal calculated cut-off level is 2.60, with a sensitivity of 100%, a specificity of 94% and an accuracy of 92%.

7.2.4 C-Peptide and Insulin

Two studies focused on early insulin and C-peptide levels as predictors for GDM. The first study (T. Bitó et al., 2005) measured insulin levels (OGTT: fasting and two-hour) before the 16th week of gestation. GDM diagnosis was made with a 75g OGTT at different points during the pregnancy (< 16 weeks, 24-28 weeks, 32-34 weeks of gestation). Elevated fasting and/or two-hour serum insulin levels showed good predictive value for GDM at 32-34 weeks of gestation. Due to that, the study group recommends treating the women with elevated fasting and serum insulin levels in the same way as women with glucose intolerance. The second study (Fatema Nargis et al., 2016) compared hs-CRP and C-peptide of 28 GDM cases with 71 healthy controls. Women with the later development of gestational diabetes showed higher levels of C-peptide. It should be noted that both studies had slightly small groups of participants.

7.3 Conclusion

Approximately 57% of the studies investigating SHBG as an early predictor for GDM concluded that first trimester SHBG levels were significantly different between pregnancies affected by GDM and those that were not. However, the remaining studies did not support this association. This inconsistency may be due to differences in study design, samples sizes, or population characteristics. Therefore, while SHBG shows potential as a predictive marker, further large-scale, standardized investigations are needed to determine its true utility in early GDM screening.

The Quantose-IR test demonstrated validity as predictive tool for GDM in the first trimester, however, according to Eid et al. (2022), the one-hour glucose tolerance test appeared to be an even stronger predictor. In contrast, the TyG index did not show significant differences in first-trimester values between GDM and non-GDM pregnancies. Based on these findings, both tools may have limited usefulness as standalone early predictors for GDM, although Quantose-IR warrants additional research due to its novel methodology and potential.

Regarding HOMA-IR, most studies reported a strong positive correlation between its values and the development of GDM. Nevertheless, it is important to note that only three studies were included in this analysis. To obtain more robust and generalizable results, further studies with larger cohorts and diverse populations are necessary.

8. Adipocyte-derived markers

8.1 Introduction

This chapter focuses on adipocyte-derived markers, specifically adipocytokines. Sixteen of the studies reviewed addressed this topic. Adipose tissue is now recognized as an active endocrine organ that plays a significant role in both the physiology and pathophysiology of the human body. One of its key functions is the production of various endocrine factors, including adipocytokines (also known as adipokines). These protein-based hormones and cytokines are released by cells within white adipose tissue and are involved in wide range of processes, such as lipid and glucose metabolism, insulin sensitivity, appetite regulation, immune function and inflammation. Due to their broad range of physiological effects, adipocytokines are believed to contribute to the development of metabolic, inflammatory and neoplastic diseases. Moreover, an altered Leptin-to-Adiponectin-Ratio has been observed in individuals with hypertension, dyslipidaemia and impaired glucose tolerance. Given their role in the pathogenesis of metabolic disorders, the potential impact of adipocytokines on pregnancy-related complications – particularly gestational diabetes – has been investigated. These studies further explored the potential of adipocytokines as early biomarkers for gestational diabetes (Gutaj et al., 2020; Mancuso, 2016).

8.2 Summary of all investigated adipokine

Adiponectin

Adiponectin is the most extensively studied adipokine in pregnancy (Khoramipour et al., 2021). It is primarily produced by adipocytes within white adipose tissue. Research has demonstrated its antidiabetic, anti-inflammatory and cardioprotective properties. Accordingly, reduced adiponectin levels are associated with obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular diseases and certain cancer. Women generally exhibit higher adiponectin levels than men, likely due to higher estrogen levels, which influence adipose tissue. Most studies (Gutaj et al., 2020) have shown that in uncomplicated pregnancies, adiponectin levels in the first trimester do not significantly differ from those in non-pregnant women but decrease towards the end of pregnancy.

Leptin

The name *leptin* is derived from the Greek word *leptos*, meaning “thin” (Al-Hussaniy et al., 2021). Leptin shares structural similarities with pro-inflammatory cytokines, such as Interleukine-6. Its receptors are expressed on various cell types and leptin levels correlate

directly with the amount of adipose tissue. Leptin plays a critical role in regulating food intake and energy consumption, acting as a signal of energy availability to the brain. When adipose tissue decreases, circulating leptin levels decline, resulting in increased hunger and energy conserving neuroendocrine responses. Conversely, elevated leptin levels in obesity can lead to leptin resistance (Dornbush & Aeddula, 2023). During pregnancy, leptin levels rise due to its production by both maternal adipose tissue and the placenta. A significant proportion of placental leptin is released into the maternal circulation, contributing to pregnancy-associated weight gain (C. N. Boyle & Le Foll, 2020; Briffa et al., 2015).

Visfatin

Visfatin is named for its dominant source: visceral adipose tissue. Individuals with obesity exhibit increased amounts of visceral fat and consequently, elevated visfatin levels. Visfatin concentrations also rise during pregnancy, peaking between the 19^{th} and 26^{th} weeks of gestation. Interestingly, in obese women with normal pregnancies, visfatin levels remain stable. The precise physiological range of visfatin during pregnancies remains unknown (Gutaj et al., 2020).

Omentin

Like visfatin, omentin is mainly secreted by visceral adipose tissue, though it is also produced in the heart, lungs, ovaries and placenta. There are two isoforms - omentin-1, the predominant circulating form and omentin-2. Lower omentin-1 levels have been observed in individuals with obesity and diabetes mellitus. Since maternal omentin levels do not decrease postpartum, it is likely that the placenta does not significantly contribute to the circulating omentin concentrations (Gutaj et al., 2020).

Chemerin

Chemerin is primarily produced in the liver, subcutaneous fat and visceral adipose tissue, with smaller amounts secreted by various organs, including the placenta (Gutaj et al., 2020). Like other adipokines, chemerin levels are elevated in individuals with obesity (Buechler et al., 2019). It is strongly associated with metabolic, inflammatory and cardiovascular conditions. During pregnancy, chemerin concentrations rise progressively and peak at term (Gutaj et al., 2020).

Irisin

Irisin is a relatively novel adipokine. Initially identified as a myokine secreted by muscle tissue in response to exercise, it has the ability to induce the transformations of white adipose tissue into brown fat (Boström et al., 2012). A Spanish research group later discovered that white adipose tissue is also a source of irisin secretion, classifying it as an adipokine as well (Roca-Rivada et al., 2013). Additional sources include the pancreas, liver, female reproductive system, placenta and neonatal cord blood serum (Gutaj et al., 2020). Irisin plays a role in glucose regulation, neurological and musculoskeletal homeostasis and may help reduce the risk of certain cancers and cardiovascular diseases (S. Liu et al., 2022). In eumenorrheic women, serum levels of irisin are higher in the luteal than in the follicular phase, suggesting involvement in ovulatory regulation. Irisin levels progressively increase throughout pregnancy (Gutaj et al., 2020).

Resistin

Resistin is primarily produced by peripheral blood mononuclear cells (PBMCs), macrophages and bone marrow cells. Additional sources include trophoblastic placental cells and the pituitary gland. Elevated resistin levels are connected with inflammatory conditions (Acquarone et al., 2019). Resistin reduces insulin sensitivity by impairing glucose uptake in adipocytes and raising plasma glucose concentration. While adipose tissue secretion of resistin remains unchanged during pregnancy, circulating resitin levels increase, likely due to placental contribution (Gutaj et al., 2020).

8.3 Collected studies

8.3.1 Leptin, visfatin, omentin, chemerin, resistin

The studies investigating a variety of adipocytokine levels in pregnancies complected by GDM and normoglycemic pregnancies were performed on three different continents: Australia (Melbourne), Europe (Denmark, Netherlands, Romania and the United Kingdom) and North America (US).

Two Australian studies (Abell et al., 2019; Abell et al., 2017) investigated several biomarkers in the same study population. All pregnant participants were overweight or obese and according to a validated risk prediction tool, at high risk for GDM. Blood samples were drawn between twelve and fifteen weeks of gestation. From the 103 participants 90 performed an OGTT and 13 a Glucose Challenge test (GCT) between 26 to 28 weeks of

gestation. GDM was diagnosed according to the ADIPS (Australasian Diabetes in Pregnancy Society) in 25 women. In fact, low levels of high molecular weight (HMW) adiponectin were associated with GDM.

According to Khoramipour et al. (2021) there are three molecular forms of adiponectin within the human body: low weight molecular (LWM), moderate weight molecular (MWM) and high weight molecular (HWM). Each of these has special activities. The here investigated HMW adiponectin for example has associations with glucose uptake and central obesity.

After adjusting for maternal age, pre-pregnancy BMI and past history of GDM, Abell et al. (2017) reported that reduced omentin-1 levels and increased Interleukine-6 (IL-6) levels were also significantly associated with GDM. Due to their findings the study group predicted that adding HMW adiponectin, omentin-1 and IL-6 to early risk prediction tools concerning ADIPS defined GDM could boost the sensitivity. In the second study publication, Abell et al. (2019) not only looked into the improvement of risk prediction tools according to the ADIPS but also to the IADPSG (International Association of Diabetes and Pregnancy Study Groups). In an univariable regression analysis HMW adiponectin was connected to a reduced risk for ADIPS and IADPSG GDM. Moreover, they compared a validated risk prediction tool with the tool inclusive the adipocytokines identified above. The reference biomarker model is based on fasting glucose and maternal factors. As described above, HMW adiponectin enhanced the biomarker model according to the ADIPS criteria. However, according to the IADPSG criteria, no significant improvement was found. Thus, authors proposed further studies to investigate the promising biomarker adiponectin and its use in risk prediction tools according to IADPSG criteria.

Lain et al. (2008) investigated the possible different concentrations of adiponectin, resistin and IL-6 in women with and without GDM. Blood samples of nulliparous women between 9.3 +- 2.6 weeks of gestation were gained. GDM was diagnosed between twenty-four and twenty-eight weeks of gestation with a two-step approach (50g GCT and a 100g three-hour OGTT). Thirty participants with GDM were compared to twenty-nine controls. The only statistically significant difference between the groups was their smoking-status. Adiponectin levels were significantly lower in women, who later developed GDM. Resistin and IL-6 concentrations did not differ among the two groups.

The second American study group (Francis et al., 2020) studied ten different adipocytokines and their association to GDM. They gained their data from a nested case-control study within the prospective Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies-Singletons Cohort. Adipocytokine levels were measured between ten and fourteen weeks of gestation. The measurements were compared between 107 participants with GDM and 214 matched controls. Throughout pregnancy women with GDM had higher FABP4, chemerin and IL-6, and lower soluble leptin receptor (s-OBr) concentrations than the controls. Furthermore, increased leptin levels and decreased adiponectin and HMW adiponectin levels were measured in GDM cases.

FABP4 stands for FA binding protein 4. Recent research ((Prentice et al., 2019) has shown its role in maintaining glucose homeostasis. Further, lower FABP4 levels have been linked to enhanced metabolic health. The soluble leptin receptor or short sOBr, as described from Schaab and Kratzsch (2015), is the primary binding protein for leptin and one of four leptin receptor isoforms. Due to its important role in leptin signalling, it is jointly responsible for the leptin bioavailability. People with type 1 diabetes mellitus or obesity showed different blood values of sOBr compared to healthy individuals.

Leptin, adiponectin and adiponectin/leptin ratio and their association to BMI and GDM were the aim of a Danish study group (Thagaard et al., 2017). The 2590 study participants were divided into three groups: normal weight, moderately obese (BMI equal and above 30 kg/m^2) and severely obese (BMI equal and above 35 kg/m^2). Blood samples were drawn between six to fourteen weeks of gestation. Significant associations between adiponectin/leptin ratio and GDM were only seen in normal weight and moderately obese women. Leptin on the other hand was only significantly connected to GDM in severely obese women. Adiponectin was overall significantly associated with GDM. One notable limiting factor is that according to the Danish National Guidelines, screening for GDM is only performed in women with GDM risk factors:

"… GDM in previous pregnancies, BMI ≥ 27 kg/m2, family history of diabetes mellitus, glycosuria, or previous delivery of infant with birth weight ≥4500 g. …" (Näslund et al. 2017: 1806 as cited in Obstetrik – DSOG

Schuitemaker et al. (2020) analysed soluble Frizzled-Related Protein 4 (sFRP4), adiponectin, chemerin and leptin in pregnant women between seventy to ninety days of gestation. Secreted Frizzled-Related Protein 4 (sFRP4) belongs to a group of five secreted

Frizzeld-Related Proteins, which are all modifying the Wnt-signalling-pathway. Through regulating the insulin secretion from islet cells, sFRP4 is part of the modulation of the glucose resistance. Previous studies showed that sFRP4 increased in the second trimester in women with GDM. The study population consisted of fifty women who developed GDM and one-hundred matched controls. GDM screening via 75g OGTT only took place in women with risk factors (GDM in previous pregnancies, family history of DM in a first degree relative etc.). Women with GDM had increased chemerin, leptin and logarithmic transformed sFRP4 levels whilst adiponectin serum concentrations were lower in the case group.

In contrast to the previous studies, Florian et al. (2021) concentrated on the predictive qualities of adipocytokines for GDM in women not at risk. The Authors examined the serum samples from sixty-eight women between eleven to thirteen weeks plus six days of pregnancy. 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), adiponectin and leptin were analyzed.

Prentice et al. (2014) define CMPF as a furan fatty acid metabolite. Furan fatty acids are integrated in phospholipids and cholesterol esters. Elevated levels of CMPF were detected in subjects with diabetes mellitus type 2 and GDM.

After a 75g OGTT between twenty-four to twenty-eight weeks of gestation twenty-one women were diagnosed with GDM. Increased Leptin levels and a decreased adiponectin/leptin ratio were associated with GDM. However, adiponectin and CMPF did not differ significantly between the case and control group. The higher leptin levels in women with GDM were independent from maternal age, pre-pregnancy BMI and smoking habits.

The focus of the study group from the United Kingdom (Ferreira et al., 2011) were visfatin and adiponectin. One hundred women with GDM were compared to three hundred controls. Serum samples were drawn between eleven to thirteen weeks of gestation. For GDM diagnosis, a two-step-approach was used: plasma glucose was measured between twenty-four to twenty-eight weeks gestation, if measurements were above 6.7 mmol/L, an OGTT was performed within the next two weeks. Maternal age, pre-pregnancy BMI and GDM in past pregnancies were higher in the case group. Further, more women with South Asian origin were in this group. Elevated visfatin and decreased adiponectin concentrations were identified in women with GDM compared to the controls.

8.3.2 Adiponectin

Half of the here discussed studies concentrate solely on adiponectin as an early biomarker. The other studies investigate a panel on serum markers including adiponectin. The studies were conducted in India, Ireland, Italy, United Kingdom, Canada and US.

Due to the promising prediction quality of adiponectin in Western literature Madhu et al. (2019) aimed to investigate adiponectin as an early biomarker for GDM in Indian women. They included four-hundred-fifty women. Blood samples were drawn between eleven to thirteen weeks of gestation. GDM screening was performed between 24 to 28 weeks of gestation via 75g OGTT according to the IADPSG. 45 women were diagnosed with GDM, adiponectin levels were compared to 45 matched controls. Women with GDM showed significant lower adiponectin concentrations than control women. According to logistic regression decreased first trimester adiponectin was revealed as strongest independent risk factor for GDM.

Similar results were observed by Corcoran et al. (2018). 248 women before 15 weeks of gestation with at least one risk factor for GDM were recruited. The risk factors included a BMI level $\geq$ 30 kg/m^2; maternal age over 40 years; several ethnicities – Indian, Pakistani, South East Asian, Middle Eastern, Afro-Caribbean; history of PCOS; family history of type 2 diabetes; macrosomic baby with a birthweight over 4 kg or an unexplained stillbirth in previous pregnancies. Measured biomarkers were c-reactive protein (CRP), sex hormone binding globulin (SHBG), 1,5-anhydroglucitol and adiponectin. OGTT for GDM diagnosis was taken at twenty-eight weeks of gestation. Due to different reasons OGTT results from twenty-three women were not available and therefore excluded from the study. Overall, serum measurements of forty-six women with and one-hundred-seventy-eight women without GDM were compared.

An Italian study group (F. Ianniello et al., 2013) examined adiponectin and its association with GDM throughout pregnancy in overweight/obese women. Authors compared sixteen women with and without GDM. First trimester adiponectin levels were measured between eight to eleven weeks of gestation. GDM screening consisted of a fasting plasma glucose level and three-hour 100g OGTT and was confirmed after at least two abnormal levels. Despite of the small sample size, GDM cases had significant decreased adiponectin concentrations the whole pregnancy, as well as higher body fat mas and blood pressure at the beginning.

Nanda et al. (2011) investigated the prediction ability of adiponectin, SHBG and Follistatin-like-3 at eleven to thirteen weeks of gestation for developing GDM later in pregnancy. 11464 women at the King´s College Hospital London were screened. Biomarker levels were detected in eighty women with and 300 women without GDM. Median adiponectin and SHBG concentrations were lower in the case group. Moreover, the detection rate for GDM concerning maternal characteristics increased after adding both factors.

Lacroix et al. (2013) focused on adiponectin levels in the first and second trimester. First trimester blood samples were drawn at six to thirteen weeks of gestation. From a total of four-hundred-fifty women, thirty-eight were screened positive for GDM between twenty-four to twenty-eight weeks of gestation. Adiponectin values were statistically lower in women with GDM, than in the control group.

Between 2006 and 2008 Nicholson et al. (2013) recruited women in the first trimester for their study "Parity, Inflammation and Diabetes (PID)" to investigate the possible connection of adiponectin and TNF-alpha with maternal glucose tolerance. Biomarkers in question were measured between eight to fourteen weeks gestation. TNF-alpha was screened in two-hundred-ten and adiponectin in one-hundred-seventy-four women. GDM was diagnosed via 50g GCT at twenty-four to twenty-eight weeks of gestation. Unadjusted as well as adjusted for age, ethnicity and parity, analyses revealed a negative correlation between adiponectin and a pathological GCT result, but after including BMI, the association was no longer significant.

8.3.3 Irisin

The two studies in this subchapter focused solely on the adipokine/myokine: irisin and its possible potential as an early marker for GDM. The first one (P. Wang et al., 2018) is a prospective cohort study from Harbin and Beijing, China and the second one (Erol et al., 2016) is a prospective nested case-control study from Antalya, Turkey. P. Wang et al. (2018) included women with a singleton pregnancy, that were at least 18 years old into their study cohort. Women were excluded if they had pregestational diabetes, pregnancy induced hypertension, preeclampsia, history of childbirth, alcohol abuse, chronic diseases (liver, kidney, autoimmune diseases, neurological disorders, active/chronic inflammation), termination of pregnancy at the follow-up appointment or if blood samples were lost. Fasting plasma glucose and irisin were measured at the first visit and a gestational age of at least 8 weeks. GDM was screened between 24 to 28 gestational weeks with 75g two-hour OGTT.

The diagnosis was made according to the criteria of the Ministry of Health (MOH) of China. This criteria states, that gestational diabetes mellitus is diagnosed, if at least one of the following glucose levels is met or exceeded: fasting: 5.1 mmol/L, one-hour: 10.0 mmol/L and two-hour: 8.5 mmol/L. A total of 1150 pregnant women, including 135 women with GDM formed the study population. Women with GDM had significantly lower first trimester irisin levels compared with the normoglycemic women. A likelihood ratio test showed an independent association of irisin with GDM. Moreover, irisin was calculated to have a higher ability to predict GDM compared to CRP, FPG, insulin levels and the addition of irisin to a prediction model based on known risk factors helped to better discriminate between women with GDM and without GDM.

In the second study, Erol et al. (2016) investigated first and second trimester irisin levels and their possible connection to the later development of GDM. Exclusion criteria included pregestational diabetes, multiple pregnancies, chronic hypertension, preeclampsia, acute/chronic disease, which would affect the glucose regulation, fetal anomalies, history of smoking or chronic alcohol consumption. 258 women with a singleton pregnancy, available first and second trimester blood samples as well as a childbirth past 37 gestational weeks and child with a gestational age-appropriate birthweight were enrolled in the study. GDM was screened with an 75g two-hour OGTT between 24 to 28 gestational weeks. The GDM diagnosis was made according to IADPSG criteria, if at least one of the following glucose levels was met or exceeded: fasting: 92 mg/dl, one-hour: 180 mg/dl and two-hour: 153 mg/dl. From the enrolled study population, 20 women with GDM and 30 normoglycemic women were selected. Matching criteria for cases and controls included maternal and gestational age, as well as the BMI level at the first prenatal visit. First trimester irisin levels were significantly lower in the cases compared with the controls. Women with first trimester irisin levels under 540 ng/ml had a higher risk of developing GDM, compared to women with irisin levels above it.

8.4 Conclusion

In summary, the majority of studies reviewed in this chapter have investigated adiponectin and its association with gestational diabetes mellitus. All but two studies found a relationship between lower adiponectin levels in the first trimester and subsequent development of GDM. The exceptions were: 1) the study by (Florian et al., 2021), which concentrated on women not at risk for GDM, and 2) the “Parity, Inflammation and Diabetes” study by (Nicholson et al., 2013), which reported a loss of significance after adjusting for BMI. Based on these

findings, adiponectin appears to be a promising early biomarker for GDM. However, further research is necessary to confirm its clinical utility. Increases in leptin and chemerin concentrations have also shown potential as biomarkers for GDM. Visfatin yielded inconsistent results, while omentin-1, resistin and CMPF did not differ significantly between women with and without GDM. It is important to note, however, that resistin and CMPF were each investigated in only one study. Additionally, lower sOBr levels and higher concentrations of FABP4 and sFRP4 were associated with GDM. Another promising candidate is irisin, as both studies investigating first trimester levels found significantly lower irisin concentrations in women who later developed GDM. Overall, adipocytokine levels may serve as valuable components in a comprehensive GDM risk prediction model.

9. Vitamin D and Osteocalcin

9.1 Introduction

It is important to distinguish between the two main forms of vitamin D: ergocalciferol (D_2) and cholecalciferol (D_3). The primary sources of ergocalciferol are plants and mushrooms (Martin & Campbell, 2011), while cholecalciferol is mainly synthesised in the skin through exposure to the sunlight, although it is also present in certain foods, such as salmon (Holick, 2004). Vitamin D status has been linked to various diseases and conditions, including type 1 diabetes. Unfortunately, in modern lifestyles, dietary intake and sunlight exposure are often insufficient, resulting in widespread vitamin D deficiency. The most reliable method for assessing vitamin D status is the measurement of 25-hydroxyvitamin D. Optimal levels range between 25 and 80 ng/ml (Martin & Campbell, 2011). 25-hydroxyvitamin D is the preliminary stage to the more hormonally active form, 1,25-Dihydroxycholecalciferol. The conversion from cholecalciferol to 1,25-dihydroxy-cholecalciferol involves two hydroxylation: the first occurs in the liver, producing 25-hydroxy-cholecalciferol and the second takes place in the kidneys, forming 1,25-dihydroxy-cholecalciferol (Horn, 2020a). In the studies reviewed, 25-hydroxyvitamin D levels below 50 ng/ml are often classified as deficient, levels between 50 and 75 ng/ml as insufficient and levels above 75 ng/ml as sufficient.

Osteocalcin is a y-carboxyglutamic acid-containing protein, produced by osteoblasts during bone formation. Its carboxylation depends on vitamin K and the enzyme gamma-glutamyl carboxylase, allowing osteocalcin to be incorporated into the bone matrix. During bone reabsorption, osteoclasts reduce the pH of the local environment. This acidic condition leads to the decarboxylation of osteocalcin, which is subsequently released into the bloodstream. Measuring osteocalcin levels in the blood provides insights into bone metabolic activity, making it a valuable biomarker for osteoporosis and other bone-related metabolic diseases. Moreover, decarboxylated osteocalcin plays a role in energy metabolism, including the promotion of insulin secretion (Nowicki & Jakubowska-Pietkiewicz, 2024). C. Liu et al. (2015), through a systemic review and meta-analysis, demonstrated an association between serum osteocalcin levels and type 2 diabetes mellitus across multiple studies on the subject.

9.2 Collected Studies

Fourteen studies in total fell into this category. Eleven of them laid their focus on the 25-Hydroxyvitamin D, one each additionally on metabolic vitamin D gene variants and on

osteocalcin, as well as one only on osteocalcin. The studies come from different parts of Asia, Australia, Europe and North America. The comparison of the study results could not deliver a common answer to the question, if the first trimester Vitamin D status can predict the development of gestational diabetes. Six studies (Al-Ajlan et al., 2018; Ren et al., 2020; Vivanti et al., 2020; Xia et al., 2019; Xu et al., 2018; C. Zhang et al., 2008) in total concluded, that women with diagnosed GDM, had significant lower Vitamin D levels early in the pregnancy. However, one of them, a study from France and Belgium (Vivanti et al., 2020), found no significant difference after modifying the result for 25-OH-D insufficiency. Moreover, they found no linear correlation between GDM and Vitamin D status because the risk of GDM developing did not decrease as Vitamin D levels increased. The study population included 250 GDM cases and 941 matched healthy controls. Additionally to the six significant results, one study from China (Ni et al., 2021), with 22394 participants, detected a statistically higher incidence of gestational diabetes in women with Vitamin D insufficiency (50 nmol/L ≤ 25(OH)D < 75 nmol/L) compared to women with Vitamin D deficiency (25(OH) D < 50.00nmol/L).

On the other hand, six studies (Baker et al., 2012; V. T. Boyle et al., 2016; S. Park et al., 2014; Savvidou et al., 2011; Tabatabaei et al., 2014; B. Zhu, Huang, et al., 2019) did not detect significant lower first trimester Vitamin D levels in women with GDM. The significant difference often disappeared after an adjusting for the maternal age, pre-pregnancy BMI, ethnicity or the conception season.

In addition to the Vitamin D status, a Chinese research team (B. Zhu, Huang, et al., 2019) studied the effects of the metabolic Vitamin D gene variants on the development of gestational diabetes. They could find two VDR gene variants (rs1544410 and rs731236), which were associated with a higher risk for GDM. Interactions between the genetic variants and Vitamin D status were not detected.

A Canadian study (Tabatabaei et al., 2014) explored not only 25-OH-D, but also Osteocalcin. In this study, they compared the Vitamin D and Osteocalcin level of each forty-eight women with and without GDM. The comparison did show significant difference concerning the Osteocalcin levels but not the 25-Hydroxyvitamin D levels. Even though, Osteocalcin was significantly elevated in women with GDM, it was not predictive for GDM. Papastefanou et al. (2015) focused their attention solely on Osteocalcin as an early biomarker for gestational diabetes. This study had 134 participants. Osteocalcin was significantly higher

in GDM cases, as well as an independent risk factor for gestational diabetes. The combination of osteocalcin, maternal age and maternal weight showed to be an enhanced risk prediction model.

9.3 Conclusion

In conclusion, the findings of the reviewed studies on Vitamin D were divided, with approximately half suggesting an association with gestational diabetes mellitus. Therefore, based solely on these results, vitamin D cannot yet be clearly promoted as an early biomarker, for GDM. To obtain a more definitive understanding, larger studies are needed – particularly those that include diverse populations of women across a wide range of ethnicities, ages and body mass indices. Both osteocalcin and vitamin D gene variants have shown promising potential, however, only a limited number of studies addressed these factors. As such, further large-scale research is required to validate these findings. It is also important to note, that the GDM screening methods varied across the studies, with different diagnostic criteria and glucose cut-off values, which may have influenced the comparability of results.

10. PAPP-A and beta-hCG

10.1 Introduction

The main subjects of this chapter are (beta-) Human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A). In addition, placental-growth factor (PlGF), prokineticin-1 (PROK1), soluble human leukocyte antigen (sHLA-G), pregnancy-associated plasma protein-A2 (PAPP-A2) and inhibin-A are also discussed.

10.1.1 PLGF, PROK1, sHLA-G, inhibin-A, PAPP-A2

Placental-growth factor (PlGF), as described from Yanachkova et al. (2023), is a glycoprotein belonging to the vascular endothelial growth factor (VEGF) family. Due to its primary origin in the placenta, elevated levels are observed during pregnancy. In the first trimester of an uncomplicated pregnancy, PlGF concentrations are low, but they begin to increase around the 11^{th} to 12^{th} week of gestation, peaking around the 30^{th} week. PlGF is considered a potential predictor for pregnancy complications, such as preeclampsia (Yanachkova et al., 2023).

Prokineticin-1 (PROK1), also referred to as endocrine gland-derived vascular endothelial growth factor (EG-VEGF), is a peptide involved in angiogenesis and reproductive processess. PROK1 is the major prokineticin in female reproductive function (Ulu & Jafarzade, 2023).

According to Peilong Li et al. (2021), sHLA-G is one of seven isoforms encoded by the HLA-G-gene. Four of these are cell membrane-anchored isoforms, while three are soluble. Low sHLA-G levels, as noted by Beneventi et al. (2014), have been observed in pregnancies complicated with preeclampsia, intrauterine growth restriction and spontaneous abortion.

Hansen et al. (2016) describes PAPP-A2 as a metalloproteinase that shares approximately 45% of its amino acid sequence with PAPP-A. Like PAPP-A, it plays a role in the insulin-like growth factor (IGF) system. Elevated levels of PAPP-A2 have been associated with preeclampsia. Inhibin-A is a glycoprotein hormone that belongs to the transforming growth factor-beta (TGF-β) family. Elevated concentrations of inhibin-A have been reported in pregnancies affected by preeclampsia (Neuman et al., 2020).

10.1.2 Human chorionic gonadotropin

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of an alpha and a beta subunit. The alpha-subunit is common to several hormones, including luteinizing

hormone (LH), follicle-stimulating hormone (FSH) and thyreoid-stimulating hormone (TSH). During pregnancy, various forms of hCG, including its individual subunits, can be detected in serum and urine. The majority of hCG is produced by syncytiothrophoblastic cells of the placenta, though it is also synthesized in smaller amounts by the pituitary gland, liver and colon. HCG plays a crucial role in maintaining early pregnancy by stimulating the corpus luteum to secrete progesterone. Serum hCG concentrations rise steadily during early pregnancy, typically peaking around the 10th week of gestation. Between conception and approximately the 8th, levels double every 24 hours. After peaking, concentrations decline until around the 16th week, after which they remain relatively stable until delivery. A failure in the expected doubling pattern before the 8th week may indicate a nonviable pregnancy. Ectopic pregnancies often present with a slower, non-doubling rise in hCG levels. However, due to the wide physiological range of hCG concentrations, ultrasound measurements should be used alongside serum levels to improve diagnostic accuracy (Betz & Fane, 2023).

10.1.3 Pregnancy-associated Plasma Protein A

Pregnancy-associated plasma protein A (PAPP-A) is a metalloproteinase consisting of 1547 amino acids, with a zinc-binding element at its N-terminus. During pregnancy, PAPP-A is primarily synthesized by syncytiotrophoblasts. In not-pregnant individuals, low levels of PAPP-A are produced by the ovaries, endometrium, kidney, colon and bone marrow cells. PAPP-A concentrations increase progressively throughout a healthy pregnancy and are commonly used in first trimester screening to identify risk pregnancies (Bidlingmaier, 2018). PAPP-A binds insulin growth factor binding protein 4 (IGFBP4), playing a critical role in the IGF system, which is essential for fetal growth and development (Hansen et al., 2016). IGFBP4 is an inhibitor of IGF-1, a growth factor necessary for the intestinal growth effects of glucagon-like peptide 2 (Austin et al., 2018).

10.2 Collected Studies

10.2.1 (Beta-)Human chorionic gonadotropin

The two studies were conducted in Thailand and China. Sirikunalai et al. (2016) examined the association of beta-hCG and adverse pregnancy outcomes in Thailand. Collected beta-hCG levels were divided in three groups: equal or below 0.5 MoM was classified as low, between 0.5 and 2.0 MoM was classified as normal and at least 2.0 MoM was classified as high. Between January 2007 and July 2013 8150 pregnant women could provide first trimester blood samples. 785 fell in group one, 6035 in group two and 1357 in group three. GDM as one adverse pregnancy outcome was diagnosed via three-hour OGTT. In the high

beta-hCG group a smaller number of women developed gestational diabetes compared to the other two groups.

Y. Liu et al. (2021) also showed that higher first trimester hCG levels were associated with a lower risk of developing GDM. Moreover, they detected a negative association of fT4 with GDM and a positive association of fT4 with hCG. The study population consisted of 18683 pregnant women, of whom 2214 women developed GDM. GDM screening was conducted with a 75g OGTT between twenty-four and twenty-eight weeks of gestation. Due the findings of both studies (beta-) hCG seems to have a more protective than predictive role concerning GDM.

10.2.2 Pregnancy-associated Plasma Protein A

Eight studies and one systemic review and meta-analysis of literature investigated the predictive quality of PAPP-A for gestational diabetes. The majority of them were conducted in Europe and each one in Australia and China.

Ren et al. (2020) compared the early PAPP-A concentrations of fifty-one women with and forty-nine women without GDM with each other. All women were patients in the Peking University International Hospital. GDM was diagnosed via fasting plasma glucose and 75g OGTT at twenty-four to twenty-eight weeks of gestation. PAPP-A, 25-hydroxy-vitamine-D and triglyceride values were measured between seven and eleven weeks of pregnancy. PAPP-A concentrations were significantly lower in women, who later developed GDM.

Wells et al. (2015) had a study population consisting of 1664 pregnant women from the Royal Hospital for Women, Randwick, Australia. PAPP-A levels were measured between eleven and fourteen weeks of gestation. GDM screening was conducted through two different approaches. Women at high risk for GDM had a 75 g OGTT at 15.5 +- 1.5 weeks of gestation and the remaining women had a one hour 50 Glucose-Challenge-test (GCT) between 26 and 28 of gestation. Diagnosis was made according to the Australasian Diabetes in Pregnancy 2013. Depending on the time of GDM diagnosis two groups were formed: early GDM (< 22 gestational weeks) and late GDM (≥ 22 gestational weeks). MoM PAPP-A was lower in the early GDM group compared to late GDM group. After adjusting for parity, women of both groups with MoM PAPP-A levels in the lowest quartile were at a higher risk of developing gestational diabetes.

Three of the Europe based studies were conducted in Italy. Lovati et al. (2013) collected the blood samples between 11 and 14 fourteen weeks of pregnancy as part of the Combined

screening for Down syndrome in the hospital of Pavia. GDM screening took place around 24 to 28 gestational weeks. In the period before March 2010 a three-hour 100g OGTT was used for GDM diagnosis, after that timeframe a two-hour 75g OGTT was used. 307 pregnant women were diagnosed, and 366 non-diabetic pregnant women were included as control group. PAPP-A serum concentration as well as PAPP-A MoM corrected were significantly decreased in the case group. PAPP-A MoM values also showed a descending gradient from the control group over women, whose GDM was diet treated to women with Insulin treated GDM.

The second Italian study (Beneventi et al., 2014) was also conducted in Pavia, and did not only investigate PAPP-A, but also sHLA-G. Beneventi et al. (2014) combined in their study a retrospective and a prospective case-control-study. For the retrospective study 112 women, who developed GDM were compared with the same amount on euglycemic pregnant women. Clinical data was obtained from the clinical database. For the prospective study, blood samples from 105 healthy pregnancies were compared to 18 pregnancies affected by GDM. PAPP-A and sHLA-G levels were measured between eleven to fourteen weeks of gestation and GDM was diagnosed via two-hour 75g OGTT between twenty-four to twenty-eight weeks. Results from the retrospective study showed significantly lower PAPP-A values in the case group. sHLA-G values were also decreased, but not significant. On the contrary in the prospective study both biomarkers were significantly decreased in the GDM affected pregnancies.

Quattrocchi et al. (2015) investigated the potential connection of early low PAPP-A concentrations and adverse pregnancy outcomes in Messina. The study population got divided based on their MoM PAPP-A. 164 women with MoM PAPP-A levels below 0.3 formed the case group and 1640 of those with levels equal or above 0.3 formed the control group. Blood samples were drawn at 8 to 11 weeks of gestation. Gestational diabetes as one adverse outcome, was not significantly different between the two groups.

Syngelaki et al. (2015) examined the association of early PAPP-A and placental growth factor (PlGF) levels with the development of GDM. The study population included 30438 women without and 787 women with GDM. Gestational diabetes screening was a two-step approach. In the first step, plasma glucose was measured between 24 to 28 weeks of gestation. If it was at least 6.7 mmol/l, a 75g OGTT within two weeks was conducted. Because of that approach, a OGTT was not carried out in all participants. Although, MoM

PAPP-A was significantly decreased and MoM PlGF significantly increased in the pregnancies affected by GDM, the addition of these biomarkers did not improve their prediction tool based on maternal factors.

A Turkish study group (Inan et al., 2018) studied first trimester concentrations of PAPP-A, Prokineticin-1 (PROK1), PROK1/PAPP-A-ratio in connection with adverse pregnancy outcomes. 162 pregnant women were divided into five groups according to their pregnancy outcome (Preeclampsia, Foetal Growth Restriction, GDM, Spontaneous preterm birth and unaffected pregnancies). To determine GDM, between 24 and 28 weeks of gestation a one-hour 50g GCT and three-hour 100g OGTT were performed. 18 women had at least two pathological levels and were therefore diagnosed with it. 102 women had unaffected pregnancies and formed the control group. PAPP-A levels were not significantly different between GDM affected and unaffected pregnancies. However, PROK1 and PROK1/PAPP-A-ratio were significantly decreased in women with gestational diabetes.

Two structural points were different between the Swedish study by Dereke et al. (2020), and the other studies in this subchapter: 1)The case group consisted of 99 women high at risk for GDM and therefore screened for GDM at 13.6 +- 2.8 weeks of gestation. Defined risk factors were older age, overweight and obesity, previous macrocosmic baby and Asian or Middle Eastern origin. 2) Investigated biomarker was pregnancy-associated plasma protein-A2 (PAPP-A2) and not PAPP-A. The control group consisted of 100, matched by age and BMI, healthy pregnant women. PAPP-A2 levels were significantly increased in women with early GDM compared to the controls.

Talasaz et al. (2018) conducted a systemic review and meta-analysis of studies concerning the association of first trimester PAPP-A levels and gestational diabetes. Therefore, they searched PubMED, Medline, Scopus and Google Scholar in the period from 1974 to 2017 for literature. Seventeen studies were included in the systemic review and five in the meta-analysis. Their investigations resulted in the conclusion that low first trimester PAPP-A concentrations have low predictive accuracy for gestational diabetes. However, they also concluded that PAPP-A in combination with other biomarker, could be useful for the earlier detection of GDM and therefore should be further examined.

10.2.3 (beta-)hCG and PAPP-A

This sub-chapter contains nine studies and one systemic review investigating the possible connection of both main biomarkers (beta-hCG and PAPP-A) from this chapter and the

development of GDM. All studies, but one were conducted in Europe. The exception was Di Xiao et al. (2018), who was conducted in China and compared first trimester beta-hCG and PAPP-A levels of 599 women with and 986 women without gestational diabetes. GDM was diagnosed with a 75g OGTT around 24 to 28 weeks of gestation. MoM beta-hCG concentrations were decreased, but the difference was not significant. MoM PAPP-A levels were significantly lower in the case group. Moreover, the low PAPP-A values seem to be an independent risk factor for GDM. However, its addition to an early risk prediction tool did not improve its predictive quality. The risk prediction tool is based on maternal factors.

A Turkish research group (Caliskan et al., 2020) found similar results regarding beta-hCG and PAPP-A. Firstly, beta-hCG levels were not significantly different between pregnancies affected by GDM and unaffected pregnancies. Secondly, PAPP-A levels were significantly lower in women with GDM. GDM screening was a two-step approach. First step was a 50g OGTT between twenty-four and twenty-eight weeks. If the plasma glucose levels one hour after the taking of the glucose solution were at least 140 mg/dl, the second step a 100g OGTT was carried out. Due to this approach, the study population was divided into four groups. 158 women had in the first step plasma glucose levels below the limit and therefore the control group. 42 women were above the limit of the first step, but their second step concluded no abnormal glucose levels. 59 women were diagnosed with GDM, because they had at least two abnormal plasma glucose levels in the second test and/or a plasma glucose level of at least 180mg/dl in the first test. The last group contains nineteen women with a positive first test and one abnormal level in the second test and because of that they were defined as the glucose intolerance group (GIT). In further comparison of the groups, PAPP-A measurements were similar in the GIT and the control group as well as in the second group and the control group.

The second Turkish study from this sub-chapter was conducted in Istanbul. Kavak et al. (2006) included between July 2001 to July 2004 490 women in their study population, 14 were later excluded. They evaluated the potential predictive quality of early beta-hCG and PAPP-A levels for GDM, SGA babies and hypertensive disorders in pregnancy. GDM was tested via three-hour 100g OGTT and as a result 18 women were diagnosed. Neither beta-hCG nor PAPP-A were significantly different in the gestational diabetes group compared to the remaining women.

Tul et al. (2003) were interested in the predictive quality of free beta-hCG, PAPP-A and inhibin-A for gestational diabetes. They compared blood samples of 1136 women. Blood samples were gained between ten to fourteen weeks of gestation. The women were divided according to their pregnancy outcomes (SGA, preterm delivery, GDM, hypertensive disorder and controls) in five groups. After the GDM screening via a three-hour 100g OGTT twenty-seven women were diagnosed. In this group all tested biomarkers levels were low, but the difference was not significant.

Husslein et al. (2012) studied the association of first trimester PAPP-A as well as beta-hCG and GDM treated with insulin. Pregnancies affected by gestational diabetes, which only needed diet treatment were excluded from the study. The study was conducted in Klagenfurt, Austria. 72 women with insulin treated GDM were included, as well as 216 BMI and gestational age matched controls. Blood samples were drawn around 11 to 14 weeks of gestation and GDM screening with a 75g OGTT took place between 24 to 28 weeks of pregnancy. The tested biomarker levels did not differ between the two groups.

An Italian study group (Visconti et al., 2019) examined the association between, PAPP-A, beta-hCG, nuchal translucency, a first trimester combined test (FTCT) and GDM. From the 2410 included pregnant women, 596 were diagnosed with GDM. GDM screening with a 75g OGTT were conducted around 16 to 18 weeks of gestation and/or 24 to 28 weeks of gestation according to Italian guidelines. In total 44 were diagnosed with GDM in the early timeframe. PAPP-A and beta-hCG levels did not differ between affected and unaffected pregnancies. However, the first trimester combined test values were significantly different. Moreover, women with MoM PAPP-A concentrations <1 were at risk for GDM, whereas MoM beta-hCG concentrations >2 seem to have a protective effect.

Savvidou et al. (2012) investigated possible differences concerning the first trimester nuchal translucency, crown-rump-length, beta-hCG and PAPP-A in pregnant women with type 1 DM, type 2 DM, GDM and uncomplicated pregnancies. All study participants were recruited between their first trimester visit (11 to 13 weeks of gestation) in the King´s College Hospital, London, UK. The study population consisted of 194 women with type 1 DM, 122 with type 2 DM, 779 with GDM as well as 41007 normoglycemic women as controls. GDM screening was a two-step-approach: Between 24 to 28 weeks of gestation plasma glucose was measured, if the level was at least 6.7 mmol/l a two-hour 75g OGTT within the next two

weeks was carried out. Beta-hCG levels did not differ significantly between the groups and PAPP-A levels were only significantly different in women with type 2 DM.

In contrary to the first study from the United Kingdom, Spencer and Cowans (2013) found significantly different concentrations of beta-hCG and PAPP-A in women affected by GDM. First trimester aneuploidy screening consisting of beta-hCG, PAPP-A and sonographic measurements (nuchal translucency, crown-rump-length) were taken between eleven to thirteen 11 to 13 weeks of gestation. GDM was screened in women at risk for GDM between 26 to 28 weeks of gestation with a 75 g OGTT. Defined risk factors were BMI > 30 kg/m^2, a previous macrocosmic baby weighting ≥ 4.5 kg, GDM in a previous pregnancy, family history of DM in a first degree relative and a family origin with a high prevalence of diabetes (South Asia, Black Caribbean, Middle Eastern). 7429 women were included in the study, including 870 with GDM. 20056 women with a first trimester screening, but without a GDM screening were considered as second comparison group. Sonographic measurements showed no significant difference between the three groups. MoM beta-hCG and PAPP-A levels were in the GDM group significantly lower and were therefore predictors for GDM. However, sensitivity was quite low.

Ong et al. (2000) investigated first trimester PAPP-A and free beta-hCG as potential predictors for pregnancy complications. Included pregnancy complications were miscarriage, preterm delivery, GDM, pregnancy-induced hypertension and intrauterine growth restriction. GDM diagnosis was made by a 75g OGTT if the fasting glucose level was 8.0 mmol/L or the two-hour glucose level met or exceeded 11.0 mmol/L. Due to the fact that the study population included women from two different hospitals (the Harold Wood Hospital in Essex and the King´s College Hospital in London) the reasons for taking an OGTT were different. In the Harold Wood Hospital, a OGTT was taken at about 24 gestational weeks, if the woman had a family history of diabetes, a previous delivery of at least 4.5 kg heavy neonate, previous unexplained fetal loss, weighted more than 100 kg, history of gestational/latent diabetes, glycosuria or had LGA fetus in the current pregnancy. In the King´s College Hospital a OGTT was taken at about 30 gestational weeks, if a random plasma glucose level at 28 gestational weeks was over 6.7 mmol/L. From the 5297 pregnant women included in the study, 4297 had unaffected pregnancies and formed the control group. PAPP-A levels increased, while free beta-hCG levels decreased throughout the pregnancy. Significantly lower PAPP-A serum levels were measured in pregnancies affected by miscarriage, proteinuric and non-proteinuric pregnancy induced hypertension, growth

restriction and preexisting/gestational diabetes mellitus. Significantly lower free beta-hCG concentrations were measured in pregnancies complected by proteinuric pregnancy induced hypertension and GDM.

Donovan et al. (2018) examined in their systemic review and meta-analysis 13 studies concerning a potential association of first trimester biomarkers: beta-hCG and PAPP-A and GDM. Beta-hCG was a subject in nine and PAPP-A in all of them. Two studies were conducted in Asia, two in Australia and nine in Europe. Median levels of both biomarkers were lower in the GDM groups. In contrast to beta-hCG studies, which showed no study heterogeneity, the PAPP-A studies exhibited a high heterogeneity. This high study heterogeneity was addressed by stratifying the data according to geographic location, biomarker assay method and timing of GDM diagnosis. When comparing studies by geographic location, the two studies conducted in Asia did not detect as low PAPP-A levels in women with GDM, in contrast to the studies from Europe and Australia. These findings suggest that the association between low PAPP-A levels and GDM may be less pronounced in Asian populations.

10.3 Conclusion

In conclusion, the collected findings on the predictive value of early PAPP-A measurements for GDM are mixed. While eleven studies reported significantly lower first trimester PAPP-A levels in pregnant women who later develop GDM, another seven did not observe a statistically significant difference. Moreover, two of the studies that did find reduced PAPP-A levels, concluded that the inclusion of PAPP-A in a GDM risk prediction tool did not improve its predictive performance. The majority of the studies examining beta-hCG found no significant differences between pregnancies affected by GDM and those without complications. However, three studies indicated that elevated beta-hCG in the first trimester may have a protective effect against the development of GDM. These findings suggest that further research is necessary to better understand the potential predictive value of PAPP-A and the possible protective role of beta-hCG in relation to GDM.

In addition to the main biomarkers, five others were considered in this chapter. PROK1 levels were significantly lower, while PlGF and PAPP-A2 levels were significantly higher in women with GDM. Results for sHLA-G were inconsistend, with one study reporting significantly lower levels GDM pregnancies, and another finding no difference. Inhibin-A levels did not differ between women with and without GDM. It is important to note that each

of these five biomarkers was evaluated in only one or two studies, limiting the generalizability of the findings.

11. Iron markers

11.1 Introduction

Iron

Iron is an essential micronutrient involved in a wide range of biological processes in all living organisms. Every cell and organ in the human body requires iron for development and metabolic functioning. Consequently, both iron deficiency and iron overload can have significant consequences for the human health. These effects are particularly pronounced in cells with high metabolic rates, likely due to the impairment of mitochondrial and cellular energetics by iron deficiency. During periods of rapid development, cells require increased oxygen, making them more vulnerable to iron deficiency. For example, neonates have a total-body oxygen consumption rate that is three to four times higher per kilogram of body weight compared to adults. Pregnancy is another time of increased iron demand. To maintain adequate circulation and oxygen supply to maternal organs and the placenta, the maternal body increases the blood volume. Additionally, the fetoplacental unit requires iron for proper development and growth. Given these physiological demands, it is not surprising that the highest prevalence of iron-deficiency is found in pregnant women and young children (Abbaspour et al., 2014; Georgieff et al., 2019). The consequences of iron deficiency during pregnancy include maternal anaemia, shortened gestational period, low birth weight, and adverse effects on the child´s cognitive development (World Health Organization, 2020). To mitigate these outcomes, the World Health Organisation (WHO) recommends daily supplements during pregnancy with 30-60 mg of elemental iron and 0.4 mg of folic acid (World Health Organization, 2016).

Ferritin

Ferritin is defined as an iron-binding, shell-shaped protein (Knovich et al., 2009). In its iron-free form, it is referred to as apoferritin. Apoferritin consists of 24 subunits of either heavy (H) or light (L) type, with the ratio of these subunits varying by tissue. For example, H-rich ferritin is predominant in the heart, while L-rich ferritin is primarily found in the liver. While iron is essential, it can also be toxic due to its potential to generate free radicals that damage DNA and proteins. Ferritin helps buffer this toxicity by capturing and storing iron. It exists both intra- and extracellularly. Because of its presence in blood plasma, ferritin is a widely used marker for assessing iron status.

According to the WHO (World Health Organization, 2020), in healthy individuals over the age of five, serum ferritin concentrations below 15 µg/L indicate iron deficiency. In the presence of inflammation or infection the threshold is increased to 70 µg/L, as ferritin levels rise in response to inflammatory processes. During pregnancy the recommended cut-off for serum ferritin remains below 15 µg/L, regardless of inflammatory status.

Soluble transferrin receptor (sTfR)

As previously mentioned, free iron in the blood stream is toxic and must be transported in a redox-inactivated form. Transferrin, a glycoprotein consisting of 679 amino acids, binds iron and delivers it to various tissues (Gomme et al., 2005). On the surface of cells, iron loaded transferrin (Tf) binds to transferrin receptor-1 (TfR1), a type II transmembrane glycoprotein expressed on nearly all cells. This complex is then internalized into the cells. Once inside the cell, iron is released and either utilized or stored via ferritin. The transferrin-transferrin-receptor complex then returns to the cell surface, where transferrin is released. The transferrin-receptor is subsequently cleaved, producing a shortenend monomeric form known as soluble transferrin receptor (sTfR) (Harms & Kaiser, 2015).

Cells regulate iron uptake in two primary ways: by storing excess iron in ferritin and by modulating the expression of transferrin receptors. When iron supply is reduced the expression of transferrin receptors increases, leading to elevated sTfR levels. Therefore, high sTfR concentrations are observed in individuals with iron deficiency and autoimmune haemolytic anaemia. In contrast, individuals with aplastic anaemia typically present with a decreased sTfR levels. For this reason, sTfR is also used as a biomarker to assess iron deficiency (Harms & Kaiser, 2015).

11.2 Collected studies

In this chapter the results of seven studies from four different continents were evaluated. Three studies were conducted in Asia (China, Iran, Lebanon), two in Australia and one each in Europe (Denmark) and the United States of America. The first study is from China (B. Zhu, Liang, et al., 2019) and the study population is part of the Ma'anshan Birth Cohort (MABC) Study. In this study 3289 pregnant women with a OGTT at 24 to 28 weeks were included. The aim was to invest iron-related factors (serum iron concentration, haemoglobin, the use of iron supplements) in early pregnancy and their possible association with GDM. Blood samples were taken at fourteen and 24 to 28 gestational weeks. In the final analysis of the first trimester 3006 pregnant women were included. The serum iron

concentrations did not differ significantly between the GDM and non-GDM group. However, haemoglobin levels were significantly higher in the GDM group. There were no significant associations between iron supplements and the development of GDM, but there was significant association between the use of pre-pregnancy iron supplements and gestational diabetes mellitus.

In the study performed in Yazd, Iran, Soheilykhah et al. (2017) included 1358 pregnant women with an 75g OGTT between 24 to 28 gestational weeks. Haemoglobin, ferritin, iron concentrations and total iron-binding capacity (TIBC) were measured between 12 to 16 gestational weeks. Pre-natal iron supplements kept going and after four months according to the national policy 50 mg of elemental iron were taken as iron supplements. 281 pregnant women developed GDM. In the GDM group the women were significantly older, had higher pre-pregnancy BMIs and serum ferritin concentrations. Haemoglobin levels, iron levels and total iron-binding capacity did not differ significantly.

Zein et al. (2015) evaluated 104 pregnant women with an 75g OGTT at 24 to 28 gestational weeks in Beirut, Lebanon. The women were divided into four groups according to their quartile ferritin levels in the first trimester. The GDM risk did not differ between the groups. However, the women from the fourth group with ferritin values above 38,5 µl/l had significant higher 2-hour OGTT levels.

Both Australian studies (Khambalia, Aimone, et al., 2016; Khambalia, Collins, et al., 2016) have the following overlaps: the study population consist of pregnant women, who attended the first trimester Down syndrome screening between January and October 2007 in New South Wales, Australia, same blood samples and most of the authors. The first study (Khambalia, Collins, et al., 2016) was published in September 2015 and its aim was to evaluate the association of first trimester iron deficiency with pregnancy and birth outcomes. One of these pregnancy outcomes was GDM. A total of 4420 pregnant women with an average gestational age of twelve weeks at entry point were included. The collected blood samples were analysed for ferritin (SF), soluble transferrin receptor (sTfR) and CRP. Total body iron was calculated from SF and sTfR levels. Iron deficiency (ID) was determinant using its definitions: SF < 12 µg/l, sTfR ≥ 21.0 nmol/l and TBI < 0 mg/kg. The prevalence of ID based on the previous definitions was 19.6, 15.3 and 15.7 percent respectively. ID defined by SF and TBI showed a significantly association with a decreased risk for GDM. However, ID defined by sTfR did not show such an association.

In the second Australian study (Khambalia, Aimone, et al., 2016) did evaluate the risk of GDM in association with high maternal iron status, dietary iron intake and iron supplements in an in-house study and a systemic review. The study population of the in-house study did consist of the same women of the previous Australian study. They included 129 GDM cases and 3657 controls in the in-house study. Blood samples were analysed for ferritin, sTfR and CRP. Women with GDM had significantly higher concentrations of CRP, serum ferritin and were less likely to have an iron deficiency. In the meta-analysis the included nineteen publications from PubMed, MEDLINE, EMBASE and CHINAHL between January 1995 to July 2015. The publications consist of two randomly controlled trials, three cohort studies and fourteen case-control studies (including the in-house study). The two trials looked at possible association between first trimester iron supplements and risk of GDM and did not find significant association. The three cohort studies looked at dietary iron intake and risk for GDM and two found a significant association for dietary haem iron intake but not for non-haem iron. From the fourteen case-control studies only three had their blood samples taken in the first trimester. Two of that three evaluated significant association between higher serum ferritin and GDM.

The American study (Rawal et al., 2017) is a case-control study within the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Fetal Growth Study. The study population consist of pregnant women from twelve US hospital centres. 107 women with GDM according to their medical files were examined and each case was matched with two controls. The matches were based on age, race/ethnicity and gestational week of blood collection. GDM was diagnosed with an OGTT according to the Carpenter and Coustan criteria and was carried out on average in the 27^{th} gestational week. Blood samples were taken at four points during the pregnancy. In the first trimester the point of measurement was between ten to fourteen weeks of gestation. The measured iron status biomarkers in question were plasma hepcidin, plasma ferritin and soluble transferrin receptor (sTfR). Early plasma ferritin concentrations were significantly higher in GDM cases. The other biomarker showed no significant differences in the first trimester between cases and controls.

The Danish study (Bowers et al., 2016) compared iron biomarkers of 350 pregnant women with GDM and 349 without GDM. The study population is part of the Danish National Birth Cohort between 1996 and 2002. GDM diagnosis was found in the medical records or evaluated over a telephone interview with documented levels according to the WHO criteria

(fasting glucose ≥ 7.0 mmol/l or 75g OGTT 2-hour glucose level ≥ 7.8 mmol/l). Blood samples to determinate serum ferritin and soluble transferrin receptors were taken at six to twelve gestational weeks. Both iron markers were significantly higher in the GDM group.

11.3 Conclusion

In conclusion, five out of seven studies investigated the association between first trimester serum ferritin concentrations and the risk of developing GDM. Four of these five studies reported significantly higher serum ferritin levels in women who developed GDM compared to those who did not. Only the Lebanese study failed to find a significant difference. Based on these findings, serum ferritin appears to be a promising candidate for inclusion in a panel of early biomarkers for GDM. However, further large-scale studies are necessary to confirm its predictive value.

In contrast, soluble transferrin receptor levels as well as total iron concentrations did not differ significantly in most of the reviewed studies. Only one study (Bowers et al., 2016) reported elevated levels of soluble transferrin receptors in women with GDM.

12. Thyroid Function

12.1 Introduction

The aim of this chapter is to identify potential associations between early thyroid hormone levels, thyroid antibodies and the development of gestational diabetes mellitus, by comparing the findings from various studies. The regulation of thyroid hormones is embedded within the self-regulatory circuit known as hypothalamic-pituitary-thyroid (HPT) axis. The process begins with the hypothalamus releasing thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary gland to secrete thyroid-stimulating hormones (TSH) into the bloodstream. TSH then binds to the thyroid-releasing hormone receptor (TSH-R) on thyroid follicular cells, triggering the thyroid gland to produces its two main hormones: tetraiodothyronine (T4) and triiodothyronine (T3). TRH, TSH and T4 are linked through feedback mechanisms that maintain hormone balance. Iodine, an essential trace element, is a key component of thyroid hormones (Horn, 2020g). The gold standard for assessing the iodine status is measuring renal iodine excretion (RIE) (Sert et al., 2020).

Thyroid peroxidase (TPO) and thyroglobulin (TG) antibodies are thyroid-autoantibodies, primarily associated with Hashimoto´s thyroiditis. TPO can induce an antibody-dependent cell-mediated cytotoxicity (Prummel & Wiersinga, 2005). However, thyroid-autoantibodies are also present in smaller amounts in some healthy pregnant women (Sert et al., 2020).

There are well-established connections between thyroid hormones and glucose homeostasis. Studies have shown that thyroid hormones, particularly T3, influence pancreatic beta-cell development and function. During the neonatal period, T3 promotes beta-cell proliferation and enhances insulin secretion in response to glucose. In diabetic animal models, T3 has a protective effect on the beta-cells, reducing cell death and improving glucose tolerance. Thyroid hormones also play a role in glucose metabolism through their actions on the gastrointestinal tract, liver, pancreas, adipose tissue, skeletal muscles, and the central nervous system. In the gastrointestinal tract, thyroid hormones enhance motility and increase glucose absorption. In the liver, they stimulate gluconeogenesis by upregulating glucose transporter GLUT2 and enzymes such as phosphoenlpyruvate carboxykinase (PEPCK), contributing to hyperinsulinemia and insulin resistance (Eom et al., 2022). In adipose tissue, thyroid hormones promote lipolysis, increasing free fatty acids, which in turn enhance glucose production. In addition, thyroid hormones increase glucose uptake and also stimulate glucagon release from pancreatic alpha-cells (Eom et al., 2022).

Centrally, thyroid hormones can modulate hepatic glucose production via the sympathetic nervous system. In the paraventricular nucleus (PVN) of the hypothalamus, triiodothyronine enhances hepatic glucose output independently of other glucoregulatory hormones. Thus, thyroid hormones influence glucose metabolism through both direct peripheral effects and central regulation, which may explain the close association between thyroid disorders and diabetes mellitus (Eom et al., 2022).

12.1.1 Changes of the thyroid function during pregnancy

During pregnancy, various hormonal and metabolic changes occur that affect both glucose metabolism and thyroid function. Insulin sensitivity decreases by approximately 50-60%, even in pregnancies not affected by GDM. In healthy pregnancies, this reduction is typically compensated by increased insulin secretion from pancreatic beta-cells. Depending on a country´s iodine status, thyroid gland size increases by about 10% in iodine sufficient populations and by 20-40% in iodine deficient populations (Eom et al., 2022). To support a nearly 50% increase in thyroid hormone production during pregnancy, the WHO recommends increasing daily iodine intake by 50%, advising a total intake of 250µg during pregnancy and lactation (WHO/UNICEF, 2007). TSH, hCG, FSH and LH all belong to the family of glycoprotein hormones. Each of these hormones consists of two subunits, with similar alpha-subunits and unique beta-subunits. Due to structural similarity, the elevated levels of hCG in the first trimester stimulate the thyroid gland to produce more thyroid hormones, leading to physiological suppression of TSH secretion (Eom et al., 2022).

12.2 Collected studies

In this chapter, the results of eight studies and one systemic review are compared and presented. Four studies originated in China, one in Turkey, one in the United Arabic Emirates, two in United States of America and the systemic review contains eleven Asian, seven European and two American studies.

The first Chinese study (Kuan Huang et al., 2019) is a cohort study situated in the Anhui province. A total of 1683 pregnant women in the timeframe between November 2008 and October 2010 were included in the study. GDM was diagnosed via a 100g OGTT at 24 to 28 gestational weeks. 26 women had a least two pathological levels at the OGTT and were diagnosed with gestational diabetes mellitus. TSH, fT4 and TPOAb concentrations were evaluated in the first trimester. The evaluation of the results showed that the isolated positive

Thyroid peroxidase antibodies were associated with an increased risk of GDM. TSH and fT4 did not differ significantly between GDM and non-GDM cases.

The second one (Yong Zhang et al., 2017) compared the results of 6031 pregnant Han Chinese women. All women had a screening for thyroid function in the first (between nine to twelve gestational weeks) and third trimester. The aim of the study was to evaluate associations between thyroid function and adverse pregnancy outcomes, such as gestational diabetes mellitus. 533 pregnant women of the study were diagnosed with GDM by a 75g OGTT. It showed that the first trimester free thyroxine levels were significantly lower in the GDM cases in comparison to the healthy pregnancies. Additionally, the divided the study population into four groups according to their first and third trimester levels. Without other adjustments the low/normal group in comparison with the normal/normal group had a significantly higher risk of developing GDM. After adjusting for age, BMI and TSH levels, there were no significant differences between the groups.

In the third Chinese study (Y. Liu et al., 2021), 18683 pregnant women from a tertiary hospital in Shanghai were included. The aim was to evaluate possible associations between hCG, thyroid hormones and gestational diabetes mellitus. Blood samples for the determination of hCG, TSH, fT4, TPOAb, FPG and HbA1c were taken between the ninth and thirteenth gestational weeks. GDM was diagnosed with a 75g OGTT during the 24 to 28 gestational weeks. The results showed that pregnant women with higher hCG levels were significantly associated with lower glucose levels during the OGTT, which results depending on the cut-off levels on a 6.4% to 11.8% lower risk of developing GDM. There were no associations between TSH and GDM, although hCG was negatively associated with TSH. However, there was a significant mediation effect of hCG on the also significant association of GDM with lower maternal fT4 levels in the first trimester.

Tang et al. (2021) compared 768 pregnant women with normal glucose tolerance and 699 pregnant women with GDM. Thyroid function tests were performed at three points in the pregnancy. All included women had a natural conception, a singleton pregnancy and gestational age and week of pregnancy determined by the last menstrual period and ultrasound. The findings resulted in a significantly higher positive antibody rate in the GDM cases, as well as significantly higher fT3, TPOAb and TgAb levels in the first trimester.

The aim of the Turkish study (Sert et al., 2020) was to find a possible relationship between thyroid function abnormalities, thyroid antibodies in the first trimester and the development

of GDM. Therefore, they determined TSH, fT3, fT4, anti-TPO, anti-TG and renal iodine excretion from 302 pregnant women. All women without risk factors for GDM and a first trimester FPG < 92 mg/dl were evaluated with a 50g GCT between 24 to 28 gestational weeks regardless of their fasting situation. A positive GCT (blood glucose ≥ 140 mg/dl) resulted in the performance of a 100g OGTT. GDM diagnosis then was achieved, when two blood glucose levels of the OGTT were above their cut-off levels. 62 women were diagnosed with GDM. Women with and without GDM did not significantly differ considering age, pre-pregnancy BMI, gravidy and parity. However, TSH and RIE were significantly higher in the GDM cases and there were significantly more positive cases of thyroid-autoantibodies (anti-TPO, anti-TG) found in the GDM group.

The study based in the United Arabic Emirates (Agarwal et al., 2006) included 80 women with GDM and 221 women without GDM in their study. TSH, fT4 and fT3 were measured between 5-18 weeks of gestation in all women, anti-TPO was only available from 255 pregnant women. The blood samples from the first trimester screening were also used to determined FPG and a 2-hour postprandial glucose test (PPG). Women with a FPG ≥ 5.3 mmol/l and/or a PPG ≥ 7.8 mmol/l underwent a 75g OGTT within two weeks. GDM was diagnosed according to the WHO criteria. Those women, who were negative the first time, underwent a second OGTT between 24 to 28 weeks of gestation. GDM and non-GDM cases did differ significantly at age, pre-pregnancy BMI, FPG, PPG, but not concerning the thyroid markers.

The study populations of the two American studies (Haddow et al., 2016; Rawal et al., 2018) were formed from a larger study whose study participants came from several American hospitals. Haddow et al. (2016) looked at associations between thyroid hormones, thyroid antibodies, maternal weight and the Development of GDM in pregnant women from the FaSTER trial. The FaSTER trial included in total 9351 singleton, euthyroid women. 272 women were diagnosed with GDM by at least two abnormal values at a 3-hour GCT. TSH, fT4 and thyroid antibodies were measured at 11 to 14 and 15 to 18.9 weeks of gestation. The results showed pregnant women with GDM are older, weight more, deliver early and a greater percentage smokes ciagrettes. In the first time period no other significant differences between GDM and non-GDM cases were found. However, between 15 to 18.9 gestational weeks women with GDM had, even after adjusting for age and maternal weight, significantly lower levels of fT4.

In the second American study (Rawal et al., 2018) the study population consisted of 107 women with GDM and 214 women without GDM within the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies. Cases and controls were matched based on well-established factors of GDM (age, race/ethnicity)/determinants of blood biomarker levels during pregnancy. GDM was diagnosed with a OGTT at an average gestational week 27 with the Carpenter and Coustan criteria. Women without OGTT results were classified as GDM cases, if they had an indication of medication-treated GDM on the hospital discharge diagnosis. TSH, fT3, fT4 and fT3/fT4-ratio were measured at four visits during the pregnancy. The visit in the first trimester was between ten to fourteen weeks of gestation. The median fT4 were significantly lower and median fT3 and fT3/fT4-ratio were significantly higher among GDM cases at first and second trimester visits. TSH did not differ significantly.

The meta-analysis by Yang et al. (2015) compared the results of each 10 cohort and case-controlled studies. All studies included pregnant women, and their point of interest were positive thyroid antibodies and the development of GDM. The authors of the meta-analysis searched in Pubmed, Wanfang and China National Knowledge Internet (CNKI). They included eleven Asian, seven European and two American studies in their meta-analysis. Nine studies only evaluated anti-TPO, ten evaluated both thyroid-autoantibodies (anti-TPO, anti-TG) and one evaluated both autoantibodies separate and therefore was later split into two studies. The studies did not have the same definition of GDM and thyroid disfunction, as well as exclusion criteria. The synthesis meta-analysis showed a significant association between thyroid antibodies and GDM, as well as anti-TPO on its own with GDM. However, the meta-analysis of the elven cohort studies did not reveal association between higher positive antibodies and a higher risk of GDM. Therefore, the authors concluded that, there is a significant but not strong association between thyroid antibodies and the risk of developing GDM and that there is no predictive value of first trimester thyroid-antibodies for GDM.

12.3 Conclusion

In summary, the study results concerning the association between thyroid markers and GDM were mixed. Among the various markers, lower fT4 levels and positive thyroid autoantibodies appear to show the greatest potential for indicating an elevated risk of GDM in the first trimester. Specifically, four studies reported significantly lower levels of fT4 in women who later developed GDM. However, another four found no significant association,

reflecting inconsistent findings in the current literature. Regarding fT3 only two out of five studies observed significantly higher levels in GDM cases. TSH showed no significant association with GDM in the majority of the studies. Thyroid autoantibodies were examined in four studies, three of these reported a significant association between antibody positivity and the development of GDM. These findings are supported by a recent meta-analysis. However, when only cohort studies within the meta-analysis were considered, the data revealed that a higher prevalence of thyroid autoantibodies did not consistently result in the development of GDM. Based on this, the authors of the meta-analysis concluded that there may be a strong, yet not statistically significant association between elevated thyroid antibody levels and GDM.

In conclusion, lower fT4 concentrations and positive thyroid antibody status show potential as early indicators for GDM risk. However, further research is needed to validate these findings, particularly in combination with other emerging biomarkers, to improve the predictive accuracy for GDM in early pregnancy.

13. Gut Microbiota

13.1 Introduction

Microorganisms inhabit the entire human body, including all body cavities. Collectively referred to as the microbiota, these microorganisms include bacteria, archea, bacteriophages, eukaryotic virus and fungi. The microbiome refers to the collective genetic material of these intestinal microorganisms. Although microbiota is present in various body regions, most of them are found in the intestines. This community, known as the gut microbiota, is highly individual, however, several factors influence its composition, including mode of delivery, infant feeding practices, lifestyle, medication use and genetics. A healthy gut microbiota is generally characterized by high taxa diversity, abundant microbial gene richness and stable functional microbiome cores. Numerous studies and observations suggest that the intestinal microbiota not only plays a key role in maintaining human health but also contributes to the development of certain metabolic and inflammatory disorders. Initial evidence for a role of gut microbiota and gut hormone secretion in glucose regulation came from epidemiological studies comparing individuals with and without total colectomy. These studies found that individuals who had undergone total colectomy had an increased risk of developing type 2 diabetes mellitus (Fan & Pedersen, 2021).

Further research into the gut microbiota has shown that a rich and balanced microbial population serves as a protective factor against metabolic disorders. A notable finding in relation to type 2 diabetes mellitus is the reduction of butyrate-producing species such as Faecalibacterium prausnitzii and Roseburia intestinalis. Butyrate, a short-chain fatty acid (SCFAs), is produced through the fermentation of plant fibers by gut microbiota. SCFAs play an essential role in regulating appetite, insulin response, inflammatory processes and maintaining colonic cell function (Crudele et al., 2023).

Additionally, microbiota-featured factors implicated in the development of diabetes mellitus included reduced levels of Akkermansia muciniohila and an increased presence of pro-inflammatory bacterial species. This dysbiosis, characteristic of the pre-diabetic state in type 2 diabetes mellitus, resembles the microbiotic profile observed in pregnant women with GDM (Fan & Pedersen, 2021). Therefore, investigating gut microbiota composition in early pregnancy may offer promising insights for predicting the development of GDM.

13.2 Collected studies

All three studies (Hu et al., 2021; Ma et al., 2020; Wei Zheng et al., 2020), which are investigated in this chapter were conducted in China and all of them sequenced the stool samples with the 16S rRNA gene amplicon to get the microbiome. Ma et al. (2020) determined if changes within the gut microbiota during the first trimester were associated with the development of gestational diabetes. They compared 98 GDM cases with 98 matched by age, gestational age and sample collection data healthy controls within the Hunan Provincial Maternal and Child Health Hospital in South China. The study cohort was further divided in a discovery (70 cases vs. 70 controls) and a validation (28 cases vs. 28 controls) set. The stool and blood samples were collected between 10 to 15 weeks of gestation. GDM screening took place between 24 to 28 weeks of gestation with a 75g OGTT. GDM was diagnosed according to the IADSPSG criteria, if at least one glucose level was elevated (fasting $\geq$ 5.1 mmol/l, 1h $\geq$ 10 mmol/l, 2h $\geq$ 8.5 mmol/l). The GDM group had significantly higher levels of BMI, waist, glucose, insulin homeostatis, high-sensitivity CRP, systolic and diastolic blood pressure, haemoglobin, triglyceride, ALT, GGT, uric acid, fasting blood glucose and fasting insulin, as well as lower values of HDL. 20% of all pregnant women in the study ate probiotic yogurt during pregnancy, however no significant differences in the daily average yogurt intake between cases and controls. Discovery and validation set differed significantly only by AST and urea levels, otherwise they were comparable.

Concerning the alpha diversity of the gut microbiota, women with GDM had lower richness and diversity, but higher dominance. Beta analysis showed an increased individual diversity in the GDM group. Women who later develop GDM had significantly higher abundance of *Eisenbergiella, Tyzzerella and Lachnospiraceae NK4A136.* On the contrary, *Parasutterella, Parabacteriodes, Megasphaera, Dialister, Ruminococcaceae UCG-002, -003, -005, Eubacterium xylanophilum group and Eubacterium eligens group* were reduced in the GDM group. For exploring potential clinical paths, they investigated a possible association between differential taxa and maternal clinical features. Two of the dominant species (*Eisenbergiella and Tyzzerella 4)* in the case group were correlated positively with fasting blood glucose. On the contrary, the microorganisms *Parabacteroides, Parasutterella and Ruminococcaceae UCG 002* were correlated negatively with fasting blood glucose levels. Furthermore, *Dialister,* which was dominant in the controls, was negatively correlated with fasting insulin, daily oil and yogurt intake. Another step was the exploration of co-occuring

and co-excluding networks of differential genera for healthy controls and GDM cases. The positive association between genera of *Ruminococcaceae UCG 003 and Eubacterium xylanophilum group* were no longer obtained and replaced by a new positive correlation between Ruminococcaceae UCG 002, Ruminococcaceae UCG 003 and Lachnospiraceae NK4A136. Moreover, the strong negative correlation between *Parasutterella and Ruminococcaceae UCG 002* in the control group disappeared in the GDM group and the positive correlation between *Proteobacteria and Firmicutes phyla* in the control group turned into a negative one in the GDM group. For the analysis of the connected predictive quality of gut microbiota and clinical data for development of GDM, the study authors established a linear discriminant analysis (LDA) consisting of set with 5 OTUs *(Parabacteroides, Ruminococcus 2, Ruminococcaceae UCG-014, Alloprevotella and uncultured Ruminococcaceae) and* 2 clinical indices (GLU, GGT). The generated receiver operating characteristic (ROC) curves presented that the model showed accuracy and efficacy in the detection of GDM in early pregnancy with areas under the ROC curves (AUC) for the discovery and the validation sets by being 0.736 (95%-confidence interval 0.663-0.808) and 0.696 (95%-confidence interval 0.575-0.818). Furthermore, the relative abundance of uncultured-Ruminococcaceae was significantly higher in the controls in both sets. The level of fasting glucose was significantly higher in the GDM group. Overall, the model showed a higher accuracy and specificity in both sets, compared to other models.

The second study (Hu et al., 2021) in this chapter investigated possible association between gut bacterial biomarkers in early pregnancy and the following risk of GDM development in Chinese pregnant women. Therefore Ping Hu et.al. created a nested case-control study within the Tongij-Shuangliu Birth Cohort study. They compared 201 GDM cases with 201 matched healthy controls. Matching criteria contains age, gestational week and date of stool sample collection. The stool samples were collected between 6 to 15 weeks of gestation, and GDM screening took place between 24 to 28 weeks of gestation. GDM diagnosis was apprehend through 75g OGTT according to the IADPSG criteria. Women with GDM had a generally higher BMI and fasting glucose and were more likely to be current or former alcohol drinkers. Furthermore 13 cases had GDM in a previous pregnancy, while none of the controls had gestational diabetes in previous pregnancies. The microorganisms: *Rothia, Actinomyces, Bifidobacterium, Adlercreutzia, Coriobacteriaceae and Lachnospiraceae spp.* were significantly reduced in the GDM group. Five of the beforehand written bacteria are SCFAs-producing bacteria. Whereas *Ruminococcaceae spp. and Veilonellaceae* were significantly

overrepresented in the GDM group. The overrepresentation of *Staphylococcus* relative to *Clostridium roseburia and Coriobacteriaceae* as reference microorganisms were positively correlated with all three measured glucose levels of the OGTT. To comprehend the predictive quality of the microbiota-featured values they compared the relative abundance of 41 microbial taxa between cases and controls. Increased volume from 6 of these microbial taxa were associated with reduced risk of developing GDM. The bacteria in question were: Actinobacteria, families of Coriobacteriaceae and Gemellaceae, genus 26 of Coriobacteriaceae, genus 74 of Gemellaceae and an unknown species of Coprococcus. In comparison with conventional risk factors of GDM, the 6 microorganisms showed a reduced predictive quality. However, in combination with the risk factors a significantly increased C-statistic from 0.69 to 0.75 was detected.

Wei Zheng et al. (2020) studied the changes of the gut microbiota from the first to second trimester and their relationship with the later development of gestational diabetes. The study was conducted in Beijing, China. 31 pregnant women with GDM were compared to 103 healthy controls. Stool samples were collected in the first trimester between 8 to 12 weeks of gestation (timepoint T1), as well as in the following months until the GDM screening between 24 to 28 gestational weeks (timepoint T2). GDM diagnosis was apprehend through a 75g OGTT. Cases and controls were similar in age, gravidity and parity. Women with GDM had higher pre-pregnancy BMI, total cholesterol and total triglyceride levels compared to the healthy controls. There was a comparable increase in the *Firmicutes/Bacteriodetes ratio* in both groups between the two timepoints. With the help of a linear discriminant analysis (LDA) they identified multiple different taxa between the cases and controls at both timepoints. At T1, a collection of 10 taxa were detected to have a higher relative abundance in the controls in comparison to the GDM group. The microorganisms in question were *Prevotella, Coprococcus, Streptococcus, Pentococcus, Desulfovibrio, Intestinimonas, Veilonella and the parent taxa of Streptococcus (that is the family Streptococcaceae, the order Lactobacillales and class Bacilli).* A reduced amount of *Caprococcus and Streptococcus* were found in the GDM group at both timepoints. On the contrary, the ratios of *Megasphaera and Eggerthella* increased in the GDM group. The microbiota of the control group showed a greater number of different species between the two timepoints (49 different taxa), then the GDM group (only 7 different taxa). To determinate the potential of the gut microbiota in the first trimester as an early biomarker for GDM, a random forest analysis

was performed to establish at 3-point panel. The established panel consisted of *Caprococcus, Intestinimonas and Veilonella* and showed a medium performance with an AUC of 0.743.

13.3 Conclusion

The foundational framework of the three studies is largely consistent. All employed the same GDM screening protocol and utilized identical stool sample gene sequencing methods. Additionally, each study was conducted in China, providing a shared geographical and demographic context. A key finding across all three studies was that incorporating gut microbial feature into prediction models – alongside conventional GDM risk factors – enhanced the models prediction accuracy. However, despite these similarities the studies diverged in their identification of which specific microbial features should be included. Notably, studies two and three both identified the bacterium *Caprococcus* as a potential protective factor against gestational diabetes. This overlap suggests a promising lead, yet the overall inconsistency in microbial markers highlights the need for further investigation.

Given the potential of gut microbiota as an early biomarker – particularly in Asian populations – it is essential to conduct larger-scale studies that include more diverse populations. Such studies would help clarify which microbial features are most relevant and generalizable across different ethnic groups, ultimately improving the reliability of microbiome-based GDM prediction models.

14. Metabolomic, Genomic and Transcriptomic Data

14.1 Metabolomics

14.1.1 Introduction

Small molecules involved in metabolism, commonly referred to as metabolites, collectively make up the metabolome. The study of metabolites, along with their functions, interactions and dynamic changes, is known as metabolomics (Rinschen et al., 2019). As defined by the Encyclopedia Britannica, metabolomics is part of the broader field of "omics" sciences, which aim to investigate biomolecule and their associated molecular pathways. Other major "omics" disciplines include genomics, proteomics and transcriptomics (Rogers, Kara. Britannica. https://www.britannica.com/science/omics). Initially, the primary value of metabolomic research was seen in the identification of possible biomarkers such as disease-specific metabolites. However, recent discoveries have revealed that metabolites are not merely by-products of biological processes. Rather, they can actively influence gene expression and protein function, allowing them to interact across all "omics" levels (Rinschen et al., 2019).

Two key analytical techniques are predominantly used in metabolomic studies: mass spectrometry (MS) and nuclear magnetic resonance (NMR). These methods are applied in both targeted and untargeted approaches. In targeted metabolomics, one or more pre-selected known metabolites are quantified. In contrast, untargeted metabolomics involves analysing biological samples for a broad spectrum of metabolites, identifying patterns and comparing differences between groups (Xie et al., 2023).

To deepen understanding of diseases mechanism, researchers also map metabolic pathways. Linking metabolomic data with genomic, transcriptomic and proteomic information. This integration has led to the development of several metabolic pathway databases. One widely used resource is the Kyoto Encyclopedia of Genes and Genomes (KEGG), which includes genomic, chemical and phylogenetic data. KEGG enables researchers to search for metabolites by category, identifying their metabolic pathways and trace their up- and downstream interactions, helping build a more comprehensive picture of their role in health and disease.

Due to the physiological fluctuations in glucose levels during early pregnancy – including an initial increase followed by a decrease and rising insulin resistance - it is difficult to define a clear glucose threshold for early GDM diagnosis. This has prompted interest in

metabolomic research as a means of identifying early biomarkers for GDM (Xie et al., 2023). In addition to improving early detection, metabolomics may also provide deeper insights into the pathophysiology of GDM (Mokkala et al., 2020). Metabolic profiles related to GDM have been found to involve various pathways, including amino acid metabolism (e.g., branched chain -, aromatic and glutamic acids), lipid metabolism (e.g., triglycerides, phospholipids, cholesterol, fatty acids), uric acid metabolism and intestinal microbiota-related metabolism (Xie et al., 2023).

14.1.2 Collected studies

This sub-chapter consist of two European and five Asian studies. Two of them investigated metabolomic profiles in overweight/obese pregnant women, three concentrated on the amino acid metabolism and two on the metabolic risk profiles of overweight/obese pregnant women.

Mokkala et al. (2020) investigated the metabolomic profiles of overweight (BMI ≥ 25 kg/m2)/obese (BMI ≥ 30 kg/m2) women with and without the later development of GDM, as well as the potential of specific metabolites to be early biomarkers for GDM. The study was conducted in southwest Finland and included 357 women with serum metabolites profiles in the first trimester. GDM screening took place between 25.0 to 27.1 gestational weeks and was diagnosed if at least one value was above the determined cut-off levels of a 2h-75g OGTT (fasting ≥ 5.3 mmol/L, 1h ≥ 10.0 mmol/L and 2h ≥ 8.6 mmol/L). 82 women were diagnosed with GDM. A higher percentage of pregnant women with GDM, than without complications were obese. In total 78 lipid variables in the first trimester were different between GDM complicated and uncomplicated pregnancies. In the GDM group, concentrations of all sized VLDL particles, free cholesterol to total lipid ratio in VLDL particles, medium- and small-size HDL particles, total cholesterol and cholesterol ester in very large-sized HDL particles, triglycerides and ratio of MUFAs (monounsaturated fatty acids) to total fatty acids were elevated. In contrast, the levels of very large-sized HDL particles, total cholesterol and cholesterol ester in VLDL particles, free cholesterol to total lipid ratio in very large-sized HDL particles, estimated degree of unsaturation of fatty acids, the ratio of n-6 long-chain PUFAs (polyunsaturated fatty acids) and PUFAs to total fatty acids were lower in women who developed gestational diabetes. Additionally, elevated levels of glucose, lactate, pyruvate, two branched-chain amino acids (isoleucine and leucine), alanine, aromatic amino acid: phenylalanine and GlycA were detected in women with GDM. The most predictive value for the development of GDM later in pregnancy,

showed the concentrations of small-sized HDL particles, followed by glucose, GlycA, leucine and pyruvate levels. In a risk prediction model combined of these variables, an enhanced AUC was established. The highest predictive accuracy for gestational was found in the combination of glucose, GlycA, leucine and small-sized HDL particles.

Tian et al. (2021) aim was to identify possible serum metabolites as early indicator for GDM. Therefore, they recruited Chinese women from the Hunan Province in their first trimester. GDM screening took place between 24 and 28 gestational weeks with a 2h-75g OGTT and was diagnosed if at least one glucose level was above the cut-off levels (fasting ≥ 5.1 mmol/L, 1h ≥ 10.0 mmol/L, 2h ≥ 8.5 mmol/L). The final study population included 51 women with gestational diabetes and 51 healthy matched controls. Matching criteria were age and the timing of blood collection. In the GDM group early pregnancy weight, BMI, blood pressure, the number of women with GDM in their history, haemoglobin and LDL levels were significantly higher. Triglyceride, total cholesterol and HDL values did not differ significantly between the two groups. In total, 44 metabolites differed between the cases and controls. After the correction for multiple hypothesis testing, 26 highly significantly different metabolites could be found. In the control group higher levels of 4-oxoproline, dihydrothymine, 1,5-anhydro-D-glucitol, leu-leu, metval, hexadecanedioic acid and calcitriol were detected. On the contrary, in the GDM group elevated values of L-glutamic acid, L-pyroglutamic acid, L-cysteinesulfinic acid, xanthine, 2-methylhippuric acid, pantothenic acid and incadronic acid were obtained. In a followed KEGG enrichment analysis the majority of the metabolic pathways was related to the amino acid metabolism, because of that, the study authors concluded that the amino acid metabolism is important for the development of gestational diabetes.

In the second Finnish study, Nevalainen et al. (2016) evaluated association between early maternal serum concentrations of 10 amino acids an 31 acylcarnitines. The study authors wanted to investigate this special metabolite profile, which was found in women with type 2 diabetes mellitus (T2DM), because of the pathophysiological similarities between GDM and T2DM. From 31.146 pregnant women, whom at a first trimester combined screening at the Oulu University Hospital, 69 randomly selected women with GDM and 295 randomly selected normoglycemic controls were included in the study population. GDM screening in Finland conducted with a 2h-75g OGTT between 12 to 16 or 24 to 28 gestational weeks. An earlier OGTT is carried out in women with an elevated risk potential for GDM development. This includes women with a BMI ≥ 35, GDM in prior pregnancy, first trimester glucosuria,

polycystic ovary syndrome or a first-degree relative with type 2 diabetes mellitus. Women from the GDM group of this study were all diagnosed in the second trimester, if at least one glucose level was above the cut-off level (fasting ≥ 5.3 mmol/L, 1h ≥ 10.0 mmol/L, 2h ≥ 8.6 mmol/L). Concerning the basic characteristics, it was observed that women in the case group were significantly older, weight more and had a significantly higher BMI, as well as gestational age at sampling. Women with the development of GDM, had significantly lower levels of PAPP-A in the first trimester, the other combined screening markers (beta-hCG, nuchal translucency) did not differ significantly between the two groups. The comparison of the metabolic profiles showed that arginine levels were elevated and, on the contrary, glycine and C5OH levels were decreased in GDM cases. They then combined the PAPP-A, arginine, glycine, C5OH and risk factors (maternal age, BMI, smoking status) in various formations with each other and calculated the detection rates of GDM. The highest detection rate was achieved by the combination of all of them. However, in the absent of PAPP-A, the detection rate was almost as good.

Jiang et al. (2020) wanted to evaluate the performance of amino acids levels as early predictor for GDM development. Their study population consisted of 431 pregnant women, of whom 65 women were diagnosed with GDM between 24 to 28 gestational weeks with a 2h-75g OGTT. The diagnosis was made if at least one of the glucose levels was above the cut-off points defined by the IADPSG. Women with GDM were older, had higher BMI, fasting plasma glucose HOMA-IR, triglyceride and GGT levels. In total 18 amino acids were analysed. Alanine, isoleucine and tyrosine were significantly lower in women with GDM. These associations remained significant even after adjusting for age, parity, family history of diabetes, BMI, GGT, triglyceride, fasting plasma glucose and fasting serum insulin. To investigate the predictive value of amino acids and conventional risk factors (age, BMI, triglyceride, GGT, fasting plasma glucose and fasting serum insulin) for GDM, a ROC analysis was executed. The combination of all the conventional risk factors reached an AUC of 0.692. Each here significant amino acid on its own achieved an AUC of 0.604 (alanine), 0.594 (isoleucine) and 0.588 (tyrosine). The highest AUC of 0.737 was calculated by the combination of the conventional risk factors plus isoleucine and tyrosine. This new model did also improve the net reclassification improvement (NRI) for GDM.

Leng et al. (2016) focused their attention on alanine aminotransferase (ALT) as a possible early predictor of GDM. The reason for this focus, is due to the important role of the liver in the glucose metabolism and the association of hepatocytic enzymes (specifically ALT) with

insulin resistance and type 2 diabetes mellitus. And as prior established, type 2 diabetes and GDM share a few similar risk factors, as well as pathogenesis. 17359 pregnant women were included in the study. GDM was diagnosed with a two-step approach in 1332 women. The two-step approach includes a 1h-50g GCT, followed by 2h-75g OGTT if the GCT result is ≥ 7.8 mmol/L. The final GDM diagnosed was achieved if at least one of the following cut-off points in the OGTT was exceeded: fasting ≥ 5.1 mmol/L, 1h ≥ 10.0 mmol/L, 2h ≥ 8.5 mmol/L. Women in the case group were significantly older, shorter, had higher BMI and blood pressure levels, were more likely to be multipara, Han-ethnicity, smokers before pregnancy and have a first-degree relative with diabetes. Significantly higher median ALT levels were detected in women with GDM. Elevated ALT levels (ALT ≥ 22 U/L) and BMI values ≥ 25 kg/m2 were associated with the risk for developing gestational diabetes. In an additional analysis with further adjustment for the plasma glucose levels at the GCT time, ALT levels between 22 to 40 U/L were still associated with GDM, however, ALT ≥ 40 U/L were no longer associated. In comparison with the reference group (ALT < 22 U/L, BMI < 25 kg/m2), elevated ALT levels increased the Odds Ratio of overweight/obesity for GDM risk, and vice versa.

Two Asian studies, one from Taiwan (Yen et al., 2019) and one from Japan (H. Sasaki et al., 2020) took a deeper look on the metabolic profile in pregnant women and its possible association with gestational diabetes. Yen et al. (2019) investigated if overweight and obesity are associated with the appearance of certain metabolic risk factors in the beginning of pregnancy and the risk of developing GDM. Further to determinate if these metabolic risk factors may be a potential connection between overweight and/or obesity and gestational diabetes. Overweight/obesity was defined by a BMI ≥ 24 kg/m^2. The study population included 429 pregnant women with a BMI lower than 24 kg/m2 and 98 with a BMI at a minimum of 24 kg/m2. GDM screening took place between the 24^{th} and 28^{th} week of gestation with a 2h-75g OGTT. The GDM diagnosis was made according to the American diabetes association (ADA) if at least one glucose level was above the defined cut-offs (fasting plasma glucose ≥ 5.1 mmol/L, 1h ≥ 10.0 mmol/L, 2h ≥ 8.5 mmol/L). Plasma glucose, HbA1c, total cholesterol, HDL-C, LDL-C, plasma triglyceride (TG) and C-peptide were measured between 8.7 and 11 gestational weeks. Insulin resistance was defined by a HOMA-IR ≥ 25 percentile. 74 women were diagnosed with GDM. In the GDM group 54 women had a BMI below 24 kg/m^2 and 20 women were part of the overweight/obesity group. In other words, the 12.6% of the normal weight group and 20.4% of the overweight/obesity group

developed GDM. In general, women with GDM were older, had a higher history of GDM in previous pregnancies as well as a higher family history of diabetes and had higher levels of plasma glucose and HbA1c. Additionally, women with GDM in the normal weight group had higher HOMA-IR and plasma triglyceride levels. In contrast, women with GDM in the overweight/obesity group had higher blood pressure levels. Significant interactions between HbA1c, diastolic blood pressure, BMI group and GDM were detected. Therefore, the comparison of odds ratios of GDM for HbA1c and diastolic blood pressure in the two weight groups, showed significantly higher odds ratios in the overweight/obesity group. In both groups, there was a positive association of the GDM incidence rate and the numbers of risk factors. Pregnant overweight/obese women tend to have an accumulation of metabolic risk factors in the early pregnancy and these accumulations of metabolic risk factors is connected to an increased risk for GDM.

H. Sasaki et al. (2020) evaluated the metabolic status of pregnant women with and without adverse pregnancy outcomes. Blood samples for determination of different metabolic biomarkers were measured in the first and second trimester. 80636 pregnant women were included in the study, 76025 had uncomplicated healthy pregnancies and 4611 had endocrine disorders (43 with diabetes mellitus type 1, 78 with diabetes mellitus type 2, 2315 with gestational diabetes, 354 with dislipidemia and 1821 with other endocrine disorders, including complications of the four disorders ahead). In Japan all pregnant women get screened for d. GDM and “overt diabetes in pregnancy” with the following stepwise method. The first step is the measurement of a random plasma glucose level in the first trimester. If the random plasma glucose level is above 200 mg/dl, 75g OGTT should be planned. However, before the planning fasting blood glucose levels, HbA1c and diabetic retinopathy should be examined, so as not to overlook the differential diagnosis “overt diabetes in pregnancy”. The second step contains a 50g-GCT (with a cut-off level of 140mg/dl) or the measurement of the random blood glucose level for second time (cut-off level 100 mg/dL) between 24 to 28 gestational weeks. Women with positive screening results, excluding those with a diagnosed “overt diabetes in pregnancy”, will then receive a 75g OGTT. The GDM diagnosis is made according to the IADPSG criteria. “Overt diabetes in pregnancy” is classified as diabetes mellitus type 2. HbA1c levels were higher in the GDM, type 1 DM and type 2 DM group compared to the healthy pregnancies. In addition, women with GDM showed increased levels of triglyceride compared to the healthy cases. However, there were no significant differences concerning the cholesterol levels in the comparison of GDM cases

and healthy controls. In general, women with a higher BMI, had higher HbA1c, total cholesterol, LDL-C and triglyceride levels and lower HDL-C levels. Furthermore, women with an increased BMI were at higher risk to have diabetes or endocrine disorders.

14.1.3 Conclusion

All studies reviewed identified potential early biomarker for GDM through metabolomic analysis. However, their results varied significantly.

The first Finish study (Mokkala et al., 2020) found the highest predictive accuracy for later GDM diagnosis using a combination of small-sized HDL particles, glucose levels and the two amino acids GlycA and leucine. In contrast, the second Finish study (Nevalainen et al., 2016) achieved the highest detection rate using a combination of arginine, glycine, C5OH, PAPP-A and conventional risk factors such as maternal age, BMI and smoking status. Notably, elevated arginine levels demonstrated the highest predictive value as a single biomarker.

In the first Chinese study (Tian et al., 2021) women with GDM exhibited higher levels of L-glutamic acids, L-pyroglutamic, L-cysteinsulfinic acid, Xanthine, 2-methylhippuric acid, pantothenic acid and incadronic acid. Conversely, the control group showed increased levels of 4-oxoproline, dihydrothymine, 1,5-anhydro-D-glucitol, leu-leu, metval, hexadecanedioic acid and calcitriol. The second Chinese study (Jiang et al., 2020) reported the highest AUC for for GDM prediction when combing conventional risk factors with the amino acids isoleucine and tyrosine. The third Chinese study (Leng et al., 2016) took a different approach, focusing on alanine aminotransferase (ALT). The study found the highest GDM risk among women with ALT levels ≥ 22 U/L and BMI ≥ 25 kg/m2.

Yen et al. (2019) demonstrated that overweight and obesity are connected to expression of several metabolic risk factors in early pregnancy and subsequently also to the risk of developing gestational diabetes. H. Sasaki et al. (2020) evaluated the metabolic status of pregnant women and their possible association to adverse pregnancy outcomes. Increased levels of HbA1c and triglyceride were found in women who later developed GDM.

Overall, these findings suggest that metabolomics holds considerable promise for inclusion in future GDM risk prediction models. However, due to variations across studies – including differences in ethnic populations, methodology, and targeted metabolites – larger, more diverse studies are necessary to identify which specific metabolites offer the most reliable predictive value.

14.2 Transcriptomics (RNA) and Genomics

14.2.1 Introduction

The main focus of the following studies is the investigation of RNA, DNA and other genetic markers as potential early biomarkers for gestational diabetes mellitus. Transcriptomics and genomics are branches of the "omics" sciences. Transcriptomics involves the examination of all RNAs, while genomics focuses on the structure and function of the genome. Common analytical techniques include next generation sequencing, microarray analysis and RNA sequencing. These investigative methods enable more personalized medicine and contribute to a better understanding of disease pathways, as well as the discovery of new biomarkers (Rogers, Kara. Britannica. https://www.britannica.com/science/omics). RNAs can be categorized into coding and non-coding RNAs. In addition to their role in translating DNA into proteins, RNAs also perform regulatory functions. These regulatory RNAs include, for example, micro RNA (miRNA) and piwi-interacting RNAs (piRNA) (Dai et al., 2020). Micro-RNAs, a group of non-coding RNAs typically comprising up to 20 nucleotides, are involved in gene regulation and participate in numerous biological pathways. Previous studies have shown the downregulation of specific miRNAs in cancer patients, as well as their involvement in cardiovascular, immune-related, neurodegenerative and other diseases. Moreover, microRNAs play a role in glucose metabolism (Margaritis et al., 2021). Due to this involvement, miRNAs have been investigated as potential biomarkers for type 1 and 2 diabetes mellitus, as well as, GDM (Yoffe et al., 2019). The wide-ranging roles of transcriptomics have also prompted recent investigations into its connection with the development of adverse pregnancy outcomes (Del Vecchio et al., 2021).

14.2.2 Collected Studies

Del Vecchio et al. (2021) examined the cell-free DNA and RNA content in maternal and cord plasma samples and investigated possible associations with adverse pregnancy outcomes. The studied adverse pregnancy outcomes were gestational diabetes, hypertensive disease of pregnancy, foetal growth restriction (FGR), placental abruption, collectively termed ischaemic placental disease. 160 women were included, 99 of those had uncomplicated pregnancies and the remaining 61 women developed adverse pregnancy complications. 17 out of the 61 women were diagnosed with GDM. GDM screening was a two-step approach between 24 to 28 gestational weeks consisting of a 50g GCT and a followed fasting 3h-100g OGTT, if values in the GCT were above 135 mg/dl. In the GDM cases the placental fraction of cell-free DNA increased significantly in the first trimester

compared to unaffected pregnancies. This trend persisted through the second and third trimester, however, not significantly anymore. Another non-placental tissue-of-origin, the pancreatic cell-free DNA fraction was also significantly increased in the first trimester in women with gestational diabetes. The pancreatic cell-free DNA was also found in the cord blood of women with GDM. The study authors concluded therefore, that this a possible sign for a pancreatic dysregulation in the babies. The total CG methylation percentage did not differ significantly between normal, GDM affected, and preclampsia/hypertensive disease affected pregnancies. Plasma cell-free RNA was conducted at all trimesters, delivery and of the umbilical cord. The placental signature was elevated in women with GDM, however, not significantly. The comparison of BMI groups showed a lower trend of the placental fraction of cell-free DNA and RNA in the first trimester in women with BMI above 30 kg/m2, then women with a BMI between 18.5 to 24.9 kg/m2 and women with BMI between 25 and 29.9 kg/m2 were found in between the first two groups. To focus on specific genes that were expressed different in pregnancy, they used RNA-seq. Three genes (CSH1, CSH2, CGA) were only expressed in women with adverse pregnancy outcomes. To analyse certain genes expressed in the first trimester some more, a qRT-PCR was used. In the qRT-PCR S100 calcium-binding protein A8 (S100A8) was significantly increased in women with APOs. Further, matrix metallopeptidase 8 (MMP8), membrane spanning 4-Domains A3 (MS4A3) and BCL2CL15 were significantly upregulated in women with GDM and preeclampsia/hypertensive diseases in pregnancy. In contrast, alkaline phosphatase biomineralization was found significantly downregulated in women with APOs compared to uncomplicated pregnancies. With cell-free RNA sequencing possible biomarkers for the early detection of adverse pregnancy outcomes were identified and combined in a panel. The panel was able to detect around 90 precent of the true positive values (APOs). According to a model the most recurring factors were SRPK1, S100A9, NAMPT, MS4A3, MMP8, HAGLR, ALPL, ASCL1 and KLHL2. The panel solely for GDM consisting of the genes SRPK1, S100A8, S100A9, and MS4A3 could identify in a logistic regression model 64 precent of the true values. Due to the overlapping of factors for preeclampsia and gestational diabetes and the lower percentage of positive values in the logistic regression model solely for GDM, the authors concluded that an early detection of GDM according to the detected genes could be difficult. However, the model with cell-free DNA could identify 80 precent of the positive GDM pregnancies and showed therefore more promise.

Yoffe et al. (2019) investigated in a case-control study the potential of circulating miRNA as an early biomarker for gestational diabetes. Blood samples were taken between 9+0 and 11+6 gestational weeks. 43 pregnant women from Italy and Spain were included in the study. In the Italian cohort women were screened for GDM with a 75g OGTT between 24 to 28 gestational weeks according to the IADPSG. Women in the Spanish cohort had an O´Sullivan 50g Screening test at the 24th week of gestation, if the glucose levels were above 7.8 mmol/L a 100g OGTT at 26 gestational weeks was performed. GDM then was diagnosed if two glucose values were elevated (fasting ≥ 5.3 mmol/L, 1h ≥ 10.0 mmol/L, 2h ≥ 8.6 mmol/L, 3h ≥ 7.8mmol/L). 23 women in total were diagnosed with GDM. NanoString nCounter human miRNA assay was used to determinate the miRNA profiles of the subjects. Two miRNAs – miR-223 and miR-23a were found to be significantly upregulated in the GDM group. In a second analysis, the study authors additionally divided the results according to the country of residence. In the Spanish cohort only miR-223 was significantly elevated in the GDM. However, in the Italian cohort both miRNAs from above were significantly increased in the GDM group. In follow-up cohort with 20 women from Italy to validate their findings, a RT-qPCR was used to review the levels of miR-223 and miR-23a. Both miRNAs were elevated in the GDM cases. This new cohort was divided 50:50 in uncomplicated pregnancies and pregnancies with the development of GDM. To evaluate the predictive quality of miRNA, three different linear regression models, which sort the samples in GDM and control samples according to the expressed values of the miRNAs, were used. In one model the values of miR-223 and miR-23a were used for the determination, in the other two one of each was used. The model with miR-223 got by comparison of the AUC a slightly better result. A permutation test, which compared the accuracy of the models with a random classifier, was performed to validate the results. All models showed significant AUC and accuracy values. To discover the main source of the miRNA plasma values, three GDM-related tissues - placental, visceral adipose and abdominal subcutaneous adipose tissue - were examined. Therefore, samples from six women with GDM and eight women without it, were collected after the delivery. Analysis technique was once more the NanoString nCounter human miRNA assay. In total, 798 human miRNAs were determined, almost 400 of them were expressed in all investigated tissue types. All samples showed high levels of miR-223 and miR-23a and there were no differences between GDM and control samples.

Zhou et al. (2021) investigated the genetic variants, which are associated with beta-cell function or insulin resistance in diabetes mellitus type 2 and bile acid metabolites and their potential influence on development of GDM. From a large cohort of 22302 Chinese women, 230 with GDM and 217 with uncomplicated pregnancies were selected. GDM was screened using a two-step approach. The first step was a 1h-50g-GCT at 24 to 28 gestational weeks at primary care hospitals. Women with glucose levels ≥ 7.8 mmol/L were referred to the GDM clinic within the Tianjin Women and Children Health Centre. In the GDM clinic a 75g OGTT was taken to determinate GDM. GDM was diagnosed according to the WHO GDM criteria 2013, if at least one glucose level was above the cut-off (fasting ≥ 5.9 to 6.9 mmol/L, 1h ≥ 10.0 mmol/L, 2h ≥ 8.5 to 11.0 mmol/L). Blood samples were collected at around the 10^{th} gestational week and genome-wide genotyping was performed. Women in the GDM group had higher BMI, blood pressure, alanine and aminotransferase levels. In 90 precent of the samples the following eleven bile acids: glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA) hyodeoxycholic acid (HCDA), cholic acid (CA), glycoursodeoxycholic acid (GUDCA), chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) were detected. Most of these bile acids, except TCA and CA were lower in the GDM group. Two genetic risk scores (GRSs) based on 52 and 30 independent genetic variants, which are according to previous studies connected with the beta-cell function and insulin resistance. A comparison was drawn up, which compared the associations of the genetic risk scores and the eleven bile acids with an adjustment for clinical cofounders. Five bile acid species were then found to be associated with the genetic risk scores. However, only TDCA was significant. The weighted beta-cell genetic risk score was significantly associated with higher risk of developing GDM. Another significant association could be found between beta-cell genetic risk scores and TDCA. Moreover, they detected a significant interaction of insulin-resistance genetic risk score with TCDCA on the risk of GDM was found. TCDCA ≤ 0.2 nmol/ml was significantly associated with an increased risk for GDM. In comparison with women with a low genetic risk for GDM and high concentrations of TCDCA, women with high genetic risk and low concentrations of TCDCA had an OR of 14.39 for GDM.

14.2.3 Conclusion

The American study by Del Vecchio et al. (2021) focused on cell-free DNA methylation and transcriptomics as predictors for adverse pregnancy outcomes (APOs), including GDM.

Using cell-free RNA sequencing, the researchers developed a panel to detect APOs, which successfully identified a majority of true positive cases. However, the panel´s performance in detecting GDM specifically was less effective. Due to this limitation and because of overlapping features with early biomarkers for preeclampsia, the authors concluded that early detection of GDM alone using their panel might be challenging. A separate panel based on cell-free DNA markers demonstrated higher accuracy for GDM and currently appears more promising.

Yoffe et al. (2019) investigated the potential of micro-RNAs as early biomarkers in an Italian-Spanish cohort. Two miRNAs – miR-223 and mi-23a – were found to be upregulated in GDM cases. In a linear-regression model, mi-223 emerged as the slightly better classifier for GDM.

Zhou et al. (2021) found that a weighted beta-cell genetic risk score was associated with GDM. Additionally, the bile acid TCDA (taurodeoxycholic acid) was significantly associated with this genetic risk score. In examining the interaction effects between genetic risk scores and bile acid species on GDM risk, a significant interaction was observed between the bile acid TCDCA (taurochenodeoxycholic acid) and the insulin-resistance genetic risk score. Women with a high genetic risk and low concentrations of TCDCA exhibited an elevated odds ratio for the risk of developing GDM.

15. Hormones

15.1 Introduction

This chapter discusses the hormones: anti-mullerian hormone (AMH), estriol, prolactin, progesterone and testosterone and their potential roles as early biomarkers for the development of gestational diabetes mellitus.

15.1.1 Anti-mullerian hormone

The anti-mullerian hormone is a dimeric glycoprotein belonging to the transforming growth factor β (TGF-β) superfamily. AMH plays a key role in sexual differentiation during the embryonic period. In male fetuses, it is the first molecule secreted by the Sertoli cells. Between the sixth and eighth gestational week, AMH induces the regression of the Müllerian ducts. In conjunctions with testosterone, this promotes the formation of the Wolffian ducts, which develop into the epididymis, vas deferens and seminal vesicles. AMH secretion in men continues until puberty (Bedenk et al., 2020; Shand et al., 2014).

In female fetuses, AMH is absent during this critical window, allowing the Müllerian ducts to persist and eventually develop into the internal female reproductive organs. In females, AMH production begins around the 36th gestational week and is primarily carried out by granulosa cells of pre-antral and antral ovarian follicles. Serum AMH levels increase until approximately 24.5 years of age, after which they decline and become nearly undetectable after menopause (Bedenk et al., 2020). This pattern parallels the female fertility curve (Shand et al., 2014). AMH also influences ovarian function, particularly follicular growth, and is widely used in gynaecology, especially as a marker of ovarian reserve (Bedenk et al., 2020; Shand et al., 2014).

15.1.2 Estrogens

Estrogens are steroid hormones essential for regulating various physiological processes in women (Parisi et al., 2023). The family of natural estrogens include estrone (E1), estradiol (E2), estriol (E3) and estetrol (E4) (Gérard & Foidart, 2023). A women´s age hormonal status determine the relative concentrations of each estrogen. Estradiol predominates throughout most of life. Estriol levels are highest during pregnancy, while estrone is more prevalent post-menopause (Parisi et al., 2023).

Estetrol, the most recently identified estrogen, is producted by the fetal liver during pregnancy. Consequently, it is detectable in maternal plasma and urine during this period (Gérard & Foidart, 2023). Estrogen levels generally rise throughout pregnancy and are essential for its maintenance (Karvaly et al., 2024).

Estrogen and their receptors also contribute to body weight regulation and insulin sensitivity (Hur et al., 2017). Compared to premenopausal women, age-matched men show higher rates of insulin resistance. Postmenopausal declines in estrogen levels are associated with reduced protective metabolic effects (Paoli et al., 2021). Studies have linked low estriol levels during pregnancy to adverse outcomes (Hur et al., 2017).

15.1.3 Prolactin

Prolactin is a polypeptide hormone composed of 199 amino acids, produced by lactotrophs in the anterior pituitary gland (Rassie et al., 2022). It was originally named for its role in promoting lactation, but is now known to have diverse physiological funcitons (Freeman et al., 2000). Prolactin receptors are found in various tissues, including pancreatic beta cells and adipocytes, which are crucial for metabolic regulation. In vitro and animal studies have demonstrated prolactin´s effect on insulin sensitivity, adipocyte function and lipid

metabolism. These studies also suggest that increasing prolactin levels, along with other lactogenic hormones, may contribute to systemic insulin resistance and reduced insulin receptor binding. However, elevated prolactin has also been directly linked with enhanced maternal pancreatic beta-cell function and increased insulin secretion. These findings suggest a complex role for prolactin and other lactogenic hormones in the development of insulin resistance and potentially GDM (Rassie et al., 2022).

15.1.4 Progesterone

Progesterone is a steroid hormone primarily produced by the corpus luteum and the placenta during pregnancy. It is also secreted in smaller amounts by the adrenal cortex, Leydig cells in men, adipose and other tissues, and the nervous system (Kolatorova et al., 2022). Progesterone is crucial for maintaining pregnancy and also plays an immunomodulatory role (Lai et al., 2024).

During pregnancy, progesterone levels rise significantly – from approximately 10-40 ng/mL in early pregnancy to 100-200 ng/mL in late pregnancy. Additional roles of progesterone include its function as an analgesic and neuroactive steroid (Kolatorova et al., 2022).

Beta-cells in both sexes express receptors for estrogen, androgens and progesterone (Mauvais-Jarvis, 2016). However, animal studies on progesterone's effect on beta-cell proliferation and insulin secretion have shown mixed results. Some of them suggested an inhibitory role, while others indicated a stimulating effect (M. Li et al., 2020).

15.1.5 Testosterone

Testosterone, a member of the androgen group, is secreted by the ovaries, testes and adrenal glands. While testosterone is the predominant androgen in men, dihydrotestosterone is more potent. In women, testosterone levels are approximately 10 to 15 times lower than in age-matched men. Androgens play essential roles sexual development and reproductive function. They also influence bone density, muscle mass, adipose tissue, hair growth, brain function and cardiovascular health, and contribute to estrogen biosyntesis (Naamneh Elzenaty et al., 2022).

Numerous studies have linked low testosterone levels with type 2 diabetes mellitus (Corona et al., 2023). Additionally, there is a well-established association between polycystic ovary syndrome (PCOS) and GDM. PCOS is characterized by hyperandrogenism, ovarian dysfunction and polycystic ovarian morphology (Choudhury & Rajeswari, 2022).

15.2 Collected Studies

Five studies from five different countries – Australia, Korea, Denmark, USA, Turkey - in total are part of this chapter. The first study (Shand et al., 2014), a retrospective cohort study, investigated the potential of the anti-Mullerian hormone as an early biomarker for adverse pregnancy outcomes. The in this study tested studies were preterm birth (< 37 gestational weeks), admission of the baby to the NICU, small for gestational age (birthweight < tenth percentile for sex and gestational age), fetal anomaly, perinatal death (stillbirth or death prior to neonatal discharge), preeclampsia, gestational hypertension and pregnancy hyptertension (preeclampsia and gestational hypertension), gestational diabetes and birth by Caesarean. The inclusion criteria included: singleton pregnancy and a birth at ≥ 20 gestational weeks at study hospital. 331 women fulfilled the criteria and were therefore included in the study. The women had different ages, ethnicities and BMIs. Blood samples were obtained in the course of the first trimester aneuploidy screening between 10+0 to 13+6 gestational weeks. The median anti-Mullerian hormone levels of the cohort were 9.7 pmol/l, the median level from women with GDM were 8.5 pmol/l. However, the difference was not significant, only women with pregnancy hypertension had significantly lower AMH values.

Hur et al. (2017) evaluated a possible association of first trimester maternal estriol levels and adverse pregnancy outcomes in Seoul, Korea. Adverse pregnancy outcomes here included preterm birth (< 37 gestational weeks), GDM (≥ 2 positive results in a 3h-100g OGTT), macrosomia (birth weight ≥ 4000g), large-/small-for-gestational age (birthweight > 90^{th} or < 10^{th} percentile), primary Caesarean sectio (excluding repetitive Caesarean sectio), low one minute APGAR scores < 5, pregnancy induced hypertension (PIH, systolic blood pressure > 140 mmHg/diastolic blood pressure > 90 mmHg after the 20^{th} gestational week). Serum estriol (uE3) values above the 95^{th} percentile were associated with an increased risk for the development of GDM, a primary Caesarean Sectio and PIH. uE3 levels ≥ 2.0 MoM were associated with GDM.

In the Danish study from Overgaard et al. (2020) prolactin and glucose status during pregnancy were measured and then possible association with GDM and PCOS were investigated. Blood samples were taken between 10 to 16 gestational weeks and 27 to 30 gestational weeks. 1043 blood samples were taken in the first time period and 1489 were taken in the second time period. From 1035 out of 2800 women blood samples from both timeslots were available. GDM was diagnosed by a 2h-75g OGTT using a plasma glucose level ≥ 9.0 mmol/L. Women with two or more of the following risk factors were offered an

early OGTT between 14 to 20 weeks of gestation and again between 28 to 30 weeks of gestation. Whereas women with only one risk factor were only offered the late OGTT. The defined risk factors were a BMI ≥ 27 kg/m2, a family history of diabetes, glucosuria during pregnancy, GDM in a previous pregnancy and previous delivery of a macrocosmic child. Women with a GDM diagnosis before the 20th gestational week were excluded from the study. Out of the 622 women with a late OGTT, 513 women had measured first trimester prolactin levels. Testosterone was only measured in the third trimester and therefore for this review not important. GDM was diagnosed in 28 out of 509 women with available data from the first trimester. The median prolactin concentration increased from 633 mIU/L at the 7th gestational week to 5223 mIU/L at the 30th gestational week. In early pregnancy prolactin and prolactin MoM levels did not differ between women with GDM and normoglycemic women. However, women with GDM had significantly higher pre-pregnancy BMI, HbA1c, fasting plasma glucose, fasting plasma insulin and HOMA-IR levels. Low prolactin levels in early and late pregnancy were associated with higher levels of HbA1c and low prolactin levels in late pregnancy were associated with GDM.

M. Li et al. (2020) measured also prolactin and additionally progesterone during pregnancy and then studied their possible associations with gestational diabetes. It was a nested case-control study with 107 GDM cases and 214 matched controls within the NICHD Fetal Growth Studies-Singelton Cohort. Matching criteria included age, ethnicity and gestational week of blood collection. GDM was screened according to the Carpenter-Coustan criteria with 3h-100g OGTT. It was diagnosed if at least two glucose levels were at or above the defined cut-off points: fasting glucose ≥ 95 mg/dl, 1h ≥ 180 mg/dl, 2h ≥ 155 mg/dl and 3h ≥ 140 mg/dl. Blood samples were taken throughout the entire pregnancy, with the collection period in the first trimester between 8 to 13 gestational weeks. Women with GDM had a higher percentage of diabetes family history and higher pre-pregnancy BMI levels. Prolactin and progesterone of the general study population increased throughout the pregnancy. In the gestational weeks 10 to 14 prolactin levels were significantly higher in cases (median 50.4 ng/ml) then in controls (median 42.1 ng/ml). Between 15 to 26 gestational weeks the difference between both groups was smaller, but still significant. However, in the late pregnancy the difference was not significant anymore. On the other hand, progesterone levels were significantly lower in cases (median 109.4 nmol/l) then in controls (median 126.5 nmol/l) between gestational weeks 10 to 14. No significant differences were found in the rest of the pregnancy.

In the last study from this chapter, Gözükara et al. (2015) evaluated the potential of maternal testosterone and dehydroepiandrosterone sulfate (DHEA-S) as first trimester predictors of GDM. To be included in the study pregnant women had to have singleton pregnancy, be not diabetic, have no family history of diabetes, have no history of previous GDM, be of white race and be non-smokers. 408 women, who fulfilled the inclusion criteria and did come to all their visits were included in the final analysis. Total testosterone, DHEA-S, PAPP-A and the free beta-subunit of hCG were measured in the first trimester. GDM screening was a two-step approach between 24 to 28 gestational weeks. The first step included a 1h-50g OGTT, if the measured glucose level was above 140 mg/dl, the test was considered positive and a 100g OGTT was performed. The second test was considered positive, if at least two glucose levels were abnormal. The cut-off values were fasting glucose < 95 mg/dl, 1h > 180 mg/dl, 2h > 155 mg/dl and 3h > 140 mg/dl. 22 women were tested positive with the second test and therefore were diagnosed with GDM. Total testosterone levels were significantly higher in women with GDM, however, DHEA-S levels did not differ significantly between cases and controls. With a linear regression analysis age, total testosterone levels and BMI were calculated as independent predictors of GDM.

15.3 Conclusion

The hormones discussed in this chapter – anti-Müllerian hormone, estriol, prolactin, progesterone and testosterone – each show varying degrees of potential as early biomarker for the development of GDM. While, (Shand et al., 2014) observed a non-significant decrease in AMH levels in women with GDM, other studies found stronger associations. For instance, elevated estriol levels ((Hur et al., 2017) and increased total testosterone levels ((Gözükara et al., 2015) were significantly linked to GDM. Research on prolactin yielded mixed results: (M. Li et al., 2020; Overgaard et al., 2020) reported no significant differences, whereas (M. Li et al., 2020) found both elevated prolactin levels and decreased progesterone levels in women with GDM.

These findings suggest that hormonal changes in early pregnancy may reflect underlying metabolic shifts that contribute to GDM development. However, inconsistencies between studies and limited sample sizes make it difficult to draw firm conclusions. The current body of reviewed evidence indicates potential, but also highlights the need for larger, well-designed studies that standardize hormone measurement timing and account for confounding factors.

Further research should aim to clarify these hormonal patterns and evaluate their predictive value in diverse populations. If validated, hormone-based biomarkers could eventually complement existing screening tools based on conventional and new risk factors and support earlier interventions for at-risk pregnancies.

16. Placental-derived markers

16.1 Introduction

This chapter focuses on follistatin-like-3 (FSTL3) and placental growth factor (PLGF). FSTL3 also known as follistatin-related gene protein, is a homolog of follistatin. Follistatin, a single-chain soluble glycoprotein, was first discovered in ovarian follicular fluid, where it demonstrated the ability to inhibit the synthesis and release of follicle stimulating hormone (FSH) from the pituitary gland. The liver is the primary source of follistatin secretion. Both follistatin and follistatin-like-3 share similar properties, acting as antagonists of the transforming growth factor β (TGF-β) superfamily. This superfamily includes activin A and B, myostatin and bone morphogenetic proteins. Among their functions, activin A and myosin negatively affect glucose metabolism (Bielka et al., 2023). However, activin A has also been shown to promote beta-cell proliferation and insulin secretion. Notably, elevated levels of activin A have been observed in pregnant women with gestational diabetes mellitus (Thadhani et al., 2010).

High expression of FSTL3 has been detected in the placenta (Bielka et al., 2023), with additional sources including adipose tissue, the reproductive system, pancreas, liver and skeletal muscle (H. Li et al., 2024). Further evidence for FSTL3´s role in the glucose homeostasis comes from a study by (Mukherjee et al., 2007), in which FSTL3 was genetically deleted FSTL3 in mice. This deletion led to an increase in the number and size of pancreatic islets, beta-cell hyperplasia, improved glucose tolerance, enhanced insulin sensitivity, and a reduction in visceral fat mass.

PLGF is a homodimeric glycoprotein and a member of the vascular endothelial growth factor (VEGF) family (Yanachkova et al., 2023). It is highly expressed in the placenta, with smaller amounts also found in tissues, such as heart, lungs, thyroid, liver, skeletal muscle and bone (Chau et al., 2017). PLGF plays a key role in the development and maturation of the placental vascular system. Due to its predominant expression in the placenta, PLGF levesl rise during pregnancy, although they vary across gestation. In healthy, uncomplicated pregnancies, PLGF levels are initially low, begin to rise around the 11^{th} to 12^{th} gestational week, and peak around the 30^{th} gestational week, after which they gradually decline (Yanachkova et al., 2023).

PLGF functions as a proangiogenic factor, and its activity is balanced by antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1), which help maintain angiogenic

homeostasis during pregnancy. In uncomplicated pregnancies, sFlt-1 levels increase during the third trimester, while in pregnancies complicated by preeclampsia or fetal growth restriction, elevated sFlt-1 levels are observed earlier. Consequently, abnormal PLGF and sFlt-1 levels have shown promise as predictive biomarkers for pregnancy complications such as preeclampsia and fetal growth restriction (Stepan et al., 2023). Some studies have also reported associations between elevated PLGF levels and GDM, although findings remain inconsistent (Yanachkova et al., 2023)

16.2 Collected studies

16.2.1 Follistatin-like-3

In this subchapter the focus is on the biomarker follistatin-like-3. The first study (Nanda et al., 2011) was conducted in the United Kingdom. Their aim was the development of a model predicting GDM based on maternal characteristics and biochemical markers at 11 to 13 gestational weeks. They further investigated early serum levels of adiponectin, SHBG and FSTL3 in women who later developed GDM and evaluate the accuracy of the risk prediction model. The study population for the development of the risk prediction model consisted of women with a singleton pregnancy without pre-pregnancy type 1 or 2 diabetes mellitus, which delivered a phenotypically normal neonate at ≥ 30 gestational weeks. Adiponectin, SHBG and FSTL3 were measured between 11 to 13 weeks of gestation. GDM screening was two-step approach. At first, a random plasma glucose level was measured between 24 to 28 gestational weeks. If the glucose level was above 6.7 mmol/L, an 75g OGTT would follow within the next two weeks. The final GDM diagnosis was made, if the fasting plasma glucose level was at least 6.0 mmol/L or the two-hour glucose level was at least 7.8 mmol/L. Women with a normal random plasma glucose level would get an OGTT, if they had persistent glucosuria, developed a polyhydramnios or a macrosomic fetus. In the screening study for the early risk prediction model 11464 pregnant women including 297 women with GDM were included. These women had routine first trimester screening between 11 to 13 gestational weeks at the King´s College Hospital in London. For the first trimester screening fetal crown-rump length, nuchal translucency as well as PAPP-A and free beta-hCG were measured. GDM women in this screening study were older, had higher BMI levels, included more women of African and South Asian ethnicity, had a higher percentage of first-degree relatives with diabetes, developed GDM or delivered a macrosomic neonate in the previous pregnancy. A logistic regression analysis revealed that in the prediction of GDM, maternal age, BMI, ethnicity, previous history of GDM and delivery of a macrosomic neonate were

significant contributions. For the evaluation of adiponectin, SHBG and FSTL3 levels as well as the risk prediction model, 80 randomly selected women with GDM and 300 women without GDM from the screening study were investigated. The women with GDM from this study population were also older, had higher BMI levels, more women of South Asian ethnicity, had a first-degree relative with diabetes, developed GDM or delivered a macrosomic neonate in a previous pregnancy. Median adiponectin MoM and SHBG MoM were significantly lower in women with GDM. However, for this chapter "the important one" the median FSTL3 MoM did not differ significantly between cases and controls.

In the second study, Thadhani et al. (2010) investigated in a nested-case-control study if early FSTL3 concentrations differ between pregnancies complicated by GDM and uncomplicated one. The study population consisted of pregnant women from the MGH Obstetrical Maternal Study (MOMS), which were recruited from the Massachusetts General Hospital and affiliated health centers between 1998 and 2005. Women were excluded if they had GDM in a previous pregnancy, had preeclampsia (systolic RR ≥ 140 mmHG/diastolic RR ≥ 90 mmHg and proteinuria), had SGA babies (< 10^{th} birth weight percentile for gestational age), had multiple gestations and had a glucose intolerance at < 20 gestational weeks. Women with a glucose intolerance before the 20^{th} gestational week were excluded, because this could represent pre-gestational diabetes. GDM screening was two-step approach. Firstly, a 50 one-hour GCT between 24 to 28 gestational weeks was conducted. Iif the blood glucose level one hour after administration met or exceeded 140 mg/dl, a 100g three-hour OGTT after 12 hours of fasting would get taken. GDM then would get diagnosed, if at least two glucose levels were abnormal according to the American Diabetes Association guidelines. The final study population included 37 women with GDM and 127 normoglycemic women. Women with GDM delivered earlier and gained less weight between the first visit and delivery compared to the controls. The GDM group contained a lower percentage of Caucasian women. First trimester FSTL3 levels were significantly lower in women with GDM. The FSTL3 levels then were divided into tertiles. The risk of developing GDM was 11.2-times higher in the first tertile then in the third tertile. After adjusting for gestational age at blood collection, maternal age, ethnicity, BMI, blood pressure and parity the odds ratio increased even more to about 14.0. A multivariate logistic regression model calculated only FSTL3 and non-white ethnicity as significant independent predictors for developing gestational diabetes mellitus.

In the Turkish study from Karageyim Karsidag et al. (2017), possible differences in FSTL3 of pregnant women with GDM compared to normoglycemic pregnant women were evaluated. The exclusion criteria included multiple gestation, miscarriage/delivery < 30 gestational weeks, history of type 1 or 2 diabetes mellitus, GDM in previous pregnancies, signs of other concurrent medical complications and/or indication for high risk in the first trimester screening test. 170 women were enrolled in the beginning, 144 completed the study and therefore formed the final study population. First trimester screening took place between 11 to 14 gestational weeks. GDM was diagnosed, if at least one glucose level of the 75g two-hour OGTT between 24 to 28 gestational weeks was abnormal according to the American Diabetes Association guidelines. 19 women were diagnosed with GDM. Women with GDM had a significantly greater weight gain throughout the pregnancy and higher HbA1c levels in the second trimester. PAPP-A, free-beta-hCG did not differ significantly lower between cases and controls. FSTL3 levels were lower in the case group, however, the difference was not significant.

16.2.2 Placental growth factor

The second subchapter contains also three studies. The main focus of these studies is the placental growth factor. Mosimann et al. (2016) evaluated first trimester placental growth factor (PLGF) and its association with GDM as well as HbA1c. The study was conducted in Bern, Switzerland. PLGF and HbA1c were measured between 8 to 14 gestational weeks. Due to limited existing data on the PLGF before the 11^{th} week of gestation, most women with measurements before that, had a second measurement between 11 to 14 gestational weeks. GDM screening took place between 24 to 28 gestational weeks by a 75g two-hour OGTT. GDM then was diagnosed if one of the following glucose levels was met or exceeded: fasting ≥ 5.1 mmol/L, one-hour ≥ 10.0 mmol/L, two-hour ≥ 8.5 mmol/L. In total 328 pregnant women including 51 women with GDM formed the study population. GDM women were significantly older and had higher BMI values. PLGF levels did not differ between women with GDM and normoglycemic women. On the contrast, HbA1c was significantly higher in women with GDM. They found no significant correlation between PLGF and HbA1c or PLGF MoM and HbA1c.

The second study of this subchapter (Eleftheriades et al., 2014) examined early PLGF levels in women with GDM and created a first trimester risk prediction model. For the GDM diagnosis a 75g two-hour OGTT between 24 to 28 gestational weeks carried out. GDM was diagnosed if at least one glucose level met or exceeded the following cut-off levels: fasting

≥ 92 mg/dl, one hour ≥ 180 mg/dl, two-hour ≥ 153 mg/dl. The study population consisted of 134 pregnant women, including 40 women with GDM, recruited at their combined screening in a private hospital in Athens, Greece. At the combined screening between 11 to 14 gestational weeks maternal history and characteristics, ultrasonic measurements (fetal crown-rump length (CRL), fetal heart rate (FHR), nuchal translucency (NT) thickness, fetal nasal bone, blood flow in the fetal ductus venosus and blood in the fetal tricuspid valve) and blood samples (free beta-hCG, PAPP-A, PLGF) were taken. Women with GDM were significantly older, gained more weight throughout pregnancy and had increased Log PLGF and PLGF levels. A multiple regression analysis showed a positive association between log10 PLGF and fasting glucose levels. In each of the two groups (GDM and control group), log PLGF and log10 MoM PAPP-A were correlated. Further, a logistic regression analysis showed that maternal age, weight and log10 PLGF were significant predictors for GDM.

Chaparro et al. (2018) evaluated placental proteins in oral fluids (gingival crevicular fluid (GCF) and saliva) and the periodontal disease status as first trimester predictors for GDM. The study was performed in Santiago, Chile. 212 healthy pregnant women between 11 to 14 gestational weeks have gotten recruited for the study. All women were between 18 and 40 years old. GDM was diagnosed if at least one glucose level of the 75g two-hour OGTT between 24 to 28 gestational weeks was abnormal. In total 14 women developed GDM. PLGF and soluble vascular endothelia growth factor receptor-1 (s-VEGFR-1)/sFlt-1 in GCF samples were determined and compared in these 14 women and 28 random control samples from the screening population. Periodontal features were compared between the GDM group and the 28 controls, as well as the 198 women without GDM from the screening population. Women in the GDM group were older, smaller, had higher glucose concentrations, and the following periodontal features: more bleeding on probing, higher probing death, higher mean attachment loss and more mean periodontal inflamed surface area. PLGF levels in GCF were significantly higher in women with GDM, however, PLGF levels in saliva did not differ between GDM cases and controls. sFlt-1 concentrations did neither differ in GCF nor in saliva. In a multiparametric model initial glycemia, PLGF levels in GCF and BMI between 11 to 14 weeks of gestation were combined and adjusted by age and periodontal disease severity. This model showed an association between glycemia and PLGF with an increased risk of developing GDM. The area under the ROC for their model combining glycemia and PLGF was 0.898. A PLGF level in GCF of 5.27 pg/nl got a 75% specificity, a 76.8%

sensitivity and 26.7% false positive rate for predicting gestational diabetes mellitus. The predictive potential of the model was not affected by the BMI.

16.3 Conclusion

Among the three studies that investigated FSTL3 levels in relation to gestational diabetes mellitus (Karageyim Karsidag et al., 2017; Nanda et al., 2011; Thadhani et al., 2010), only Thadhani et al. (2010) reported significantly lower FSTL3 concentrations in women with GDM. Consequently, this review does not support a strong recommendation for the use of FSTL3 as an early predictor of GDM. However, the potential role of FSTL3 as a biomarker cannot be entirely ruled out and warrants further investigations through larger studies, possibly employing novel diagnostic approaches. The primary limiting factors include the small number of studies, relatively small study populations and varying GDM screening methods.

The findings from the three studies assessing PLGF levels (Chaparro et al., 2018; Eleftheriades et al., 2014; Mosimann et al., 2016) are also mixed. These studies utilized different biological samples: the first two detected PLGF in blood samples, while the third assessed PLGF levels in gingival crevicular fluid and saliva. Mosimann et al. (2016) found no significant difference in PLGF levels between women with GDM and normoglycemic controls. In contrast, Eleftheriades et al. (2014) reported significantly elevated PLGF level in women with GDM. Chaparro et al. (2018), the only study analysing oral fluids, also detected increased PLGF concentrations in gingival crevicular fluid among women with GDM. Based on these findings, PLGF appears to have more promising potential as an early biomarker for GDM. Nevertheless, similar to the FSTL3 findings, conclusions are limited by the small number of studies, small sample sizes, and the use of different types of biological fluids. Therefore, additional large-scale studies are needed to validate these results.

17. Hepatokine (fetuin-A, afamin, angiopoietin-like-protein)

17.1 Introduction

Proteins secreted by the liver are referred to as hepatokines (Lanthier et al., 2022). This term was introduced in 2008, and since then, over 20 hepatokines and their metabolic effects have been discovered and studied in vitro and in animal models. One of the most extensively researched hepatokines in humans is fetuin-A (Stefan et al., 2023). Fetuin-A is a glycoprotein primarily secreted by the liver (Ochieng et al., 2018). It plays several roles in glucose metabolism. First, it inhibits receptor tyrosine kinase in the liver and skeletal muscle. Second, in combination with fatty acids it activates Toll-like receptor 4, which initiates inflammatory signaling and promotes insulin resistance in adipocytes and macrophages. Additionally, by interfering with glucose-induced insulin secretion, fetuin-A may disrupt the maturation of pancreatic beta-cells. Given these roles, it is unsurprising that numerous studies have reported associations between elevated fetuin-A levels, insulin resistance and an increased risk of developing type 2 diabetes mellitus (Stefan et al., 2023). During healthy pregnancies, fetuin-A levels increase in the second trimester (Chaemsaithong et al., 2015).

Afamin is another glycoprotein mainly secreted by the liver, from where it enters the bloodstream (Yuan et al., 2023). Smaller amounts have also been detected in follicular and cerebrospinal fluids (Pitkänen et al., 2022). Afamin, which belongs to the albumin gene family, has the ability to bind Vitamin E (Köninger, Enekwe, et al., 2018). Vitamin E is a known antioxidant, and previous studies have suggested that it may also have ant-diabetic properties (Miyazawa et al., 2019). Significant correlations between afamin and Vitamin E levels have been observed in extravascular fluids (Köninger, Enekwe, et al., 2018). In plasma, elevated afamin concentrations are significantly associated with oxidative stress, insulin resistance and metabolic syndrome (Yuan et al., 2023). Moreover, recent findings have linked higher afamin levels to type 2 diabetes mellitus (Köninger, Enekwe, et al., 2018). Due to these associations, afamin has become a focus of research in the context of gestational diabetes mellitus and preeclampsia.

Angiopoietin-like proteins (ANGPTLs) are glycoproteins secreted by various tissues, including the liver, vascular system and hematopoietic system. They share structural similarities with angiopoietins (Seyhanli et al., 2022), which are involved in angiogenesis and the maintenance of hematopoietic stem cells. There are eight known members of the angiopoietin-like protein family, designated ANGPTL1 through ANGPTL8. The first seven share characteristic N-terminal coiled coil domain and a C-terminal fibrinogen-like domain

(Horiguchi et al., 2025). ANGPTL2 is primarily secreted by adipose tissue (Y. Sasaki et al., 2015), though it is also produced in the heart, kidney, lung and skeletal muscle (Seyhanli et al., 2022). Studies in both mice and humans have shown that elevated ANGPTL2 levels are associated with inflammation, insulin resistance and adiposity (Tabata et al., 2009). Furthermore, ANGPTL2 has been identified as an independent risk factor for the development of type 2 diabetes in the general Japanese population (Doi et al., 2013).

Angiopoietin-like Protein 8 (ANGPTL8), also known by several other names - including betatrophin, TD26, RIFL, lipasin, C19orf80 (Tseng et al., 2014) – is mainly secreted by the liver and adipose tissue. It plays an important role in lipid metabolism as well as in the pathogenesis of insulin resistance and type 2 diabetes mellitus (Guo et al., 2022). Elevated ANGPTL8 levels have been observed in obese children and adolescents with insulin resistance (Wu et al., 2016), as well as in pregnant women with GDM (Gülcü Bulmuş et al., 2020; Seyhanli et al., 2022).

17.2 Collected Studies

This subchapter is divided into three sections based on the hepatokine markers: fetuin-A, afamin and angioproietin-like proteins 2 and 8.

17.2.1 Fetuin-A

The first section contains two studies, a prospective cohort study from Ankara, Turkey (Kansu-Celik et al., 2019) and a nested case-control study from Peking, China (Jin et al., 2020). Kansu-Celik et al. (2019) investigated fetuin-A, N-terminal proatrial natriuretic peptide (pro-ANP), hs-CRP and fasting glucose levels at 11 to 14 gestational weeks as potential early predictors for GDM. Pregnant women were excluded if they were under 18 years/over 40 years old, had a diagnosis of chronic disease before the pregnancy (diabetes, thyroid dysfunction, hypertension, uncontrolled endocrine illness, abnormal renal function), FPG levels above 126 mg/dl or a two-hour postprandial glucose level/GCT value above 200 mg/dl at 24 to 28 gestational weeks, a history of a positive GTT in the first trimester and having had one in a previous pregnancy. GDM screening was a two-step approach. The first step was a 50g GCT between 24 to 28 weeks of gestation. The GCT was positive, if the glucose level after one-hour met or exceeded 140 mg/dl. A positive GCT then was followed up with 100g OGTT after three days of normal diet. Finally, GDM was diagnosed if at least two abnormal glucose levels according to the Carpenter and Coustan criteria were detected. The cut-off points were fasting: 95 mg/dl, one-hour: 180 mg/dl, two-hour: 155 mg/dl and

three-hour: 140 mg/dl. Women with a negative GCT were labelled healthy and therefore in the control group. From 327 pregnant women, who completed the antenatal follow-up, 29 developed GDM and to get a 1:2 ratio of case to control 59 age- and BMI-matched healthy controls were chosen. Due to the matching there were no significant demographic, obstetric or neonatal outcome differences between cases and controls. Women with GDM had significantly lower fetuin-A levels and significantly higher hs-CRP and FPG levels in comparison with the normoglycemic women. Spearman rank correlation analysis showed an inversive correlation between fetuin-A and hs-CRP and a positive correlation between hs-CRP and FPG, but no correlation between fetuin-A and FPG levels. Fetuin-A under 166 ng/ml had the highest specificity with a diagnostic accuracy of 70.45%. hs CRP levels above 4.65 ng/ml had the highest sensitivity with a diagnostic accuracy of 88.64%.

Jin et al. (2020) focused solely on fetuin-A and its effects on insulin resistance and GDM. The study was based on the Peking University Birth Cohort in Tongzhou (PKUBC-T). Pregnant women were included if they were between 18 and 45 years old, had a gestational age under 14 weeks, were living in Tongzhou during the past half year and had no plan of moving after the delivery, and planned to have antenatal care and delivery in Tongzhou Maternal and Child Health Hospital. Exclusion criteria included pre-pregnancy diabetes as well as cardiovascular, liver, kidney and autoimmune diseases. GDM screening was between 24 to 28 gestational weeks with a 75g OGTT. GDM was diagnosed if one of the following glucose levels was met or exceeded: fasting: 5.1 mmol/L, one-hour: 10.0 mmol/L or two-hour: 8.5 mmol/L. The Homeostasis model assessment (HOMA) was used to calculate the insulin resistance (HOMA-IR) and beta-cell function (HOMA-β). Blood samples were drawn between 7 to 13 gestational weeks after an overnight fast and a second time between 25 to 28 gestational weeks at the time of the OGTT. 5477 women were included, of whom 3304 finished an OGTT, including 593 women who were diagnosed with GDM. After exclusion of women with a history of GDM, family history of diabetes, polycystic ovary syndrome, thyroid diseases, and who were smoking and alcohol drinking, 135 women with GDM were randomly selected. 135 healthy women were matched and selected, based on age (± 2 years) and gestational age at OGTT, as control group. Women with GDM had significantly higher plasma fetuin-A, fasting insulin and HOMA-IR levels in the first and second trimester compared to the normoglycemic controls. HOMA-β showed a decrease between the first two trimesters in the GDM group, but an increase in the control group. The study cohort then was divided into quartiles according to the fetuin-A concentrations among

controls in the first trimester, the second trimester and the change in fetuin-A concentration between the two trimesters. Women in the highest quartile were more likely to develop GDM compared with the lowest quartile. The calculated optimal cut-off level for the first trimester fetuin-A concentration based on the ROC results was 305.9 pg/mL with a sensitivity of 0.644, a specificity of 0.585 and an accuracy of 0.612.

17.2.2 Afamin

The second section contains three studies, a retrospective study from Fuzhou, China (X. Wang et al., 2021), a pilot study from Essen, Germany (Köninger, Mathan, et al., 2018), and a nested case-control study from Innsbruck, Austria (Tramontana et al., 2018). X. Wang et al. (2021) wanted to establish an early risk prediction model for GDM using clinical characteristics, early pregnancy peripheral blood biochemical indicators. Followed by an evaluation of the predictive accuracy. Pregnant women were included in the study, if they had a singleton pregnancy, a routine blood test, including afamin, a prenatal aneuploidy screening, containing measuring the nuchal translucency thickness, free beta-hCG and PAPP-A, biochemical test between 11 to 12 gestational weeks, an OGTT between 24 to 28 gestational weeks and no history of chronic diseases and no birth defect, miscarriage or incomplete/unavailable data. GDM screening was taken care of in form of an OGTT between 24 to 28 gestational weeks according to the American Diabetes Association (ADA) criteria from 2012. 607 women with GDM and 833 normoglycemic were included in the study. The controls were matched to the cases based on their blood sampling time. In the case group the following blood levels were significantly higher compared to the control group: white blood cells, neutrophile, lymphocyte, haemoglobin, neutrophile/lymphocyte ratio, triglyceride, total cholesterol, LDL and afamin levels. In contrast, the platelet/lymphocyte ratio and the PAPP-A concentrations were significantly lower in women with GDM. With a multivariate logistic regression analysis maternal age, early pregnancy afamin, triglyceride and platelet/lymphocyte ratio were detected as independent risk factors for GDM. The combination of the independent risk factors formed the risk prediction model and its area under the curve (AUC) from the ROC was 0.748.

Köninger, Mathan, et al. (2018) included for their first-trimester cohort the frozen samples of 110 women with singleton pregnancies. All samples were from pregnant women, who had their delivery in the University Hospital of Essen between 2003 and 2014. 59 of them were diagnosed with GDM and the remaining 51 women formed the control group. Eight women of the GDM group had polycystic ovary syndrome. GDM was diagnosed if at least

one glucose level of a 75g 2h-OGTT between 24 to 28 gestational weeks was elevated. Before 2011 the glucose cut-off levels were as followed: fasting: 95 mg/dl, one-hour: 180 mg/dl and two-hour: 155 mg/dl. After 2011 and until the end of the study the defined cut-off levels were: fasting: 92 mg/dl, one-hour: 180 mg/dl and two-hour: 153 mg/dl. 36 of the women with GDM were diagnosed before 2011 and 23 after 2011. The definitions for getting an 75g OGTT changed also during the duration of the study. Before 2013 only women with an elevated risk for GDM (obesity, family history of diabetes/macrosomia in a previous pregnancy or a pathological 50g OGTT) got an 75g OGTT. Since 2013 and until the end of the study, all women got an 50g OGTT. Followed by 75g OGTT if the glucose levels met or exceeded 135 mg/dl in the 50g OGTT or the presence of risk factors. Therefore, not all women from the control group had an 75g OGTT. In this study a mid-trimester cohort consisting of 105 women with singleton pregnancies were also studied, however, due to blood sampling after 16 gestational weeks, these results are not important for the review and get therefore not discussed. Women with GDM of the first-trimester cohort had significantly elevated afamin levels compared with the normoglycemic women.

The Austrian research group (Tramontana et al., 2018) evaluated the possible correlation between first trimester afamin levels and three-dimensional placental bed vascularisation in pregnant women and its potential for predicting adverse pregnancy complications. The investigated adverse pregnancy complications included gestational hypertension, preeclampsia, intrauterine growth restriction (birth weight below the third percentile), pre-term birth (before the completion of 34 gestational weeks) and GDM. 382 women with pregnancy complications and 382 pregnant women without complications as controls were included in the study cohort. The two groups were matched based on their BMI. Additionally, 220 healthy women with low-risk pregnancies were included. The 382 cases included 76 with gestational hypertension, 33 with preeclampsia, 91 with intrauterine growth restriction, 39 with pre-term birth and 170 with GDM. GDM was diagnosed with 75g OGTT between 24 to 28 gestational weeks, if at least one glucose level met or exceeded the following cut-off levels: fasting: 5.1 mmol/L, one-hour: 10.0 mmol/L and two-hour: 8.5 mmol/L. Blood samples for detecting afamin levels were drawn between 11 to 14 gestational weeks. Women, who developed preeclampsia or GDM had significantly increased serum afamin levels compared to the women without complications. The myometrial vascular index differed not significantly between women with GDM and women without

complications. In a prognostic model for development of GDM in low-risk pregnancies, first trimester afamin and BMI levels were shown to have a significant prognostic value.

17.2.3 Angiopoietin-like protein 2 and 8

The third section contains two studies, a nested case-control study from Beijing, China (Yan Zhang et al., 2016) and a prospective study from Jiangsu, China (Huang et al., 2018). Yan Zhang et al. (2016) investigated the potential of angiopoietin-like protein 2 as early biomarker for GDM. Women were excluded from the study, if they were under 18 years/over 40 years old, had a multiple pregnancy, pregestational diabetes (type 1 or 2), drug and/or alcohol abuse, an uncontrolled endocrine disease, renal failure or other conditions, which would influence the glucose regulation. Blood samples for afamin level were drawn within the combined test at a maximum of 13 gestational weeks. GDM screening was a two-step approach. Firstly, a random blood glucose level was taken ≤ 13 gestational weeks. Secondly, a 50g GCT between 24 to 28 gestational weeks was taken. Women whose glucose level met or exceeded 7.8 mmol/L had then a 75g OGTT within one to two weeks of the GCT. GDM was diagnosed, if at least one glucose level met or exceeded the following cut-off levels: fasting 5.3 mmol/L, one-hour: 10.0 mmol/L and two-hour: 8.6 mmol/L. From the 1116 women included in the study, 89 women developed GDM. 178 healthy pregnancies were then randomly selected as controls. One woman was later excluded, due to a history of chronic inflammatory disease, leaving a final control of 177 women. Women with GDM had higher pre-pregnancy BMI, higher systolic blood pressure levels, higher fasting blood glucose levels and were more likely to have a history of GDM and a family history of type 2 diabetes mellitus. First trimester ANGPTL2 concentrations were also significantly higher in women with GDM compared to normoglycemic women. Furthermore, it was calculated that women with elevated early plasma ANGPTL2 levels were 2,90-times more likely to develop GDM.

Huang et al. (2018) evaluated if angiopoietin-like protein 8 on its own or in combination with other early risk factors could be an early predictor for GDM. Due to a preliminary study, a minimum sample size of 405 pregnant women was calculated. Exclusion criteria included multiple pregnancies, pre-pregnancy diabetes, pregestational hypertension and a stillbirth. Women with FPG levels ≥ 7.0 mmol/L were considered to have a pre-existing diabetes and were therefore excluded from the study. GDM screening was taken care of between 24 to 28 gestational weeks with a 75g OGTT. GDM was diagnosed if, according to the IADPSG criteria, at least one of the following glucose levels was met or exceeded: fasting: 5.1

mmol/L, one-hour: 10.0 mmol and two-hour: 8.5 mmol/L. The final study cohort consisted of 474 pregnant women, including 88 women who developed GDM. GDM women were significantly older, more likely to be hepatitis B surface antigen positive, had higher BMI, GGT and FPG levels in the early pregnancy compared to women without GDM. First trimester ANGPTL8 concentrations were also significantly higher in women with GDM. An univariable logistic regression analysis showed that maternal age, BMI, FPG, GGT, HBsAG positivity and ANGPTL8 were associated to the risk of developing GDM. After including these variables in a model, maternal age, FPG and ANGPTL8 were independently associated with the risk of GDM. The area under the curve (AUC) for ANGPTL8 was 0.706 and significantly greater than those for BMI, HBsAG positivity and GGT. The calculated optimal cut-off level of ANGPTL8 was 2792.33 pg/ml with a sensitivity of 54.55%, a specificity of 79.27%, a positive predictive value of 37.5% and a negative predictive value of 88.4%. Through including ANGPTL8 in a risk prediction model of conventional risk factors (maternal age, parity, family history of diabetes, history of macrosomia, BMI, GGT, HBsAG and FPG) the area under the curve increased significantly.

17.3 Conclusion

The results of the two studies in the first section (Jin et al., 2020; Kansu-Celik et al., 2019), which focused on fetuin-A levels, point in opposite directions. Kansu-Celik et al. (2019) reported significantly decreased fetuin-A levels in women with GDM, whereas Jin et al. (2020) found significantly increased levels in the same group. This discrepancy highlights the need for further studies with larger samples sizes to clarify these finding.

In the second section, all three studies (Köninger, Mathan, et al., 2018; Tramontana et al., 2018; X. Wang et al., 2021) consistently reported significantly increased afamin levels in women with GDM compared to normoglycemic controls. This uniformity is promising and suggests that afamin may serve as a potential early biomarker for gestational diabetes mellitus. However, additional studies are required to confirm and strengthen this potential.

The third section includes two studies, each focusing on a different angiopoietin-like protein. Yan Zhang et al. (2016) found significantly elevated ANGPTL2 levels in women with GDM, while Huang et al. (2018) observed significantly increased concentrations of ANGPTL8 in the same group. Given the limited number of studies for each angiopoietin-like protein, further research is necessary to substantiate the suggestion that both ANGPTL2 and ANGPTL8 may serve as early biomarkers for GDM.

In summary, all biomarkers discussed in this chapter demonstrate potential, with afamin currently supported by the strongest body of evidence. A key limitation, however, remains the relatively small number of available studies.

18. Urine biomarkers

18.1 Introduction

This chapter focuses on the potential of different biomarkers measured in a urine sample, offering insight into a non-invasive screening method. The first study investigated levels of myo-inositol and D-chiro-inositol in urine, while the second one examined the urinary metabolic profile.

Myo-inositol and D-chiro-inositol are two of nine stereoisomers of inositol. Inositol, a carbocyclic sugar polyalcohol (Kiani et al., 2021), is present in both plants and animal tissue, and is also produced in the human body (Egarter, 2019). As a second messenger, inositol plays a crucial role in various physiological processes (Egarter, 2019). Its deficiency has been linked to several disorders such as fetal neural tube defects, metabolic disorders and polycystic ovary syndrome. The different stereoisomers are formed through epimerization of hydroxyl groups. Both myo-inositol and D-chiro-inositol have been identified as components of inositol phosphglycan second messengers, which participate in an insulin signaling pathway (Murphy et al., 2016). Myo-inositol can be converted into D-chiro inositol through epimerization triggered by increased insulin release during metabolic stress (Kiani et al., 2021). Previous studies have reported elevated urinary levels of these two isomers in individuals with type 2 diabetes mellitus and PCOS - both disorders involving insulin resistance (Baillargeon et al., 2006; Hong et al., 2012).

As discussed in chapter 15, metabolomics it the study of all metabolites, including their functions and interactions (Rinschen et al., 2019). Unlike the studies covered in that previous chapter, the research discussed here specifically investigated the metabolic profile of urine samples (Piras et al., 2022). Urine collection offers several advantages: it is non-invasive, patient-friendly and allows for easy collection of large volumes. Additionally, urine contains high concentrations of metabolites that can vary under different metabolic conditions (Khamis et al., 2017; Saude et al., 2007).

18.2 Collected studies

This chapter discusses two nested case-control studies – on conducted in Los Angeles, USA (Murphy et al., 2016) and the other one in Modena, Italy (Piras et al., 2022). The first study (Murphy et al., 2016) compared the urinary levels of myo-inositol and D-chiro-inositol urinary in women with and without GDM. Urine samples were collected at three times, twice during the pregnancy (between 6 -14 and 22-32 gestational weeks) and once postpartum.

Exclusion criteria included preexisting diabetes/abnormal glucose tolerance, renal/hypertensive disease, PCOS, multiple pregnancy, spontaneous/elective abortions, smoking and the use of systemic steroids, beta-mimetics or oral hypoglycemic agents. GDM screening followed a two-step approach. All women underwent a one-hour 50g GCT between 24 to 28 gestational weeks. Those with glucose levels that met or exceed 140 mg/dl, proceeded to a three-hour 100g OGTT. GDM was diagnosed based on the Carpenter and Coustan criteria, if at least two glucose thresholds were exceeded. A GCT result above 200 mg/dl was also considered diagnostic. Of the 375 women enrolled, 35 developed GDM. A control group of 59 normoglycemic women was matched by age, gestational age, ethnicity and prepregnancy BMI. The early myo-inositol/creatine and D-chiro-inositol/creatine ratios were significantly higher in the GDM group compared to the control cases. These differences nearly disappeared in the late pregnancy and postpartum. L-chiro-inositol-creatine and myo-inositol-D-chiro-inositol ratios were similar in both groups.

Piras et al. (2022) analysed urinary metabolomic profiles in obese women to evaluate their potential for predicting GDM in the first trimester. Inclusion criteria required women to be at least 18 years old, have a BMI level of at least 30 kg/m^2 and carry a singleton pregnancy. Exclusion criteria included a multiple pregnancy, intake of dietary supplements/herbal products affecting the body weight, contraindications for exercise and a birthing wish outside of the birth Center. Women were enrolled before or within the 12^{th} gestational week. The first screening for GDM was done with a two-hour 75g OGTT between the 16^{th} and 18^{th} gestational week. A negative first test was followed by a second one between 24 to 28 weeks of gestation. The OGTT was labelled positive and subsequently GDM was diagnosed, if at least one glucose level exceeded the glucose thresholds according to the ADA. Women included in the study got a caloric restriction and exercise program for the time of pregnancy. 29 women developed GDM, and the remaining 25 women of the study population were taken as controls. The comparison of these two groups, revealed increased levels of tryptophan, trigonelline, hippurate and threonine, and reduced levels of 1-methylnicotinamide, 3-hydroxykynurenine, glycocholate, isoleucine, kynurenine, and valine in the women affected by gestational diabetes. All differences except glycocholate (p value of 0.05) had a p value below 0.05. Altered metabolic pathways related to these metabolites were also identified. Logisitic regression analysis yielded an AUC of 0.796 for predicting GDM based on the nine significant metabolites.

18.3 Conclusion

In summary, both studies found significant differences in urinary biomarkers between women with and without GDM. Murphy et al. (2016) demonstrated that urinary myo-inositol/creatine and D-chiro-inositol/creatine ratios were elevated between 6 and 14 gestational weeks in women with gestational diabetes. These results suggest increased inositol excretion in affected individuals. Piras et al. (2022) focused on the metabolomic profiling in obese women and identified several metabolites with altered levels those diagnosed with GDM. Although these were the only two studies in this thesis focusing specifically on urinary biomarkers, and both had relatively small sample sizes, the findings highlight the potential of urinary biomarkers for early GDM detection. Further research with larger study populations, more diverse populations - including a broader range of BMI categories and ethnic background – is necessary to validate these results.

19. Conclusion

In the last chapters, a total of 182 studies concerning 16 different biomarker groups were summarized and analysed. The first chapter highlighted that **elevated triglyceride, total cholesterol and LDL-C levels along with decreased HDL-C levels** are associated with GDM in Asian women. In contrast, the findings for Caucasian women were less clear, due to mixed results. The studies concerning fatty acids did also show mixed results, as two out of three studies indicated significantly **higher fatty acids levels** in women affected by GDM. One study focused on sphingolipids and found an association between **higher levels of the sphingolipid C18:1Cer** and GDM. In the second chapter, most of the studies did not find significant differences in the inflammatory marker hs-CRP between pregnancies affected by GDM and those that were unaffected. Although, TNF-α was significantly increased in one study, it was not considered a useful predictor for GDM, an opinion suppored by another study**.** Conversely, **interleucin-6** was significantly elevated in GDM pregnancies, however, it was the only study in this review to investigate that marker.

Among maternal ultrasound measurements, **increased adipose tissue thickness and neck circumference** showed promise as early predictors for GDM. However, three out of seven studies found that pre-pregnancy BMI had a higher predictive value than these two measurements. Nuchal translucency thickness and uterine artery pulsatile were not significantly different between uncomplicated pregnancies and those complicated by GDM. An **elevated fetal heart rate, decreased fetal growth and lower placenta vascularization indices** in the first trimester were significantly associated with GDM. Early measurements of **HbA1c and fasting plasma glucose** proved to be a helpful screening tools for pregnant women at risk for GDM. However, HbA1c measurements lost significance in women affected by PCOS. The results for glycosylated fibronectin and early OGTT or GCT were mixed. If OGTT and GCT are to be used as early screening tools, new cut-off levels are necessary to minimise false positive and negatives.

57% of studies examining SHBG found **elevated levels** in women affected by GDM. **HOMA-IR** showed a strong positive connection with GDM, while the Quantose-IR and the TyG index did not perform well as early screening tools. In the group of adipocyte-derived markers, most studies focused on **adiponectin, which was found to be decreased** in women with GDM. Other markers, such as **sOBr and irisin (decreased) as well as leptin, chemerin, FABP4 and sFRP4 (increased)** were also associated with GDM. Visfatin yielded mixed results, while omentin-1, resistin and CMPF showed no significant

differences between women with and without GDM. The relationship between Vitamin D levels and GDM development was confirmed by only half of the studies that evaluated it. **Elevated osteocalcin and the presence of the Vitamin D gene variants: rs1544410 and rs731236** were associated with a higher risk of developing GDM. A slim majority of studies reported **decreased PAPP-A levels** in women with GDM. However, in two studies the addition of PAPP-A did not improve the predictive ability of a risk prediction tool based on known risk factors. Free β-hCG levels were mostly not significantly different, but three studies identified a protective effect of elevated free β-hCG levels. **Decreased PROK1 levels and increased PLGF and PAPP-A2 levels** were associated with GDM, while inhibin-A showed no significant differences, and sHLA-G yielded mixed results. **Elevated ferritin levels appear** to be promising early predictors of GDM, while other iron parameters, such as the soluble transferrin receptor, total iron and total iron binding capacity were less successful.

Among thyroid markers, **the presence of thyroid antibodies** showed the highest potential as early predictors. Decreased fT4 levels were found in half of the studies, while fT3 levels were elevated in 40% of the studies. TSH levels did not show significant differences between pregnant women with and without GDM. The inclusion of **microbial features** in a risk prediction tool enhanced its predictive value. However, the studies could not agree on which specific feature to use. Two out of three studies found that the presence of *Caprococcus* was protective against GDM. Similar findings emerged from the **metabolomics** studies. Elevated levels of the transcriptomics: **miR-223 and miR-23a** were associated with GDM. Promising results were also observed in a **cell-free DNA panel as well as in the combination of a high genetic risk score and decreased TCDCA levels**.

In the hormonal marker group, **increased levels of estrogen and total testosterone,** along with **decreased levels of progesterone**, were associated with GDM. Prolactin yielded mixed results, and anti-Mullerian hormone showed no significant differences. Decreased FSTL3 values were associated with GDM in only one out of three studies. Elevated PLGF serum levels were in half of the studies and interestingly, **elevated PLGF levels in the gingival crevicular fluid** were also associated with GDM. Hepatokines, such as **afamin, ANGPTL2 and ANGPTL8** were positively associated with the development of GDM, while fetuin-A showed mixed results.

Regarding urinary biomarkers, two studies were discussed in this thesis, both of which identified significant differences between women diagnosed with GDM and normoglycemic women. The first study demonstrated **increased inositol excretion** in women affected by GDM. The second study found **several altered metabolites levels**, including elevated concentrations of tryptophan and threonine, and reduced levels of 1-methylnicotinamide and valine

In summary, the findings of this review show that early prediction of GDM is complex and likely requires a combination of different markers. While no single biomarker has emerged as a reliable predictor on its own, several candidates - particularly early HbA1c, adipokine, afamin and ferritin - demonstrated promise. The use of non-invasive sampling methods and novel techniques such as metabolomics or transcriptomics may further improve early screening strategies. To confirm these results, further research should include larger, ethnically diverse populations and follow standardized diagnostic criteria. The goal is to develop a practical and reliable screening tool that allows for early identification and better management of GDM.

20. Discussion

In conclusion, several biomarkers reviewed in this work show promising potential for identifying gestational diabetes as early as the first trimester. Based on the evidence presented, the most effective approach to identifying women risk for GDM may be a combination of multiple biomarkers and ultrasound measurements. However, to determine an optimal panel and establish appropriate cut-off values, larger studies incorporating diverse biomarker profiles are necessary.

Given that the predictive value of certain biomarkers appears to vary by ethnicity and BMI, future research should include diverse study populations that cover a broad range of ethnic backgrounds and BMI categories. This approach may help identify populations-specific differences in biomarker performance and improve the generalizability of findings.

This review also revealed several limitations. One major limitation was the variation in the definition and diagnosis of gestational diabetes mellitus (GDM) across the analysed studies. Due to differing international guidelines and the varying timeframes in which the studies were conducted, inconsistencies were observed in both screening methods and glucose thresholds. As outlined in this work, screening approaches ranged from one- to two-step methods, typically conducted between 24 to 28 weeks of gestation. In some cases, a two-step process was used that included an initial screening in the first trimester, followed by a second test the second trimester if needed. The specific tests varied as well, including the 50g Glucose Challenge Test (GCT), the two-hour 75g Oral Glucose Tolerance Test (OGTT), and the three-hour 100g OGTT. Depending on the test and the guidelines followed, both the diagnostic criteria and glucose cut-off levels differed significantly.

A second limiting factor was the relatively small number of studies available for certain biomarkers. In some cases, this limited the strength of the conclusions that could be drawn. Similarly small samples sizes in several studies further weakened the statistical power of their findings. To address both the second and third limitations, further research should focus on conducting larger, well-powered studies to validate and expand upon the findings presented in this review.

Despite these limitations, this review has several notable strengths. The inclusion of a large number of studies enabled a broad evaluation of various biomarkers and screening techniques. As a result, a pool of promising early risk predictors for GDM emerged, contributing to the foundation for future models of early risk assessment. Additionally, the

broad time span across which the studies were conducted, allowed for a comprehensive overview of developments and trends in GDM research over time.

Moreover, this review incorporated five different sampling methods - blood, oral fluid, urine, stool and ultrasound imaging – enhancing its scope. The inclusion of non-invasive sampling techniques further broadens the potential for feasible and patient-friendly screening strategies.

In summary, while this review did not identify a definitive biomarker or panel for early GDM prediction, it provides a valuable foundation for future research in this area. Thes findings underscore the importance of large-scale, diverse and methodologically consistent studies to advance the field and improve early detection of gestational diabetes.

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