Diploma Thesis

Distinct placental hormone secretion and GDM risk in pregnancies with male vs female fetuses

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 23/04/2023 *Sarah Schwarz m.p.*

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List of Abbreviations

Acrp30	adipocyte complement-related protein of 30 kDa
AFABP	adipocyte fatty acid-binding protein
AMPK	AMP-activated protein kinase
AP2	adipocyte protein 2
APN	adiponectin
AUC	area under the curve
BMI	body mass index
COX-2	cyclooxygenase 2
CVD	cardiovascular disease
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulfate
E1	estrone
E2	17β-estradiol
E3	estriol
ERα	estrogen receptor-α
ERβ	estrogen receptor-β
FABP4	fatty acid binding protein 4
FPG	fasting plasma glucose
FSH	follicle-stimulating hormone
GBP28	gelatin binding protein of 28 kDa
GDM	gestational diabetes mellitus
GLUT4	glucose transporter 4
GnRH	gonadotropin-releasing hormone
GSIS	glucose-stimulated insulin secretion
HBGM	home blood glucose measurement
hCG	human chorionic gonadotropin
hCS	human chorionic somatomammotropin
hGH	human growth hormone
hPL	human placental lactogen
HOMA	Homeostasis Model Assessment
IADPSG	International Association of the Diabetes and Pregnancy Study Groups
IDF	International Diabetes Federation

IGF1	insulin-like growth factor 1
IKK	IκB kinase
IL	interleukin
IOM	Institute of Medicine
IRS-1	insulin receptor substrate 1
JAK 2	janus kinase 2
LGA	large-for-gestational-age
LH	luteinizing hormone
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MiG	Metformin in gestational diabetes
MCP1	monocyte chemotactic protein 1
mRNA	messenger ribonucleic acid
NAMPT	nicotinamide phosphorybosyltransferase
NF-κB	κ-light-chain-enhancer of activated B cells
ObRs	leptin receptors
OGTT	oral glucose tolerance test
PBEF	pre-B cell colony enhancing factor
PPARγ	peroxisome proliferator- activated receptor gamma
pGH	placental growth hormone
Pgr	progesterone receptor
PI3K	phosphadityl-inositol 3 kinase
PKC	protein kinase C
PPARα	peroxisome proliferator-activated receptor-α
PRL	prolactin
RCT	randomized control trial
RPG	random plasma glucose
SGLT-1	sodium-dependent glucose transporter-1
STAT-5	signal transducer and activator of transcription-5
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TNF-α	tumor necrosis factor-α
TNFR1	tumor necrosis factor receptor 1
WHO	World Health Organisation

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Zusammenfassung

Hintergrund: Die Plazenta ist ein fetales Organ und Produktionsort für zahlreiche Schwangerschaftshormone und bioaktive Moleküle, die dazu beitragen den mütterlichen Organismus an die Schwangerschaft anzupassen, und um den heranwachsenden Fötus mit Nährstoffen, vor allem mit Glukose, zu versorgen. Um dies zu gewährleisten, entwickelt die Mutter in Laufe der Schwangerschaft eine Insulinresistenz. An diesem Prozess sind diese plazentaren Hormone und bioaktive Moleküle beteiligt. Durch Vergrößerung der β-Zellmasse und vermehrter Insulinsekretion wird der mütterliche Blutzuckerspiegel trotzdem im Normbereich gehalten. Eine Störung dieses kompensatorischen Prozesses, führt zur Entstehung von Gestationsdiabetes. Da die Inzidenz für Gestationsdiabetes bei Schwangerschaften mit Buben höher ist als bei Schwangerschaften mit Mädchen, scheint die Entstehung der Insulinresistenz oder die Anpassung der mütterlichen Insulinsekretion vom fetalen Geschlecht beeinflusst zu sein.

Zielsetzung: Ziel dieser Literaturarbeit war es, Daten über bioaktive Moleküle zu erheben, die von der Plazenta sezerniert werden, in der mütterlichen Zirkulation messbar sind, und den mütterlichen Glukosestoffwechsel während der Schwangerschaft beeinflussen. Insbesondere wurde recherchiert, ob diese Faktoren eine Rolle bei der Entstehung von Gestationsdiabetes spielen könnten, und ob sie Unterschiede je nach fetalem Geschlecht aufweisen. Die Moleküle, zu denen die Literatur analysiert wurden, waren: Östrogen, Progesteron, hCG, hPL, pGH, PRL, APN, Leptin, TNF-α, Resistin, Visfatin, Apelin und AFABP.

Methoden: Es wurde eine umfassende Literaturrecherche durchgeführt, um relevante Daten im Zeitraum zwischen 1972 und 2021 zu sammeln.

Ergebnisse: Folgende Hormone sind bei GDM dysreguliert: hCG, APN, Leptin, TNF-α, Resistin, AFABP. Bei Schwangerschaften mit Buben besteht ein erhöhtes Risiko für GDM. Fetale Geschlechtsunterschiede wurden für hCG beschrieben. Bezüglich der anderen Hormone sind die Daten uneinheitlich.

Konklusion: Es gab eine Vielzahl von Studien, die Geschlechtsunterschiede bei bioaktiven Molekülen, die von der Plazenta in die mütterliche Zirkulation abgegeben werden, untersuchen. Allerdings sind viele der Studien nur in relativ kleinen Kohorten durchgeführt worden, und unterschiedliche Rahmenbedingungen (Zeitpunkt der Messung in der Schwangerschaft, BMI der Mutter, Ethnizität) führen zu sehr variablen Ergebnissen. Lediglich für hCG wurde von einer Vielzahl an Studien gezeigt, dass bei Schwangerschaften

mit einem weiblichen Fötus erhöhte mütterliche Serumspiegel vorliegen. Außerdem werden weitere Studien mit adäquater Studiengruppengröße benötigt, die den Zusammenhang zwischen Entstehung von Gestationsdiabetes unter Einfluss der plazentaren Hormone und dem fetalen Geschlecht parallel untersuchen.

Abstract

Background: The placenta is a fetal organ and a production site for various hormones and bioactive molecules that help to adapt the maternal organism to pregnancy in order to optimize the supply of the growing fetus, especially with glucose. Therefore, throughout normal pregnancy, insulin resistance develops. To avoid hyperglycemia, maternal blood glucose level is kept within the normal range by increasing β-cell mass and insulin secretion. Placenta derived factors are involved in this process. However, if this compensatory process is disrupted, GDM may develop. Recent studies have revealed that the development of GDM depends on fetal sex with a higher risk for women carrying a boy, suggesting that maternal metabolic adaption to pregnancy by fetal factors differs between pregnancies with boys vs pregnancies with girls.

Objective: The aim of this study was to summarize and condense studies which investigate the role of placental derived bioactive factors in GDM and potential differences in depending on fetal sex in order to elucidate the mechanisms how the sex of a baby may modulate maternal GDM risk. Literature was analyzed for the molecules: estrogens, progesterone, hCG, hPL, pGH, PRL, APN, leptin, TNF-α, resistin, visfatin, apelin and AFABP.

Methods: A comprehensive literature search was conducted to collect relevant data between 1972 and 2021.

Results: The following molecules are reported to be dysregulated in GDM: hCG, APN, Leptin, TNF-α, Resistin, AFABP. GDM risk is increased in pregnancies with boys. Fetal sex differences in placenta derived factores are reported for hCG. For the other hormones, data are still inconsistent.

Conclusion: Various studies investigated sex differences in bioactive molecules released from the placenta into the maternal circulation. However, many of the studies have only been conducted in relatively small cohorts, and different framework conditions (time of measurement in pregnancy, BMI of the mother, ethnicity) lead to highly variable results. Only for hCG, by a large number of studies, was revealed that maternal serum levels are increased in pregnancies with females. In addition, further studies are needed that examine the connection between the development of GDM under the influence of placental hormones and the fetal sex in parallel, with adequate size of study groups.

1 Introduction

1.1 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is a pregnancy complication leading to chronic hyperglycaemia in pregnant women without earlier diagnosed Type 1 diabetes mellitus (T1DM) or Type 2 diabetes mellitus (T2DM). (1) This kind of glucose tolerance disorder is recognized for the first time during the second or third trimester of gestation and normally disappears after delivery of the child. (2, 3)

According to different publications, the prevalence of GDM varies between 1 percent to 22 percent of pregnant women, depending on the observed cohorts and the diagnostic criteria used, and its frequency is rising worldwide. (4)

1.1.1 Complications and outcomes

GDM leads to short- and long-term complications that may occur during pregnancy, delivery or postpartum and can affect the mother, her fetus, neonate, child and adult offspring. (5) Evidence shows that an aggressive treatment lowers or even eliminates the risk of developing these consequences. (3) Thus, an early diagnosis and the appropriate management is crucial. (6) The table below gives an overview of maternal and fetal short- and long-term complications:

Table 1 Maternal and fetal short- and long-term complications of GDM.

	Mother	Child
Short-term	• Pre-eclampsia: gestational hypertension (≥140/90 for the first time after mid-pregnancy) and proteinuria (≥0.3g/24h) (7) • Polyhydramnion (8) • Preterm labour (3) • Caesarean section (7, 8) • Instrumental delivery (5) • Traumatic labour (5) • Hypertensive disorders (5) • Urinary and genital tract infections (5)	• Perinatal mortality (8, 9) • Stillbirth (7) • Macrosomia and large-for-gestational-age (LGA) infant (9) • Organomegaly (9) • Obstetric trauma (8) • Hypoglycaemia (8, 9) • Hyperbilirubinemia (8, 9) • Hypocalcaemia (8, 9) • Hypomagnesemia (9) • Polyglobulia (10) • Polycythemia (9) • Respiratory distress syndrome (7) • Neonatal intensive care unit admission (5)
Long-term	• Future T2DM (5) • 50 percent risk to develop GDM in subsequent pregnancy (10) • Metabolic syndrome (3) • Cardiovascular disease (CVD) (3)	• Obesity (5) • Metabolic syndrome (10) • T2DM (5) • GDM (if female) (5)

1.1.1.1 Maternal complications

Maternal hyperglycaemia in pregnancy is associated with a significantly higher relative risk of developing hypertensive disorders during gestation, preterm labour and caesarean delivery. Since caesarean section results in less birth trauma for a macrosomic infant, it is often the delivery mode of choice. But still, compared to vaginal birth, the risk of trauma may be increased for the mother. Women who were diagnosed with GDM during pregnancy,

have a predisposition to develop metabolic syndrome and CVD, and a 7-fold increased risk of developing T2DM later in life. (3)

1.1.1.2 Fetal complications

Since glucose crosses the placental barrier, maternal elevated blood glucose levels also lead to hyperglycaemia in the fetus. The fetus responds with hyperinsulinemia which results in enhanced glycogen and protein synthesis, and lipogenesis. Thus, the fetus increases fat storage. Therefore, macrosomia is one of the most common and serious complications for GDM infants. (3) Depending on the definition, macrosomia refers to neonates with a birth weight of more than 4000g, or above the 90th percentile for gestational age. The perinatal mortality of LGA infants is two to five times higher than of infants with normal birth weight. Thus, GDM is associated with an increased risk for birth complications, including birth trauma to the head and neck, fetal asphyxia due to prolonged labour, shoulder dystocia, fracture of the clavicle, Erb palsey, facial paralysis, phrenic nerve injury and intracranial haemorrhage. (3)

Moreover, the fetus may develop organomegaly, according to cellular hypertrophy/hyperplasia in liver, heart, spleen, adrenals and pancreatic islets. There is evidence that fetal hyperglycaemia delays lung maturation and GDM infants thus, have a higher risk for developing respiratory distress syndrome. (3) However, since GDM usually develops in the second trimester, teratogenic birth defects, as they may occur in the offspring of mothers with pregestational diabetes, do not occur. (9)

At delivery, as maternal glucose supply to the fetus suddenly ends, the continued increased production of insulin due to β-cell hyperplasia (9) can lead to hypoglycaemia and may have serious consequences such as seizures. (3)

Hypocalcaemia and hypomagnesemia may also occur in the neonate, most frequently between 48 and 72 hours after delivery. Explanations for the impaired calcium and magnesium hemostasis may be failure of fetal parathyroid hormone response, high calcitonin levels and alterations in vitamin D metabolism. Maternal renal losses of magnesium, associated with glycosuria, reduce the hormone secretion of the parathyroid gland and lead to hypocalcaemia. (9)

Hyperglycaemia stimulates metabolism which elevates oxygen demand. Due to the resulting decreased fetal oxygen tension, erythropoietin production increases, which stimulates erythropoiesis. Polycythemia, i.e. a venous haematocrit of more than 65 percent, which is associated with hyperviscosity of the blood, impaired blood flow, formation of microthrombi

and hyperbilirubinemia. Birth trauma (hematoma formation and the destruction of red blood cells) may also contribute to the development of hyperbilirubinemia. (9)
Besides these immediate effects, GDM also bears long-term consequences for the offspring: According to recent studies, infants of women who had GDM have a lifelong increased risk to develop glucose intolerance, T2DM, obesity and/or metabolic syndrome. (3)

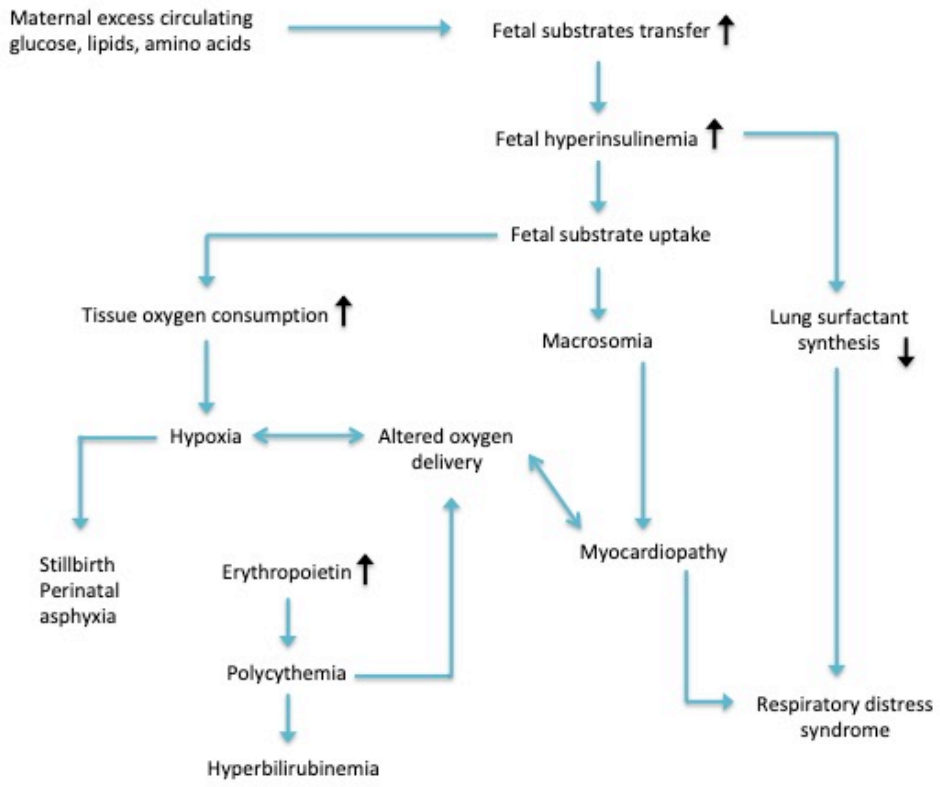

Figure 1 Adverse consequences of GDM (Modified from (11)).

1.1.2 Risk evaluation and diagnosis

During the initial consultation with the obstetrician, the pregnant woman is classified in terms of her risk for GDM or diabetes mellitus. If she is at higher risk, she should be screened for a glucose metabolism disorder as early as possible. This can be determined through a fasting plasma glucose (FPG) measurement, spontaneous glucose measurement, HbA1c determination and/or performance of an oral glucose tolerance test (OGTT). (12)

Criteria representing high risk for GDM are:

- GDM in a previous pregnancy
- pre-diabetes in the medical history (impaired glucose tolerance and/or fasting glucose ≥ 100 mg/dl)
- congenital fetal malformation in a previous pregnancy
- birth of a neonate >4500g
- stillbirth
- habitual miscarriage (>3 miscarriages in a row)
- diabetes symptoms
- obesity (body mass index (BMI) ≥ 30 kg/m^2)
- age over 35 years
- metabolic syndrome
- vascular disease (coronary heart disease, insult, peripheral artery disease)
- family history of T2DM in first degree relatives
- ethnicity (Arab, South- and Southeast Asian and Latin American women). (12)

If diabetes-specific symptoms or clinical abnormalities occur (increased thirst, polyuria, glucosuria, macrosomia), a test should be performed immediately, even if the previous findings are normal and regardless of the gestational week. (12)
All pregnant women must be screened for GDM in the 24th to 28th week of pregnancy using a 75g OGTT. Women with previously diagnosed GDM or diabetes or if the FPG value is 92 mg/dl or higher are exempt, as these women are already in need of treatment and further glucose loading is not required. The OGTT is included in the Mutter-Kind-Pass and is mandatory for receiving full childcare benefits. (12)

1.1.2.1 75g OGTT

The test should start between 6.00 and 9.00 a.m., as the glucose tolerance varies during the day and women have to be fasted. It is crucial to perform the OGTT after at least eight hours of fasting. (12) The time of the patient's last meal the night before should be noted. (13) Nutritional changes, restrictive eating, a reduction in the intake of carbohydrates and extraordinary physical activity should be avoided before taking the test. (12)
The first blood test is performed in fasting state. Therefore, 5ml of venous blood is drawn into a fluoride tube. (13) Then, the pregnant woman is advised to drink a solution of 75g glucose powder dissolved in 300 ml water within five minutes. (12) After one and after two hours, 5ml of venous blood is taken again. (13)

During the test the women should remain seated and avoid laying down as well as physical activity. (12) Drinking, eating and smoking is not allowed for the duration of the test. It is crucial that the OGTT is performed by a lab technician at a health care centre. (13) For GDM diagnostics, blood glucose values should only be measured directly in venous plasma using a quality-assured method or measured in venous whole blood and converted to venous plasma values with a factor of 1.11 (+11 percent). (12)
The diagnostic thresholds are shown below, with GDM being diagnosed at one pathological value (12):

Table 2 Assessment of the 75g OGTT according to World Health Organisation (WHO) and International Association of the Diabetes and Pregnancy Study Groups (IADPSG) recommendations.

Time	Venous plasma values (mg/dl)
fasting	≥ 92
1 h	≥ 180
2 h	≥ 153

1.1.3 Therapy

Women diagnosed with GDM need to be informed about the risks of adverse pregnancy outcome and how the consequences of hyperglycaemia may be prevented. (14)
Adequate GDM treatment targets the normalization of blood glucose values, which can be achieved through lifestyle interventions such as medical nutrition therapy, physical activity and cessation of smoking. (8) The patient must receive training regarding blood glucose self-monitoring. (12) If blood glucose values are not within the target range (Table 2) after initiating lifestyle modifications, pharmacological therapy using insulin, as a first choice, and oral hypoglycaemic agents must be started. (8, 12) According to the International Diabetes Federation (IDF) guideline, the time interval between these two therapy stages should be 1 to 2 weeks. (14) If fasting plasma glucose levels exceed 110 mg/dl, immediate therapy with insulin is recommended. If blood glucose values are repeatedly between 90 and 95 mg/dl fasting and/or between 130 and 140 mg/dl postprandially, fetal biometry should be used to evaluate whether insulin therapy is necessary. (12)

1.1.3.1 Diabetological care

1.1.3.1.1 Home blood glucose measurements

All pregnant women with GDM are advised to self-monitor blood glucose levels. (14) Home blood glucose measurements (HBGMs) should be performed at least 4 times daily in the fasting state/pre-prandial and postprandial, preferably one or two hours after each meal. (7, 12) Target blood glucose levels are shown in the table below (12):

Table 3 Target blood glucose levels in GDM patients.

Time	Targets (mg/dl)
Pre-prandial or fasting	65-95
1 h after meal	< 140
2h after meal	< 120

1.1.3.1.2 Medical nutrition therapy

The dietary program in women with GDM should restore euglycemia, prevent ketosis and lead to optimal weight gain during pregnancy. (5) The daily caloric intake should be calculated individually, taking the woman's pre-gravid weight, nutritional status, normal activity level and activity level during pregnancy into account. (9) Normal weight women should target a caloric intake between 24 and 30 kcal/kg with a macronutrient distribution of 40 to 50 percent carbohydrates, 30 to 35 percent fat and 20 percent protein. (12) In addition, 30 g of dietary fiber should be consumed daily and an adequate supply of minerals and vitamins (iron, folic acid, vitamin D, calcium, vitamin B, magnesium and iodine) should be ensured. (12) It is recommended that women with GDM eat smaller meals, but more frequently, which means three meals and three or four snacks in between. (5) Post-prandial glucose excursions can be avoided by consuming carbohydrates with low glycaemic index. (14) Buffering glucose peaks after a meal is extremely important since these critically contribute to the development of diabetic embryopathy. (9)

The optimal body weight before pregnancy and its maintenance throughout gestation contributes to maternal and neonatal health. (8) The woman's weight must be monitored at each follow-up visit or independently documented by the patient on a weekly basis. (12) Weight gain during pregnancy should follow the recommendations of Institute of Medicine (IOM), which are presented in the table below (12):

Table 4 IOM weight gain recommendations for pregnancy.

BMI	BMI limits WHO (kg/m^2)	Recommended weight gain during pregnancy (kg)	Recommended weight gain/week (kg/week) (2nd and 3rd trimester)
Underweight	< 18.5	13-18	0.51
Normal weight	18.5-24.9	11-16	0.42
Overweight	25.0-29.9	7-11	0.28
Obese	≥ 30.0	5-9	0.22

1.1.3.1.3 Exercise

Physical activity is not only beneficial in healthy pregnancy but also in pregnancy complicated by GDM. For women with GDM a moderate amount of exercise is a useful adjustment to therapy when nutritional therapy alone does not lead to the expected results. An increase in insulin sensitivity due to exercise may be the explanation for this beneficial effect. (14) Therefore, it is recommended to include at least 30 minutes of exercise into the daily life. (13) Choosing sports that are compatible with pregnancy and correspond to the woman's current training condition is crucial. (12) Women who are used to exercising can continue this during pregnancy, but the intensity and type of training may need to be modified, for instance in avoiding exercises with excessive abdominal muscular contraction. If the women's lifestyle was previously mainly sedentary, starting with arm exercises may be the right choice. (14)

1.1.3.2 Pharmacological therapy

1.1.3.2.1 Insulin

NPH insulin is mainly used as basal insulin. (12) It is administered before night rest if fasting hyperglycaemia occurs. (13) The use of long-acting insulins like glargine or detemir is also safe during pregnancy. Using glargine instead of NPH shows no difference in birth weight and also comparable risk of neonatal complications and malformations. Maternal outcomes such as pre-eclampsia and pregnancy hypertension are comparatively rare. For insulin detemir, the risk of macrosomia or neonatal hypoglycaemia is also comparable to NPH insulin. (12)

To control postprandial glucose peaks rapid- or ultra-rapid acting insulin should be administered before meal. (8) Therefore, insulin lispro and aspart should be preferred to human insulin, also because of its easier handling. Glulisine should currently not be used, due to insufficient data available. Comparison of aspart to human insulin shows no differences in macrosomia or frequency of caesarean deliveries. Lispro compared to human insulin was associated with a lower incidence of jaundice and less maternal hypoglycaemia, while higher incidences of macrosomia and high birth weight were reported in the lispro group. (12)

1.1.3.2.2 Oral hypoglycaemic agents

There is evidence that metformin and glibenclamide (glyburide) can be safely used in pregnancy causing no harm to the fetus. (13) However, long term safety has not been systemically examined. (14)

Even though metformin is able to cross the placenta, it does not appear that its use causes congenital anomalies. (8) The MiG study revealed that its effect is comparable to insulin, but in 50 percent of cases, women need an adjustment of therapy with insulin to meet the requested blood glucose values. (7) Metformin can be considered to use if insulin is not available, not practical, or refused by the treated patient. (13)

Studies have shown that glibenclamide (glyburide) is not able to cross the placenta, or only in low amounts. (7) According to randomized control trials (RCTs), the effect of glyburide is comparable with insulin, but its use seems to lead to an increase in macrosomia. (8)

To conclude, insulin remains the treatment of choice but in some cases metformin and glibenclamide (glyburide) may be safe and effective alternatives in GDM treatment. (14)

1.1.3.2.3 Obstetric care

Continuous follow-ups after GDM diagnosis, including the monitoring of maternal (metabolic) and embryonic (ultrasonographic) parameters, are essential. (8) Clinical appointments should be followed between one and three weeks. (12) Important parameters that have been used for maternal follow-ups are HBGMs and body weight. (8) At follow-ups, ultrasound, including biometry, Doppler ultrasound and the evaluation of amniotic fluid, should be performed. (12) Ultrasonographic parameters include estimated fetal weight, head circumference, abdominal circumference, femoral length and polyhydramnios. (8)

Furthermore, the growth curve should be monitored to discover asymmetric growth. In case of hyperglycaemia in early pregnancy, an early organ screening by ultrasound should be aimed to exclude malformations, especially in heart and kidney. Paying attention to the increased risk of developing gestational hypertension, pre-eclampsia and infections is crucial. The ideal date of birth and delivery mode should be determined. Exceeding the birth date should be avoided in pregnant women with insulin dependent GDM. If labour should be induced between weeks 38+0 and 40+0 can be decided individually. Insulin requirement, ultrasound findings (child weight, Doppler, amniotic fluid) and maternal diseases such as pre-eclampsia and the previous pregnancy history should be included in the decision. A caesarean section should be recommended at an estimated birth weight of 4500g or more. If the estimated birth weight is between 4000 and 4499g, the pregnant women should be informed about an increased risk of shoulder dystocia. (12)

1.1.4 Glycaemic control during labour

Not only chronic, but also acute maternal hyperglycaemia during labour leads to fetal hyperinsulinism which may result in neonatal hypoglycaemia. (15) What is more, several studies have shown that besides neonatal hypoglycaemia, maternal hyperglycaemia during delivery is associated with birth asphyxia and non-satisfactory fetal heart rate tracings. (5) Thus, maternal blood glucose levels must remain within the target range of 80-130 mg/dl during delivery. (12) Two-hourly measurements are usually sufficient. (10)

1.1.5 Postpartum care of women with GDM

If blood sugar levels are normal after delivery (fasting < 100 mg/dl and independent of food intake < 200 mg/dl), no further dietary therapy or blood sugar self-monitoring is necessary. (12) However, women who have been diagnosed with GDM must perform a postpartum OGTT. This test should be performed, depending on the local healthcare arrangements, between 0 to 6 weeks after delivery. (14) Kautzky et. al, in an Austrian paper, recommends performing an OGTT between 4 and 12 weeks postpartum. (12) If the test indicates diabetes, therapy must be initiated (lifestyle interventions, diabetes therapy). In case of prediabetes, which means impaired glucose tolerance (two-hour value 140-199 mg/dl) or increased fasting glucose (100-125 mg/dl), a diet and increased physical activity needs to be prescribed. Women with non-pathological findings should be examined for diabetes every 2 to 3 years using OGTT or at least by measuring fasting glucose and HbA1c. (12)

Furthermore, currently non-diabetic women need to be advised on the high risk of developing diabetes in the future and which lifestyle modifications may useful to prevent it. If the patient plans a further pregnancy, she needs to be informed about the need of pre-pregnancy counselling due to an increased risk of developing GDM again. (14)

2 Methods

In this thesis publications investigating placental hormones and bioactive factors and their influence on glucose metabolism, insulin resistance and GDM were collected. Studies investigating the connection between GDM risk and fetal sex and articles examining fetal sex differences in placental hormones and factors were also included. A comprehensive literature research was carried out during the period from April 2020 until September 2021 using data available at the University Library of Medical University of Graz and online database PubMed. Furthermore, information was taken from GDM guidelines. The books and articles included in this thesis were published between the years 1972 and 2021. The literature lists of the relevant publications were additionally reviewed to find further literature that was not discovered through the database search tools.

At the beginning of the literature research, general information on GDM was collected by entering the word “GDM” or “gestational diabetes mellitus” alone, or in combination with “complications”, “diagnosis” or “therapy” in the search.

For the main section of the thesis the terms “gestational diabetes mellitus”, “GDM”, “pathophysiology”, “pregnancy”, “insulin resistance”, “glucose metabolism”, “placental hormones”, “estrogen”, “progesterone”, “human chorionic gonadotropin”, “human placental lactogen”, “placental growth hormone”, “prolactin”, “adipokines”, “adiponectin”, “leptin”, “tumor necrosis factor-alpha”, “resistin”, “visfatin”, “apelin”, “adipocyte fatty acid-binding protein”, “fetal gender” and “fetal sex” were entered in the search alone or in combination. Out of 2985 publications found through the database research, 226 were included in the thesis. Studies with larger cohort sizes were weighted more heavily.

3 Results

3.1 Glucose regulation during pregnancy

During pregnancy the maternal organism undergoes various changes in the endocrine and metabolic system. For instance, the temporal adaption of insulin production and sensitivity is crucial during gestation and therefore, insulin sensitivity changes with gestational age to supply fetal glucose requirements (16, 17): In early pregnancy insulin sensitivity increases in order to absorb and store larger amounts of glucose as energy reserves for later in gestation. (1) The glucose transport across the placenta is a passive process and depends on a concentration gradient between the fetal and the maternal circulations. Fetal β-cells enforce this gradient through their high basal insulin secretion and relative glucose insensitivity which keeps fetal blood glucose levels low. (18)

Later in pregnancy, the mother becomes insulin resistant and maternal insulin levels increase of up to 50 percent. (9) This is a result of the production of local and placental hormones, such as estrogen, progesterone, leptin, cortisol, placental lactogen and placental growth hormone. (1) Decreased insulin sensitivity leads to a subtle increase in maternal blood glucose concentrations, which facilitates glucose transport across the placenta to support the rapid growth of the fetus. (1, 16) This enhanced glucose transport may even cause fasting hypoglycaemia in the mother. (5)

In this state of insulin resistance, the maternal body starts endogenous glucose production, intensifies the development of hyperglycaemia and lipolysis, which leads to increased concentrations of circulating free fatty acids. (1)

Thus, on one hand the maternal organism has to provide the fetal energy requirements and on the other hand it has to maintain maternal glucose homeostasis. (16) Evidence in animals has shown that during healthy pregnancy, insulin resistance is countered with pancreatic β-cell hyperplasia, hypertrophy and increased glucose-stimulated insulin secretion (GSIS). (1) This mechanism is also important to prevent excessive glucose delivery to the fetus. The balance between maternal insulin resistance and enlarged β-cell mass provides optimal glucose supply to the fetus throughout pregnancy. Shortly before parturition until the postpartum period, the maternal β-cell mass begins to shrink to its size before pregnancy. (18) Furthermore, insulin sensitivity returns to pre-gravid levels within a few days after birth, suggesting that placental hormones play a central role in the altered glucose homeostasis and insulin sensitivity in pregnancy. (1)

If maternal β-cells cannot compensate for the increased insulin demand, caused by the insulin resistance of normal pregnancy, GDM develops. (9)

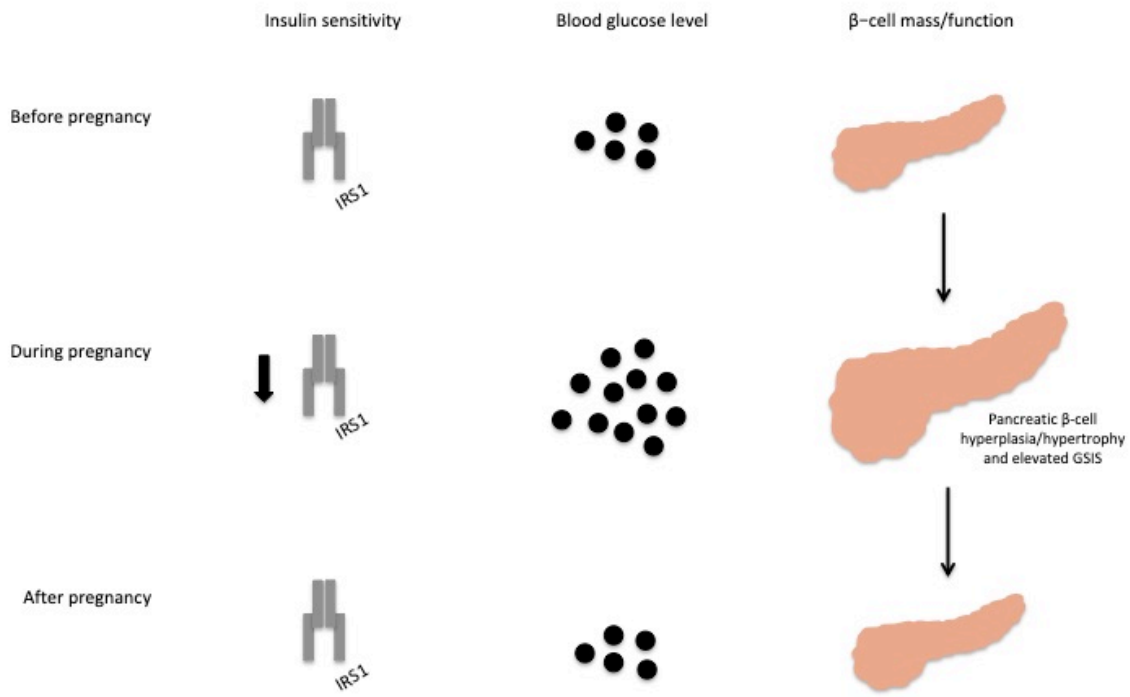

Figure 2 β-cell function, blood glucose, and insulin sensitivity in normal pregnancy (Modified from (1))

3.2 Hormonal effects in GDM

3.2.1 Steroid hormones

3.2.1.1 Estrogens

The most important estrogens in humans are estrone (E1), 17β-estradiol (E2) and estriol (E3). (19) In early gestation, until the placenta is formed, the secretion of estrogens is accomplished by the corpus luteum. Later, the placenta takes over hormone synthesis until term. (20) After the 9th week of pregnancy, estrogen production increases sharply and hormone concentrations rise three to eight times higher than in non-pregnant women. (21) During pregnancy, mainly E3 is synthesized. (19) The precursors of estriol synthesis, dehydroepiandrosterone (DHEA) and DHEA-S (dehydroepiandrosterone sulfate), are

produced in the fetal adrenal cortex and are finally converted by the placenta into E1, E2 and E3. (19, 21)

During pregnancy, estrogens have numerous functions, such as the stimulation of uteroplacental blood flow, formation of gap junctions between myometrial cells and an increasing in endometrial prostaglandin synthesis. These hormones also stimulate the growth of the mammary gland directly and by stimulating prolactin (PRL) secretion. Estrogens increase receptor-mediated uptake of LDL cholesterol, which is required for steroid hormone synthesis in the placenta, and thus, indirectly stimulate placental progesterone production. (19, 22)

3.2.1.1.1 The diabetogenic action of estrogens

Estrogen and its receptor are known to play an important role in the regulation of body weight and insulin sensitivity. (20) The steadily high estrogen levels during pregnancy have also been considered to influence β-cell function and insulin resistance, but
the exact effect of estrogens on the adaption of β-cell response to insulin resistance and thus, their impact on the development of GDM are mainly unknown. (20, 23)

However, estrogen receptors are expressed on β-cells and their activation by E2 promotes insulin synthesis, glucose-dependent insulin secretion and β-cell survival. (20) Other studies revealed E2 as protective against insulin resistance, also because it strengthens insulin binding. (24, 25) E3 shows a weak estrogenic effect but it can act as an anti-estrogen by inhibiting the binding of E2 to the estrogen receptor. Throughout normal pregnancy serum E2 and E3 concentrations are steadily rising, with E3 reaching much higher levels than E2. This hormone increase occurs in the second trimester of pregnancy, at the time when GDM clinically manifests. The study of Hur et al. revealed that elevated unconjugated early second trimester maternal serum E3 levels, i.e. > 95th percentile of the screened population or unconjugated E3 ≥ 2.0 Multiple of Median (MoM), are related to higher GDM risk and predict the development of GDM. The fact that E3 inhibits E2 receptor binding, which results in insulin resistance, may be an explanation. (20) A study by Kleiblova et al. showed another potential role of estrogen and its receptors in the regulation of insulin resistance during gestation: The activation of ERα was prevented whilst the activation of ERβ increased the development of insulin resistance. In this study, women with GDM showed lower numbers of ERα and ERβ in subcutaneous fat. Thus, a decreased expression of ERα may promote the emergence of insulin resistance in GDM patients. (26) A different approach to this issue was made by Qi et al., who investigated the relationship of cord blood hormone

concentrations and GDM and discovered that cord blood concentrations of E2 are lower in GDM when compared to normal, healthy pregnancy. (27)

3.2.1.2 Progesterone

The steroid hormone progesterone is an important pregnancy hormone. It is produced by the corpus luteum during the normal menstrual cycle. After fertilization and the following implantation, the corpus luteum continues to secrete progesterone. This secretion is stimulated by human chorionic gonadotropin (hCG), which is produced by the syncytiotrophoblast of the implanted conceptus. The continuous secretion of progesterone prevents menstruation and is required to provide an uterine environment suitable for the developing embryo. After 6 to 8 weeks of pregnancy, placental hCG production decreases and thus, also the progesterone secretion of the corpus luteum. From then on, the placental trophoblast takes over progesterone production and secretion. (28) Progesterone levels now rise exponentially until delivery. (22, 28)

Progesterone ensures the maintenance of pregnancy by preventing regressive changes in the endometrium. (19) It also reduces the uterine tonus and inhibits the muscular activity of the uterus to prevent contractions. (19, 29) Thus, progesterone counteracts the labour-promoting effects of estrogen, prostaglandins and oxytocin and prevents rejection of the fetus by inhibiting the maternal immune system to fight fetal antigens. (28) Moreover, progesterone prepares the mammary gland for lactation. Rising progesterone levels in early to mid-gestation increase maternal food intake and induce adipogenesis and fatty acid synthase expression in pre-adipocyte precursor cells, leading to weight gain and fat deposition. (30) Shortly before birth, progesterone stimulates the production of oxytocin receptors in the uterine musculature, which are relevant for triggering contractions. (19)

3.2.1.2.1 The diabetogenic action of progesterone

Typically, GDM clinically manifests in the third trimester of pregnancy, when progesterone levels peak. (31) These high hormone concentrations surely contribute to a reduced peripheral effectiveness of insulin. (24, 31) Increased progesterone levels at advanced gestation contribute to the development of insulin resistance by diminishing insulin-binding, glucose transporter 4 (GLUT4) expression, glucose transport in skeletal muscle and adipose tissue and insulin-induced gluconeogenesis in the liver. (30) Interestingly, progesterone receptors (Pgr) were identified on pancreatic β-cells, but direct effects of progesterone on

insulin secretion and islet-cell proliferation still remain a debatable topic. (31) A publication by Picard et al. suggests that hormonal alterations during pregnancy - especially of progesterone - affect the inadequate adaptation of insulin secretion to the increased needs of gestation. (31, 32) Authors revealed that fasting plasma glucose levels of female (but not male) Pgr-knockout-mice (Pgr -/-) were significantly lower than in the control group (Pgr +/+ mice). The Pgr -/- mice also showed a better glucose clearance after a glucose challenge test and thus, an increased resistance to hyperglycaemic stress than the Pgr +/+ mice. This enhanced glucose clearance was due to higher insulin levels, which result from increased proliferation of β-cells. Based on these observations in mice, it seems possible that progesterone contributes to impaired glucose metabolism during pregnancy. (31, 32) They also revealed that GDM is associated with increased circulating progesterone values in mice, that in female diabetic *db/db* mice the progression of diabetes is promoted by the hormone and that an antagonist to the hormone receptor decreases blood glucose values in female wild type and *db/db* mice. (32)

Moreover, Coughlan et al. detected that placentas of women suffering GDM release more progesterone than those of normal pregnant women, which may also confirm progesterone as a diabetogenic hormone. (33)

3.2.2 Peptide hormones

3.2.2.1 Human chorionic gonadotropin

Human chorionic gonadotropin (hCG) is a placental glycoprotein hormone, which is synthesized in rapidly increasing amounts by the syncytiotrophoblast, starting right after implantation of the fertilized ovum. (19, 34, 35) Prior to this, it is already formed by the eight-cell embryo and is secreted in high local concentrations when the blastocyst reaches the uterus. Thus, hCG is one of the earliest embryonic signals and is suggested to be involved in the regulatory process of implantation. (36) HCG is a heterodimer and consists of two peptide chains, i.e. an α-chain, which is similar in all gonadotropins, and a β-chain which is hormone-specific and determines the function of the respective hormone. (35) (37) As a substitute for luteinizing hormone (LH) during pregnancy, hCG ensures the production of progesterone in the corpus luteum by interacting with the ovarian LH/chorionic gonadotropin receptors. (19, 35, 36, 38) This is necessary to maintain pregnancy and support fetal growth. Without this crucial function of hCG, the uterine mucosa and the blastocyst would be rejected. (19, 35) Serum hCG levels initially double approximately every 48 hours

until maximum levels are reached in the 10th week of pregnancy. (19, 37) Thereafter, hCG steadily decreases until a plateau after the 18th week of pregnancy, which remains stable until term. As mentioned above, hCG stimulates progesterone but also estrogen synthesis in the corpus luteum until the placenta takes over steroid hormone production between the 8th and 10th week of pregnancy. It also induces the production of DHEA, DHEA-S and other steroids in the fetal adrenal cortex. (19)

3.2.2.1.1 The diabetogenic action of hCG

Contradictory data have been reported on the relation between hCG and GDM. (39) Visconti et al. detected lower GDM risk in women with first trimester β-hCG ≥ 2.0 MoM. (39) Spencer et al. and Ong et al. described a significant decrease in free β-hCG in the first trimester of pregnancy in women who subsequently developed GDM. (40, 41) A meta-analysis by Donovan et al. investigated whether abnormal levels of prenatal screening biomarkers, such as hCG, are associated with the risk of developing GDM later in pregnancy. Comparing nine different studies, they concluded that women with GDM had lower first trimester β-hCG levels than women with normal glucose metabolism during pregnancy. (42) In a very recent study by Liu et al., women in early pregnancy showed lower glucose levels during an OGTT when they had higher hCG levels. However, this correlation was not shown for fasting glucose levels or HbA1c. HCG levels were also negatively correlated with GDM risk and it was therefore concluded that higher hCG values in early gestation are related to lower GDM risk. (43) Additionally, Sirikunalai et al. also reported that women with high first trimester β-hCG values have a reduced risk of developing GDM. (34) However, this relationship did not apply to the second trimester, which was also reported by a further study. (34, 44) Moreover, Räty et al. reported that women with GDM even showed lower midtrimester hCG levels than women being normoglycemic during pregnancy. (45) Tul et al. and Savvidou et al. did not identify a significant association between first trimester hCG levels and the development of GDM. (46, 47) Ma et al. investigated if the increase of hCG during pregnancy causes inflammation and decreased insulin sensitivity leading to GDM. As reported earlier, low amounts of hCG/LH receptors seem to be expressed in non-gonadal tissue, such as in human primary adipocytes and in murine 3T3-L1 adipocytes, suggesting non gonadal functions of LH/hCG. The study confirmed again that hCG/LH receptors are expressed in small quantities in insulin-sensitive murine 3T3-L1-adipocytes and in murine C2C12 myocytes. Additionally, treatment with hCG caused a reduction in insulin-responsive gene expression, such as GLUT4, and 3T3-L1 cells also showed abnormalities

in insulin-stimulated glucose uptake. Regarding inflammation, hCG treatment lead to a higher expression of monocyte chemotactic protein 1 (MCP1), which is a proinflammatory cytokine, and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB). Thus, these findings suggest that higher hCG levels during gestation may play a role in the development of GDM as they lead to impaired insulin sensitivity and also cause inflammation in adipocytes. (38)

3.2.2.2 Human placental lactogen

Another placental peptide hormone is human placental lactogen (hPL) which is also called human chorionic somatomammotropin (hCS). (48, 49) This hormone is structurally related to human growth hormone (hGH) and placental growth hormone (pGH) to which it is 85 percent homologous, and also to PRL, to which it possesses a homology of 13 percent. (48, 49, 50) This similarity exists because hPL and both growth hormones derive from the same gene cluster on the long arm of chromosome 17 (q22-q24). This group of genes consists of five genes, of which hGH-N codes for hGH, hGH-V codes for pGH and hPL-A, hPL-B and hpL-L code for hPL. (22, 49) HPL is produced by the placental syncytiotrophoblast which. (22, 23, 49) Although these hormones are structurally related, they possess significant differences in expression and function. (22, 49) Similar to hGH and PRL, hPL acts by binding to the hGH and PRL receptor but has predominantly lactogenic effects. (48, 49, 50) After the 6th gestational week hPL is released into the maternal and fetal bloodstream. It increases 10-fold in the second half of pregnancy until finally plateauing in the last 4 weeks. HPL is required to adapt the maternal glucose and fat metabolism to ensure the continuous supply of glucose and amino acids to the fetus and thus, supports fetal growth. (29, 48, 49) In fact, maternal hPL values are related to fetal and placental weight and size. (23, 50) It causes various changes in maternal metabolism such as an increase in blood sugar levels, an increase in lipolysis and differentiation of the mammary gland. (51) In high concentrations, similar to PRL, hPL can induce growth and milk production in the mammary gland. (29) HPL also correlates with insulin-like growth factor 1 (IGF1) levels during pregnancy and is thought to be a main factor to stimulate its synthesis. (49) Other functions of hPL are the stimulation of fetal erythropoiesis, fetal amino acid uptake in the muscle, fetal protein and IGF1 production and glycogen synthesis. (49, 51)

3.2.2.2.1 The diabetogenic action of hPL

As mentioned above, hPL plays a key role in regulation of maternal nutrient metabolism during pregnancy. (49) It appears to act as an insulin agonist and antagonist. (25) It is known to stimulate β-cells to secrete insulin directly and after glucose load. (22, 49) Additionally, hPL binds to PRL receptors on β-cells, thereby increasing β-cell mass and thus participating in the increased production of insulin which is physiologically required during pregnancy. (22, 25) These effects have been shown in isolated human and rodent islets and also in insulinoma cells. In whole body PRL receptor knockout mice, decreased β-cell mass and GSIS was reported. (22) However, it should be taken into consideration that placental lactogen in humans is derived from the hGH gene cluster and placental lactogen in rodents from the PRL gene. In addition, PRL receptors are lower expressed in human β-cells than in those of rats and thus, may have a less important impact on human β-cell adaptation during pregnancy. (16) Moreover, hPL reduces phosphorylation of insulin receptor substrate 1 (IRS-1) which leads to severe insulin resistance. (25) Additionally, hPL levels strongly correlate with maternal blood sugar level showing an increase during hypoglycaemia and decrease in hyperglycaemic states. (23) As an insulin antagonist, the presence of hPL increases blood glucose levels in women and may thus contribute to the development of GDM. (51) Since hpL is a known insulin antagonist and promotes sparing of glucose and other nutrients in the maternal circulation and consequently aggravates GDM, Mills et al., in order to investigate the regulation of hPL specifically in GDM, examined placental messenger ribonucleic acid (mRNA) levels encoding hPL synthesis in diabetic and healthy patients but did not reveal any differences. (52)

Regarding fat metabolism, hPL also stimulates lipolysis and thus contributes to the mobilization of lipids and free fatty acids. Due to the hPL-induced increase in blood lipids, free fatty acids can be used as an energy source for the mother during fasting and glucose, amino acids and ketone bodies are directly transported across the placenta to fuel the fetus. This surge in free fatty acids impairs insulin-induced glucose shift into the cell and could also partly be the reason for the development of insulin resistance caused by hPL. (23, 25, 49)

3.2.2.3 Placental growth hormone

Humans express two different types of growth hormones. As mentioned earlier, growth hormones derive from the same gene cluster on the long arm of chromosome 17, which contains five genes including GH-N encoding hGH and GH-V which encodes pGH. (53, 54)

These two hormones are thus, closely related and differ structurally only in 13 amino acids. (23, 53-55) HGH is predominantly produced in the somatotroph cells of the anterior pituitary gland and in some extrapituitary tissues and cells such as the brain, cells of the immune system, mammary glands, testis and in the placenta. Unlike hGH secretion, pGH is produced and secreted by the placenta in a non-pulsatile fashion since GH-V is expressed in the placental syncytiotrophoblast and in extravillous trophoblast cells. (53-55) However, in advanced pregnancy the dominant growth hormone in the maternal circulation is pGH and thus, becomes a substitute for hGH, which is the main growth hormone before the 15th week of pregnancy. (22, 23, 49, 53-55) Between the 5th and 10th week of pregnancy onwards, pGH can be detected in maternal plasma. (49, 53) In early pregnancy, hGH starts decreasing continuously until it can no longer be detected in the mother's plasma after the 24th gestational week. In contrast, pGH rises rapidly from the middle of pregnancy and reaches its maximum value between the 34th and 37th week. (22) One hour after the delivery of the placenta, pGH is no longer detectable in the maternal blood and the secretion of hGH is started again which is back to pre-pregnancy levels two days after delivery. (22, 49, 53)
PGH mainly acts as somatogen but also shows little lactogenic activity. (23, 54, 55)
The continuous secretion of pGH is thought to play an important role in adapting the maternal body to pregnancy. (53, 56) Regarding its somatogenic activity, pGH levels are positively related with IGF-1 production suggesting that pGH influences IGF1 secretion as their levels seem to increase in a similar manner during gestation. (22, 49, 53, 54, 56) Since suitable receptors for pGH are expressed on the placenta, pGH could also affect the development of this organ through autocrine and paracrine mechanisms. (54) Additionally, pGH increases maternal blood flow to the fetus by adapting placental blood vessels and relaxing uterine arteries. (53) What needs to be added is that pGH only carries out its functions in the maternal and probably also in the uteroplacental tissues but is not detectable in the fetal circulation und thus, does not seem to directly support fetal growth. (22)

3.2.2.3.1 The diabetogenic action of pGH

PGH has been considered to be involved in the regulation of glucose metabolism during pregnancy to ensure an increased glucose shift across the placenta to supply the fetus with essential nutrients. (23, 53) The surge of pGH around mid-pregnancy, in concert with increasing levels of tumor necrosis factor-α (TNF-α), free cortisol and progesterone and a decrease in adiponectin (APN) levels, leads to the development of insulin resistance in the mother. GH-V carries out its functions as insulin antagonist. PGH promotes lipolysis,

ensuring that during fasting, lipids can be used as energy source for the mother whilst glucose can be saved for fetal nutrient demand. (22) Since insulin resistance underlies the development of GDM, GH-V has also been suggested to play a role in its development. (53) Moreover, Barbour et al. revealed that an overexpression of GH-V causes insulin resistance in mice. (57) In another study they examined the exact underlying mechanism and discovered that pGH enhances the expression of the p85 regulatory unit of phosphadityl-inositol 3 kinase (PI3K) which leads to a severe decrease in IRS-1 induced activity of this specific kinase in skeletal muscle. (58) As a result, insulin induced GLUT4 translocation and glucose uptake is impaired. (22) A study by Liao et al. also revealed a correlation between pGH and insulin resistance during pregnancy. In a mouse model they demonstrated that higher doses of GH-V treatment caused severe fasting plasma hyperinsulinemia in the mother and also impaired insulin sensitivity. (59)

The fact that the presence of glucose inhibits pGH secretion has been confirmed in an in vitro study by Patel and colleagues. (60) Interestingly, Bjorklund et al. detected a significant correlation between hypoglycaemia and an increase in GH-V in pregnant women with insulin-dependent diabetes mellitus. (61) In a study by Liao et al. no differences between GH-V levels in GDM patients and healthy controls at 20 weeks pregnant were identified. However, part of GDM patients delivered LGA babies and those women were measured with higher GH-V levels suggesting that GH-V may play a role in the development of macrosomia. (62) Verhaeghe et al. demonstrated that pGH concentrations did not significantly differ between normal glucose tolerant and glucose intolerant women, determined through a glucose tolerance test between 24 and 29 weeks of pregnancy. (63) Männik et al. did not detect any differnces in *pGH mRNA* expression comparing GDM and healthy patients's placentas but revealed a subtle correlation between higher *pGH mRNA* levels and GDM patients who gave birth to an LGA baby. (64) Thus, in a review by Liao et al. was concluded that according to the current state of research it does not seem that GDM patients have abnormal GH-V levels during pregnancy. (53)

3.2.2.4 Prolactin

Prolactin (PRL) is a polypeptide hormone produced and secreted by the anterior pituitary gland and by non-pituitary sites such as the mammary gland, placenta, uterus, T lymphocytes and adipocytes. (50, 65, 66) Pituitary PRL secretion is inhibited through hypothalamic dopamine and mainly stimulated by estradiol. Thus, during pregnancy the high levels of estrogens are responsible for the surge in PRL. Non-pituitary PRL carries out its function

through autocrine and paracrine mechanisms without changing circulating hormonal concentrations. (50)

Besides its lactogenic effect on the mammary gland, PRL has major metabolic functions. (50, 65) For instance, it regulates body weight, appetite, adipose tissue function, β-cell proliferation and insulin secretion. (65)

PRL acts via binding to the PRL receptor, which is expressed in metabolically active cells such as in β-cells of the pancreatic islets, hepatocytes, adipocytes, macrophages, skeletal myocytes and also in cells of the hypothalamus. (50)

Maternal PRL levels start to rise at around six to eight weeks of gestation and increase progressively further until the end of pregnancy. (23, 67) This hormonal surge is caused by increasing numbers and size of maternal pituitary lactotrophs. (23) The lactotrophs normally make up 20 percent of the cells in the pituitary gland but due to hyperplasia, they increase to 50 percent of the cells at term. (68)

Additionally, progesterone and insulin have been described to stimulate PRL secretion in the uterine decidual cells. (23) The PRL synthesis of the decidua and the subsequent release of the hormone into the amniotic fluid starts at the 12th and reaches its maximum at the 20th week of pregnancy with a following decrease until term. The function of amniotic PRL still remains largely unknown but functions such as trophoblast growth, inhibition of myometrial contractility and regulation of angiogenesis have been suggested. (67)

3.2.2.4.1 The diabetogenic action of PRL

PRL is known to have a positive impact on proliferation and insulin secretion in β-cells of the pancreatic islets. Friedrichsen et al. revealed that this function of PRL is mediated by activation of the janus kinase 2 (JAK2)/signal transducer and activator of transcription-5 (STAT-5) signalling pathway. (69)

Furthermore, Le et al. demonstrated that if this signalling pathway is disrupted by a single nucleotide polymorphism occurring in the 5' untranslated region and in the promoter region of the PRL receptor gene, the risk of developing GDM increases. (70) Shaylel et al. investigated the association between PRL and glucose tolerance in pregnancy. GDM usually develops in the third trimester, when PRL levels peak. Although there is no evidence that this hormone has a direct effect on the development of GDM, reduced insulin secretion could lead to lower PRL levels, as insulin promotes the synthesis and secretion of decidual PRL. In this study, no significant difference in serum PRL levels in GDM patients and the healthy control could be identified in the third trimester. However, the highest mean serum PRL

levels were measured in the control group and the lowest in the GDM group. (68) By contrast, Ekinci et al. showed a positive correlation between higher third trimester PRL levels and GDM. Higher glucose levels after a 2-h OGTT were associated with higher PRL levels, suggesting that PRL may be independently involved in the emergence of GDM. (71) A recent prospective longitudinal study by Li et al. identified a positive correlation between high early pregnancy PRL levels and a subsequent diagnosis of GDM in advanced gestation, suggesting that PRL may play a role in the pathophysiology of GDM even although at present the exact mechanisms remain unknown. (65)

3.3 Placental Inflammation and Adipokines in GDM

3.3.1 Adiponectin

Adiponectin (APN), also called adipocyte complement-related protein of 30 kDa (Acrp30) or gelatin binding protein of 28 kDa (GBP28), is a protein consisting of 244 amino acids which is nearly exclusively produced in the white adipose tissue. This protein exists as low-molecular-weight trimer, middle-molecular-weight hexamer and high-molecular-weight 12- to 18-mer APN. (72-74) Until now, two receptors for APN have been identified: AdipoR1 which is mainly expressed in skeletal muscle and AdipoR2 which is predominantly occurring in the liver. (16, 73)

During pregnancy, hypoadiponectinemia occurs physiologically. (7) Maternal serum APN concentrations decrease steadily, resulting in a 60 percent reduction of *APN mRNA* in white adipose tissue. In comparison, as pregnancy progresses increased APN levels in cord plasma increase, suggesting that the placenta may be another site of APN synthesis. (72) In fact, some studies detected APN in the human placenta, predominantly in the syncytiotrophoblast, at term. (1, 75) However, if the placenta certainly contributes to APN synthesis still remains a debatable topic since other authors did not detect *APN mRNA* expression in the placental tissue. (4, 76)

APN has been suggested to act anti-inflammatory by suppressing lipopolysaccharide-stimulated TNF-α production by macrophages, stimulating the secretion of anti-inflammatory interleukin interleukin (IL)-10 and enhancing apoptotic cell clearance by promoting the action of macrophages M2. (4, 74) Moreover, APN is also suggested to be an insulin-sensitizing adipokine and thus is thought to play a major role in regulating glucose and lipid metabolism. (73, 74) APN also acts antiatherogenic and is considered to be involved in the prevention of fatty liver disease and liver fibrosis. (4, 73) Additionally, plasma APN levels show a positive correlation with HDL cholesterol levels and are associated negatively with triglyceride levels. (73) Importantly, the highest biological activity is attributed to the high molecular weight APN. These multimers are considered to have the most impact on regulating glucose homeostasis and may have, in comparison to total APN, a higher association with the development CVDs, insulin resistance and metabolic disorders. (74)

The levels of APN are dependent on multiple factors such as gender, age and lifestyle. (73) For instance, APN concentrations are inversely proportional to adipose tissue or rather BMI and thus appear low in overweight persons. (1, 74) Low APN levels are present in patients

with T2DM, hypertension, insulin resistance and left ventricular hypertrophy. (73) Additionally, the expression of this adipokine is also lowered through β-adrenergic stimulation, glucocorticoids and oxidative stress. (73, 74)

3.3.1.1 Adiponectin in GDM

As already mentioned earlier, APN is considered to have insulin-sensitizing effects. (72, 73, 77) APN increases insulin sensitivity through its anti-inflammatory actions that have already been spoken about in the paragraph above. (74) It also enhances glucose uptake in skeletal muscle, decreases hepatic gluconeogenesis and promotes receptor/post-receptor insulin signalling. (4, 73, 74) APN influences insulin signal transduction by activating AMP-activated protein kinase (AMPK) which subsequently promotes the activation of IRS-1. IRS-1 is normally activated by binding of insulin to the insulin receptor. This activation promotes a signalling cascade which finally leads to GLUT4 translocation and thus glucose is transported into GLUT4 expressing cells. (Figure 3). (1)

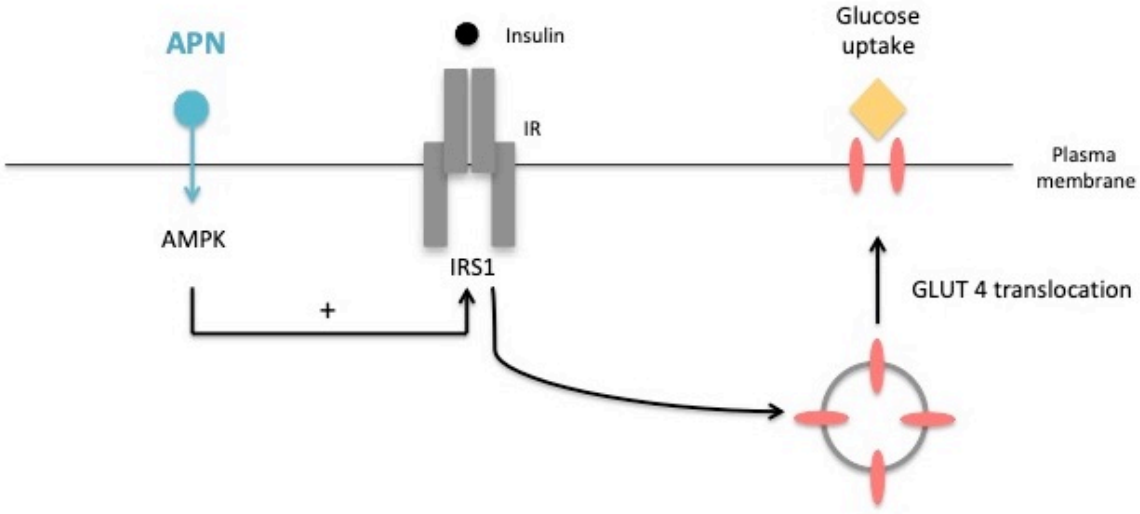

Figure 3 Insulin signalling and the role of APN (Modified from (1)).

APN also increases fatty-acid oxidation by activating gelatin binding protein of 28 kDa (PPARα) which results in a decline of triacylglycerol levels in liver and skeletal muscle subsequently leading to enhanced insulin sensitivity. (1, 73) Moreover, APN leads to enhanced insulin gene expression and exocytosis of insulin granules from β-cells and thus increases insulin secretion. (1)

Several clinical studies have revealed that women with GDM show lower concentrations of serum APN compared to women with normal glucose metabolism during pregnancy. (78-87) After reviewing 30 studies Fasshauer et al. came to the result that APN levels are lower in GDM compared to healthy controls, since the majority of studies identified higher levels whereas only 5 studies did not identify a difference. (88) Similar results have been stated in a retrospective meta-analysis by Xu et al. including 27 studies where in GDM pregnancies significantly lower maternal APN levels were detected compared to healthy controls after the impact of BMI was excluded. (95) An explanation for these low levels of APN is that GDM is a low-grade inflammatory state and released cytokines such as TNF-α and IL-6 inhibit the transcription of APN in adipocytes. (72, 73) In addition, hyperinsulinemia, which is associated with GDM, decreases APN levels (Figure 4). (72) These findings suggest that decreased APN levels have great impact on the development of GDM. (1)

Additionally, Chen et al. detected APN and its two receptors in the syncytiotrophoblast of the placenta. Lower concentrations of *APN mRNA* and higher amounts of APN receptors in placental tissue were found in normal weight women with GDM. This decreased placental APN gene expression is considered to contribute to lower plasma APN levels in GDM patients. (75) On the other hand, Lappas et al. did not detect any differences in placental APN secretion between GDM and healthy pregnancies. (89) Retnakaran et al. not only revealed a connection between low APN levels and insulin resistance in GDM but also discovered that low APN impairs β-cell function. (90) Lain et al. discovered that women with lower first trimester APN levels were more likely to subsequently develop GDM suggesting that APN concentrations in early pregnancy may be a useful predictor for GDM. (91) Moreover, two other studies revealed that women who had been diagnosed with GDM showed lower postpartum APN levels than women with normal glucose tolerance during pregnancy. (92, 93) Cortelazzi et al. detected decreased APN levels in fetuses whose mothers suffered from GDM during pregnancy in comparison to healthy fetuses with the same gestational age and independent of birth weight. (83) Thus, in a review by Miehle et al. was concluded that decreased APN levels seen in GDM patients are possible to contribute to the development of more severe insulin resistance in this disease. However, according to the authors more studies are needed to investigate the direct effects of this adipokine on the pathophysiology of GDM. (72)

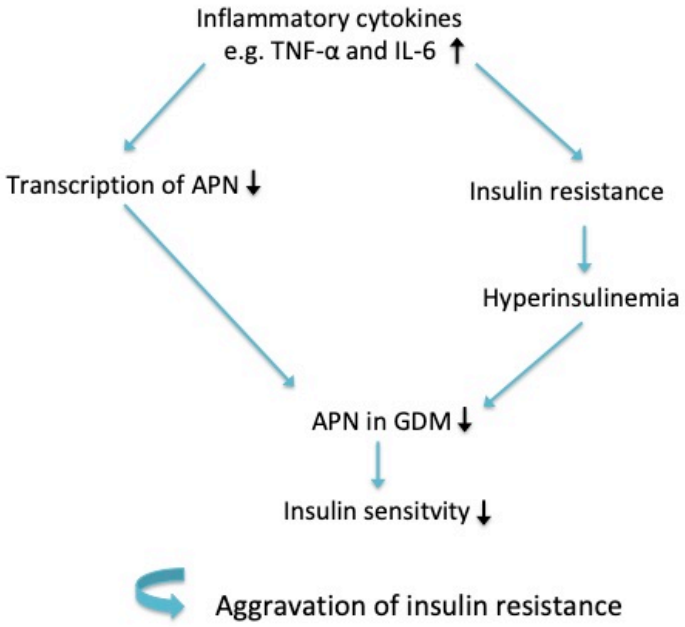

Figure 4 The role of APN in the pathophysiology of GDM (Modified from (72)).

3.3.2 Leptin

Leptin is a 16 kDa protein hormone, consisting of 167 amino acids, which is encoded by the *ob* gene. (73, 74, 94) Leptin is mainly synthesized by white adipose tissue but also the placenta, ovaries, mammary epithelium, bone marrow and lymphoid tissues are leptin sources. (17, 73, 74) It is delivered across the blood-brain-barrier and acts by binding to leptin receptors (ObRs) in the central nervous system, which are predominantly localised in hypothalamic neurons of the arcuate nucleus. (72-74, 88) At present, six splice variants of ObRs have been identified, from which only four exist in humans: the long (ObRb), the short (ObRa and ObRc) and the secretory (ObRe) isoforms. The ObRb receptor is thought to be the most relevant one, since it plays a major role in intracellular signal transduction. Besides intracellular signalling routes such as PI3K, AMPK and mitogen-activated protein kinase (MAPK), JAK/STAT signalling is the most important signal transduction pathway of leptin. (73)

Leptin is also called “satiety hormone” since it has great impact on the regulation of food intake and energy balance by binding to ObRs of appetite-modulating neurons. (4, 72, 74) Additionally, it plays a central function in the regulation of endocrine functions and the

reproductive system since it promotes gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus and LH and follicle-stimulating hormone (FSH) secretion from the pituitary gland. (72, 74) During pregnancy leptin plays an important role in implantation but also promotes hCG synthesis in trophoblast cells. Furthermore, leptin increases mitogenesis and amino acid uptake of throphoblasts and thus, promotes placental growth. (72) Leptin acts insulin-sensitising as it enhances insulin secretion in β-cells, glucose transport and utilization and also glycogen synthesis. Leptin also stimulates fatty acid metabolism, regulates inflammation, immune response and angiogenesis and also activates the sympathetic nervous system. (72, 74)

Leptin levels are, among others, under the regulation of insulin and cortisol but also do positively correlate with fat mass and BMI. (4, 17, 72) In other words, weight gain and hyperinsulinemia lead to increased leptin concentrations. (76) It has also been shown that ob/ob mice with deficits in leptin show hyperphagia and tend to develop obesity. High leptin levels are also associated with reduced insulin sensitivity. Vice versa, weight loss, fasting and hunger lead to decreased leptin concentrations and has insulin-sensitising effects. (72, 74) Interestingly, severely overweight individuals become leptin resistant and exogenous supply does not reduce body weight. (4, 88)

During pregnancy leptin levels are steadily increasing reaching concentrations that are two to three times higher than in the non-pregnant state. (17, 72, 95) This rise starts from earliest stages of pregnancy, indicating that this surge is not only due to gestational weight gain. (72) The highest values are detectable in the 28th week of pregnancy, at which point a plateau is reached. Leptin levels decline again shortly after birth reaching soon pre-gravid leptin concentrations. (4, 72) One explanation for the occurring surge in leptin during pregnancy is that maternal fat stores are broken down and the released lipids can be transported across the placenta to supply the fetus. (72) Elevated leptin levels during pregnancy are thought to be mainly due to placental leptin production which is upregulated by IL-6 and TNF-α and also, to some extent, to fetal leptin production which starts in the early second trimester. Moreover, in pregnancy the maternal body becomes resistant to leptin which promotes rising leptin levels. (17, 72, 95) There is evidence in rats that leptin transport into the brain is decreased during pregnancy which may contribute to the development of leptin resistance. (72) Shedding of the placental membrane leads to a release of soluble leptin receptors to which leptin is also able to bind. Soluble leptin receptors then can capture free leptin, which reduces its bioavailability, and delays leptin clearance in the bloodstream, which may also explain the high leptin concentrations during pregnancy. (17)

3.3.2.1 Leptin in GDM

Generally, pregnancy is a state of low-grade inflammation, which is even more severe in GDM pregnancies since proinflammatory cytokines are elevated. Increased leptin levels in GDM have been considered to be responsible for this surge in cytokines such as TNF-α, IL-6 and CC-chemokine ligands levels resulting in a chronic inflammatory state. TNF-α is a molecule well known for its role in causing insulin resistance. This developed chronic inflammatory environment amplifies hyperleptinemia even more. Hyperinsulinemia in GDM additionally enhances leptin synthesis in adipocytes. A vicious circle is created which aggravates the inflammatory situation (Figure 5). (72)

As mentioned above, leptin levels are constantly rising in normal pregnancy and hyperleptinemia is thought to be even more severe in GDM. (4) Hyperleptinemia is believed to interfere with insulin-dependent glucose transport to adipocytes and also to suppress insulin secretion in pancreatic β-cells. (96, 97) In fact, several clinical studies revealed that patients with GDM, at different gestational weeks, showed higher leptin levels than controls. Study sizes ranged from 51 to 134 participants. (98-106) Gao et al. and Qiu et al. also consider hyperleptinemia a biomarker in early pregnancy to predict GDM development. (102, 107) D'Anna et al. discovered that women who subsequently developed GDM had higher leptin concentrations in amniotic fluid than healthy controls and that those values are directly related to amniotic fluid insulin levels. (108) On the other hand, other studies did not detect any differences in maternal leptin between GDM pregnancies and pregnancies with normal glucose tolerance. These studies included between 34 and 119 participants. (85, 109, 110) One small study with 38 participants described reduced leptin levels in GDM to healthy controls. (111) In a review by Fasshauer et al. including 27 studies investigating differences in leptin concentrations between GDM patients and healthy controls, 15 studies identified no difference between the two groups, 11 identified higher leptin levels in the GDM group and 1 study detected lower leptin levels in the GDM group. Thus, they concluded that data concerning leptin concentrations and GDM are inconsistent, but it is likely that leptin is dysregulated in GDM and circulating levels of this adipokine predict GDM development. (88) However, in a systematic review and meta-analysis by Xu et al. including 27 studies was revealed that maternal leptin levels in GDM patients are significantly higher in comparison to controls. (95)

Since leptin and its receptor are expressed in the placenta, various studies investigated differences in *leptin mRNA* and receptor expression between GDM and healthy pregnancies.

(88) Several studies revealed increased levels of mRNA for leptin and the leptin receptor in placentas of GDM patients. (112-115) However, two authors did not detect any differences. (63, 116) Kleiblova et al. discovered that *leptin mRNA* is upregulated in visceral but not in subcutaneous fat tissue of pregnant women with GDM compared to glucose tolerant controls (63) whilst Kuzmicki et al. did not detect any differences in leptin mRNA expression, neither in visceral nor in subcutaneous fat tissue. (116)

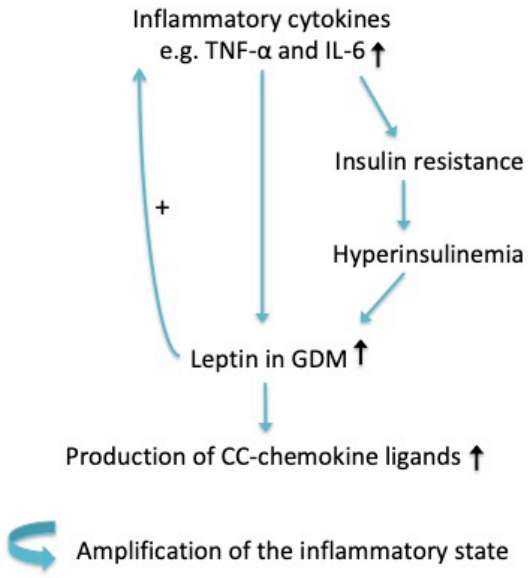

Figure 5 The role of leptin in the pathophysiology of GDM (Modified from (72)).

3.3.3 TNF-α

TNF-α is a proinflammatory adipokine that is produced by adipose tissue, monocytes and macrophages. (4, 88) TNF-α plays a major role in inflammation and autoimmune diseases. (88) It affects cell differentiation and leukocyte recruitment, acts proinflammatory by binding to TNFR1, promotes oxidative stress and thus cell degeneration. TNF-α also increases prostaglandin synthesis by stimulating cyclooxygenase 2 (COX-2) and, by activating caspases, promotes the apoptosis of inflamed cells. (4)

TNF-α mRNA and proteins in human adipose tissue are usually at low levels. (88) However, its concentration has a positive correlation with obesity and declines after body mass reduction. (4) High TNF-α levels are also related to insulin resistance in adiposity and the

development of T2DM, hyperinsulinemia, aging, sepsis, muscle damage, burns and preeclamptic pregnancy. (4, 23, 33, 94)

During gestation, TNF-α is also synthetized and secreted by the placenta. (94) *TNF-α mRNA* and proteins have been detected in early and late pregnancy in the placenta and also in cells of the uterus. (117) In early stages of pregnancy, TNF-α levels are low but show a peak in the third trimester. (94) Interestingly, insulin sensitivity changes in the exact opposite way during pregnancy. (1, 94) The greatest part of placental TNF-α (94 percent) is secreted asymmetrically into the maternal circulations and only a small amount (6 percent) is released to the fetal side. It is believed that the placenta is the most important source for TNF-α production during gestation since its levels are immediately decreasing when the placenta is removed after delivery. (94)

3.3.3.1 TNF-α in GDM

Since low-grade inflammation has been associated with obesity, T2DM and GDM, pro-inflammatory cytokines such as TNF-α are known to play a central role in the pathophysiology of insulin resistance. (1, 118) TNF-α impairs glucose transporter gene expression and insulin signalling and thus decreases GLUT4 expression on insulin sensitive cells, such as adipocytes, skeletal and cardiac muscle cells. (1, 33, 118) It does so by interfering with insulin receptor autophosphorylation. (23) Moreover, after binding to the pro-inflammatory receptor, TNF-α acts by activating protein kinase C (PKC) through IκB kinase (IKK) which hence blocks IRS-1 activation. (1) TNF-α also promotes insulin resistance by increasing serine phosphorylation of IRS-1 which affects the connection to the insulin receptor. (1, 23) It has been shown that in late pregnancy, tyrosine phosphorylation of the insulin receptor and IRS-1 in the muscle cell is disrupted and serine phosphorylation is increased. (23) This means that tyrosine phosphorylation of insulin receptor and IRS-1 is suppressed which results in diminished insulin receptor tyrosine kinase activity. (4) Thus, TNF-α inhibits the action of the insulin receptor and the signalling pathway to GLUT4 translocation and thus glucose uptake is interrupted. (1) Additionally, TNF-α activity is thought to contribute to the pathogenesis of diabetes mellitus since it is involved in apoptosis of pancreatic β-cells (118): Stanley et al. discovered that TNF-α antagonism with etanercept leads to a decline in glucose levels but does not affect insulin sensitivity in an observation period of six months in patients with metabolic syndrome. (119)

According to current knowledge, TNF-α levels are thought to increase during pregnancy and show a peak in the third trimester. (4) Interestingly there is evidence that serum

concentrations are even higher in GDM pregnancies. (4, 88) Fasshauer et al. performed a review where, among others, TNF-α involvement in GDM was investigated. It was revealed that in ten out of eleven studies in GDM women in the second or third trimester significantly higher TNF-α values were measured than in normal glucose tolerance pregnancies and thus condensed that this adipokine is elevated in GDM and that this circumstance contributes directly to the development of this disease. (88) Similar results have been stated in a retrospective meta-analysis by Xu et al. including 27 studies where in GDM pregnancies significantly higher TNF-α levels were found compared to healthy controls after the impact of BMI was excluded. (95) Based on these results, it can be assumed that TNF-α plasma concentrations are increased in GDM pregnancies. (88, 95)

Lappas et al. investigated TNF-α release from placental tissue, fat tissue and skeletal muscle between GDM and healthy pregnancies and did not identify any differences in these tissues between the two groups. (120) In a small study by Kirwan et al. with 15 participants, TNF-α is considered a significant predictor for the development of insulin resistance in late pregnancy. Based on these findings, they also question the hypothesis that placental-derived reproductive hormones are responsible for insulin resistance during pregnancy. (94) By contrast, Georgiou et al. performed a small case-control study of 14 cases and 14 controls regarding GDM predictive screening biomarkers. They were not able to find a significant association between first trimester TNF-α values and the risk of subsequently developed GDM. (121) Radaelli et al. discovered that TNF-α receptor expression is elevated in the placental tissue of GDM patients in comparison to normal glucose tolerant controls. (122) In a study by Kleiblova et al. any differences in placental TNF-α expression and *mRNA* production in visceral and subcutaneous fat tissue could not be detected between GDM and healthy pregnancies. (26)

3.3.4 Resistin

Resistin, an acronym for “resistance to insulin”, is a 12.5 kDa polypeptide hormone consisting of 108 amino acids. (73, 77, 123) This signalling molecule is expressed in adipocytes and stromal cells of fat tissue, peripheral blood mononuclear cells, macrophages, islet cells of the pancreas, skeletal muscle cells, bone marrow cells and in hepatic cells. (23, 73, 77, 123) During pregnancy, resistin is also synthetized in trophoblastic cells of the placenta and its levels increase steadily and reach highest levels especially in the third trimester. Interestingly, the majority of resistin production during pregnancy is due to synthesis in placental tissue rather than abdominal and subcutaneous fat tissue. (73, 123)

Resistin is considered to play a role in inducing insulin resistance, inflammatory processes, endothelial dysfunction, thrombosis, angiogenesis, and smooth muscle dysfunction. (77, 88) Resistin values are under control of several regulatory molecules and hormones. Its concentrations are dependent of nutritional status, pregnancy, sex, gonadal status and thyroid hormones. Additionally, resistin levels positively correlate with insulin. Insulin enhances resistin gene and protein expression and hyperinsulinema increases resistin levels in obese diabetic and normal glucose tolerant individuals. Moreover, food intake increases, and food restriction decreases *resistin mRNA* expression in adipose tissue. Peroxisome proliferator-activated receptor (PPAR), a known down-regulator of resistin gene expression, is found in low concentrations during pregnancy resulting in increased resistin expression. (123)

3.3.4.1 Resistin in GDM

Resistin is known to cause insulin resistance in muscle cells, β-cells, adipocytes and hepatocytes. It interferes with the insulin signalling cascade in β-cells of the pancreatic islets which results in impaired glucose-induced insulin secretion. It decreases GLUT4 translocation and thus glucose uptake in skeletal muscle cells and alters glycogen metabolism and insulin sensitivity in hepatocytes (Figure 6). (123)

However, according to a review by Fasshauer et al. data about differences in circulating resistin levels in GDM patients and non-GDM patients are conflicting. (88) Due to conflicting study results, a recent meta-analysis by Hu et. al investigated the relationship between maternal serum resistin values and the risk of developing GDM. 18 studies with 1041 cases and 1292 controls were included, and overall analysis revealed that elevated resistin values during pregnancy are in fact a risk factor for GDM, with resistin levels even correlating with the severity of the disease. (124) A recently published study by Kapustin et al. reports that serum resistin levels in the first and third trimester in diet and insulin treated GDM patients do not only increase, but also associate with HbA1c values. (125) Chen et al. additionally discovered that after delivery, resistin levels were significantly lower in both study groups but one and three days after parturition, GDM women still had higher resistin levels than women who did not have GDM. (126) Takhshid et al. reported that single nucleotide polymorphism-420C/G of resistin gene more often occurs in GDM patients in comparison to non-GDM individuals. However, they were not able to find any differences in serum resistin values between GDM and normal glucose tolerance pregnancies. In this study 75 cases and 70 controls were included. (127) A study by Bawah et al. revealed that resistin levels show a significant increase between the 11^{th} and 13^{th} gestational week and

indicating that hyperresistinemia is considered a useful predictive biomarker for subsequent GDM development. (128) In a study by Gürlek et al. was demonstrated that resistin in saliva measured between the 24th and 28th weeks of pregnancy, were significantly elevated in the GDM group compared to controls. They thus consider saliva determination between gestational week 24 and 28 weeks a possible screening tool for GDM. (129)

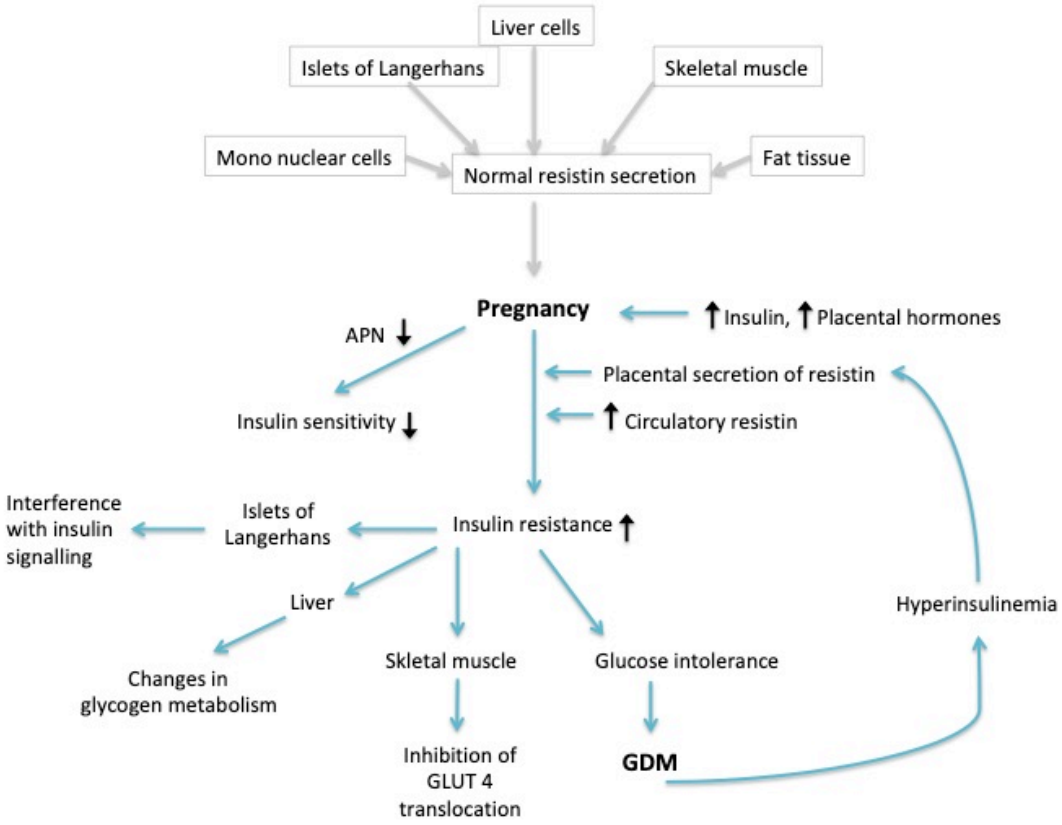

Figure 6 The role of resistin in the development of GDM (Modified from (123)).

3.3.5 Visfatin

The adipokine visfatin is protein of 52 kDa and is also termed pre-B cell colony enhancing factor (PBEF) or nicotinamide phosphorybosyltransferase (NAMPT). (72, 73, 88, 130) It derives from a gene on the long arm of chromosome 7 (7q22.2). (130) Visfatin has been known to be predominantly synthetized and expressed in human and mouse visceral adipose tissue. (72-74, 88, 130) Additionally, it has also been found in subcutaneous fat, in the placenta, myometrium, bone marrow, liver, muscle, heart, lung, kidney, immune cells such as macrophages and neutrophils, and in fetal membranes. (72, 130)

The functions of Visfatin are debatable but it is considered to play a role in energy homeostasis. (73, 130) Hyperglycemia and obesity are correlated with a rise in visfatin

plasma levels which are indeed elevated in subjects with T2DM, BMI above 30, metabolic syndrome and CVD. Visfatin is suggested to play a role in the pathophysiology of GDM. (130) It is considered to have similar effects as insulin, i.e. causing hypoglycaemia, since it has a specific binding site on the insulin receptor, which is distinct to the binding site of insulin. Thus, visfatin decreases hepatic glycogenolysis and promotes glucose utilization in cells of the adipose tissue and muscle cells. (74, 130, 131) However, the insulin-like functions of this adipokine still remain controversial. (88) Moreover, there is some evidence that visfatin plays a further role in glucose metabolism since it impairs the function of β-cells and interferes with the regulation of genes that are involved in oxidative stress. (88, 130) In addition, it acts antiapoptotic and may play a part in inflammatory response and the circadian rhythm. (72, 88) Thus, treatment of fetal membranes with recombinant human visfatin leads to a significant release of IL-1β, IL-6 and TNF-α. (72)

Visfatin is also present in breast milk and during pregnancy it is released from the amniotic epithelium. (72, 130) Pregnant women of normal weight show a surge in visfatin plasma values between the 19th and 26th week of pregnancy whereas the lowest levels are measured between the 27th and 34th week. (72)

3.3.5.1 Visfatin in GDM

Several publications observed higher visfatin serum values GDM cases as compared to healthy controls. (131-134) According to Souvannavong-Vilivong et al. this rise in visfatin is a compensatory mechanism to glucose intolerance since visfatin, as described above, may have insulin mimetic functions. (131) Furthermore, Bawah et al. revealed that in GDM, hypervisfatinemia occurs between the 11th and 13th week of pregnancy and thus should be considered a useful biomarker to predict GDM development in advanced gestation. (128) Interestingly and in contrast to that, Park et al. stated that decreased visfatin levels in the circulation may be predictive for GDM. (135) Tsiotra et al. discovered lower visfatin levels in obese GDM patients compared to normal weight and glucose tolerant women during pregnancy. (136) Feng et al. reported that besides enhancing insulin secretion, visfatin also promotes β-cell proliferation. Since failure of β-cell mass adaption during pregnancy is known to result in glucose intolerance, insufficient levels of visfatin during gestation may play an important role in the development of GDM. (137) To studies of Lobo et al. and Görkem et al. were not able to reveal any differences between serum visfatin values between overweight patients with GDM and normal glucose tolerant individuals. (138, 139) After

reviewing several studies regarding visfatin levels in GDM and non-GDM pregnancies Fasshauer et al. also came to the result that data is highly controversial. (88)

Due to these discrepancies, Zhang et al. performed a systematic review and meta-analysis concerning the relationship between visfatin levels and GDM. They included 26 studies that reported either up-regulation, down-regulation and no difference in visfatin levels between GDM and normal glucose tolerance pregnancies in their meta-analysis and came to the result that there are no significant differences in visfatin plasma levels between non-GDM and GDM patients. Nevertheless, the authors speculated that visfatin, not independantly but in interaction with other adipocytokines such as adiponectin, leptin and resistin, could contribute to GDM development. Since visfatin is strongly related to fat mass, the higher visfatin levels in GDM patients that were reported in some studies may result from heterogeneity of obesity between cases and controls. (140) In a more recent systematic review and meta-analysis by Pace et al., where the association between serum visfatin values in the second and third trimester and GDM were evaluated, similar results were obtained. (141) Interestingly, Zhaoxia et al. discovered a surge of visfatin in GDM and non-GDM subjects after the glucose load within an OGTT. Visfatin increased to a lower extent in GDM patients which may reflect impaired regulation of blood glucose values. (133)

3.3.6 Apelin

The hormone apelin derives from the human apelin gene which encodes a precursor protein called pre-proapelin. (142) Subsequently, from this precursor certain isoforms of apelin are produced: Apelin-36, apelin-17, apelin-13 and pyroglutamate-apelin-13. (73) (142) All isoforms are able to bind to the apelin receptor, which is a class G protein coupled receptor. (73, 142) Apelin and its receptor can be found in several tissues such as heart, endothelium, lung, adipocytes, gastrointestinal tract, central nervous system, mammary gland and placental tissue. (73, 142, 143)

Apelin plays a role in cardiovascular system where it acts vasodilatory, hypotensive and positive inotrope, and also regulates fluid homeostasis by acting antidiuretic. (88, 142) Additionally, it plays an important role in angiogenesis, stress response, neuroprotection, glucose homeostasis, digestive absorption and motility, and satiety. (74, 142) Apelin levels positively correlate with hyperosmolality, dehydration and hypoxia whereas fasting leads to decreased apelin plasma levels. (142) In mice and human, insulin is an important regulator of apelin since it increases apelin expression and secretion. (73, 74) Additionally, altered

apelin plasma values occur in T2DM, obesity, CVDs, impaired fluid homeostasis, cancer and tumor angiogenesis. (73, 74, 142)

3.3.6.1 Apelin in GDM

As already mentioned above, apelin is involved in the regulatory process of glucose metabolism as it enhances glucose uptake and insulin sensitivity. Apelin has been reported to diminish glucose transport in enterocytes through blocking sodium-dependent glucose transporter-1 (SGLT-1). Hyperapelinemia in obese and type 2 diabetic subjects may indicate a correlation between increased apelin levels and altered glucose homeostasis. (143) However, the existing data concerning the relationship of plasma apelin levels and GDM are inconsistent. (88) A study by Aslan et al. revealed that women with GDM have increased serum apelin-36 values compared to healthy women. (144) Similar results have been observed by Guo et al. concerning second trimester apelin plasma levels. (145) On the other hand, Boyadzhieva et al. revealed decreased apelin levels in GDM patients compared to healthy controls. (146) Oncul et al. and Telejko et al. did not detect differences in plasma apelin levels between GDM and non-GDM subjects. (147, 148) A recent meta-analysis of 20 studies by Sun et al. including 1493 GDM and 1488 non-GDM women is consistent with these findings, since they did also not reveal any differences in serum apelin levels. (143) Interestingly, Oncul et al. observed lower cord blood apelin values after GDM pregnancy. (147) However, Aslan et al. determined similar cord blood levels in GDM and controls. (144)

3.3.7 Adipocyte fatty acid-binding protein

Adipocyte fatty acid-binding protein (AFABP), which is also called adipocyte protein 2 (AP2) or fatty acid binding protein 4 (FABP4), is highly expressed in the cytoplasm of mature adipocytes, where it acts as an important carrier protein for fatty acids. (74, 149-151) It also induces lipolysis by promoting the activation of hormone-sensitive lipase. (151) Besides that, it is found in macrophages, endothelial cells and also the placenta, where its expression is up regulated by hypoxia. (88, 152) During differentiation of adipocytes, AFABP expression increases and its transcription is under the regulation of peroxisome proliferator- activated receptor gamma (PPARγ) agonists, unsaturated long chain fatty acids, dexamethasone and insulin. (150, 151) In animal studies, AFABP has been shown to be involved in glucose metabolism, firstly because it increases hepatic glucose production in

mice, thereby interfering with glucose tolerance, and secondly because a deletion of the *AFABP* gene prevented the development of insulin resistance and hyperinsulinemia. (150) AFABP also plays an important role in lipid metabolism and inflammation. (152) Abnormal AFABP values may be associated with obesity, high waist circumference, hypertension, T2DM, oxidative stress, atherosclerosis and insulin resistance. (150-152) Additionally, it is considered a predictive biomarker for the development of metabolic syndrome, acute coronary syndrome and T2DM. (88, 149, 150)

3.3.7.1 AFABP in GDM

Several publications revealed that serum AFABP values are increased in GDM patients compared to healthy controls. (149, 153-157) Zhang et al. also consider AFABP a potential risk factor for GDM development. (149) According to a recents meta-analysis by Sun et al. including 31 studies with a total number of 4590 participants, AFABP is elevated in GDM and is thus suggested a useful predictive biomarker and screening parameter to detect GDM. (156) Li et al. performed a study investigating distinct AFABP expression in placental tissue and decidua comparing GDM and non GDM subjects. They detected significantly higher AFABP concentrations in the GDM group and suggested that placental hormones such as progesterone and hPL, which are constantly rising until term, may be responsible for increased decidual and placental AFABP expression in GDM patients. They also suggested that higher serum AFABP in GDM patients may derive from adipocytes and placenta. Placental hormones are also able to enhance AFABP expression in adipocytes and block adiponectin. The interaction of placenta and adipocyte derived AFABP may induce insulin resistance and GDM. (151) Oliva et al. reported decreased AFABP concentrations in omental adipose tissue in GDM compared to non-GDM subjects which may indicate that lipolysis is increased in GDM pregnancies. (158) Patro-Małysza et al. discovered an increase in AFABP umbilical cord blood values in neonates of GDM women and stated that these values are directly correlated to maternal serum AFABP concentrations but also to maternal periconceptional and periparturient BMI and maternal metabolic parameters. (159)

3.4 GDM and fetal sex

The baby's gender has previously been reported to have an important influence on the course and complications during pregnancy and delivery and is thus, also increasing the risk for adverse obstetrical and perinatal outcomes. (160, 161) Indeed, pregnancy carrying a boy is associated with higher risk for fetal macrosomia, premature rupture of membranes, pre-term delivery, lack of progress in the first and second stages of delivery, pathological heart rate patterns of the fetus, umbilical cord prolapse, true umbilical cord knot, cesarean section and lower values in the Apgar score. (160, 162) Intriguingly, it was recently noticed that besides the main risk factors for GDM including, pre-pregnancy overweight/obesity, severe weight gain during early/mid gestation, particular ethnicity, higher maternal age, history of previous GDM and positive familial anamnesis of diabetes, fetal gender may also affect the extent of maternal insulin resistance throughout pregnancy. (163, 164)

In several retrospective studies was, among others, revealed that being pregnant with a male fetus is associated with higher rates of developing GDM and thus stated that male fetal sex can be considered as an independent risk factor for adverse gestational outcome. (162, 165-168) Retnakaran et al. conducted a retrospective cohort trial with a study population of 642,987 first-time pregnant women, who had a live birth singleton pregnancy. They similarly observed that women carrying a male fetus, were more likely diagnosed with GDM in their first and second pregnancy. They also observed that women with a normal glucose tolerant first female pregnancy and then being pregnant with a boy in their second, showed a higher risk of developing GDM. Interestingly, GDM women delivering a girl after their first pregnancy were more often diagnosed with T2DM before being pregnant for the second time. (169) Similar findings, stating that fetal sex might be a previously unknown factor playing a major part in maternal glucose metabolism during pregnancy, have been published in a subsequent prospective study by the same authors. Thus, they demonstrated once again that male pregnancies were at modestly higher risk for GDM development after adjusting for classic GDM risk factors. Furthermore, pregnancies with boys were associated with higher mean adjusted glucose levels in an OGTT measured at 30 minutes, one hour and two hours after glucose load, compared to pregnancies with girls. Pregnant women carrying male fetuses also showed worse β-cell function compared to the female gender group. (170) However, there are retrospective database analyses (171, 172, 173) and prospective cohort studies (163, 174) who did not support the hypothesis that fetal sex is linked to GDM in the mother, since there were no differences regarding GDM risk between male and female

pregnancies detected. Due to the conflicting study results, Jaskolka et al. conducted a meta-analysis regarding the association of fetal sex and the risk of GDM, including 21 controversial studies with a total of 2,4 million study participants. However only 6 out of 21 studies confirmed a relationship between GDM and fetal sex they came to the result that male pregnancies have a 4 percent higher relative risk for GDM development compared to female pregnancies. Although this increase in risk seems modest, it provides important evidence that the fetus and the mother are able to influence each other's metabolism. (160) This is in line with the data published by Broere-Brown et al. in a more recent meta-analysis, where 28 studies with over 2,1 million participants were included. (175) In addition to that, Giannubilo et al. conducted a prospective observational cohort study and detected higher risk of need for insulin therapy after GDM diagnosis in male carrying women. (176)

In the ROLO RCT with 582 participants, the association between fetal sex and insulin resistance was investigated. In a secondary analysis was among others revealed that women carrying a female infant were significantly less insulin resistant in the first and second trimester as they had lower Homeostasis Model Assessment (HOMA) indices at this stage of pregnancy. (164) Xiao et al. conducted a prospective cohort trial with 299 study participants to also investigate if fetal sex is related to maternal insulin resistance throughout gestation. By contrast, after the adjustment for maternal age, parity, race, pre-gestational BMI, education, history of GDM, smoking and alcohol use, and gestational age at blood draw, a greater tendency to insulin resistance was detected in women carrying a female compared to those carrying a male infant, since there were significantly higher insulin concentrations and significantly lower glucose-to-insulin ratios, at comparable measured glucose values. (174) Similar findings have been published by Yamashita et al., who conducted a 617 participants retrospective cohort study and discovered greater insulin resistance and lower insulin sensitivity in pregnant Japanese women carrying females compared to carrying males during mid- and late pregnancy. (173)

3.5 Placental hormones and fetal gender

3.5.1 HCG

Several studies with study populations between 69 and 560 participants have shown that maternal serum hCG values are significantly elevated in advanced gestation in female bearing women in comparison to male bearing women. (177-181) Significantly elevated hCG levels in female pregnancies were also reported in multiple other studies with variable cohort sizes, in the first and/or in the second trimester. (182-190) In a very recent study by Lin et al. maternal serum hCG levels were determined on day 14 and day 21 of gestation and already at this early stage in pregnancy hCG was elevated in female-bearers compared to male-bearers. (191)

However, in two other studies no association between child sex and first trimester hCG levels has been shown. (192, 193) Additionally, Zheng et al. did not detect any gender-specific differences in hCG values between the 15th and 20th week of pregnancy. (194) Spellacy et al. measured maternal hCG values zero to four days before delivery and were not able to show any differences between male and female pregnancies. (195) These findings are in agreement with those of Al Atawi et al., where hCG was measured at different stages of pregnancy and no correlation with fetal sex could be found. (196) However, most of the studies who detected higher hCG levels in female pregnancies contained larger cohorts (188-190), with the largest including about 1,1 million samples (182), while those that did not detect a sex difference only included between 36 and 338 study participants. (192-196)

Furthermore, Steier et al. and Gol et al. measured hCG values in cord blood after delivery and discovered significantly elevated concentrations in the case of a female compared to a male baby. (197-199) In addition, Yuen et al. discovered that the concentration of hCG was measured significantly higher in the cord blood of female newborns during the 29th and 36th week. (200)

3.5.2 Progesterone

Wuu et al. were able to detect lower progesterone values in women being pregnant with girls compared to boys at the time of 16 and 27 gestational weeks. (201) Moreover, Adamcová et al. analyzed, among others, 17-OH-pregnenolone in pregnant women, at the time of 37 weeks, and detected significantly higher values in maternal plasma but not in mixed cord blood when carrying a male fetus. (202) By contrast, several other studies observed no

differences in maternal progesterone depending on fetal sex. (177, 179, 193, 195-197, 203, 204), suggesting that maternal progesterone levels do not depend on fetal sex.

In umbilical cord however, Hagemenas et al. revealed higher progesterone values in female fetuses. (204) By contrast, Antonipillai et al. and Shaxted et al. did not discover any sex-specific differences in progesterone values in either umbilical artery or umbilical vein serum. (205, 206) Another study by Hagemenas et al. did not detect a difference between umbilical vein progesterone values and fetal sex, however female fetuses showed a more distinct difference between umbilical artery and umbilical vein progesterone concentrations than male fetuses. (207) The same authors also conducted a study to evaluate the correlation between fetal gender and placental progesterone synthesis but did not identify any differences. (208)

3.5.3 Estrogens

Järvelä et al. observed that E2 values between the 6th and 10th week of pregnancy are significantly increased in women bearing girls compared to those bearing boys. (203) Similar results have been published by Toriola et al., who detected 9 percent higher E2 values in pregnancies with girls versus pregnancies with boys. (209) However, Lutterodt et al. analyzed E2 levels in the first trimester, Vlková et al. between 8- and 21-weeks' gestation and Al Atawi et al. in the first, second and third trimester and after delivery and all studies did not detect any differences according to fetal gender. (193, 196, 210) Additionally, Haning et al. did not detect any significant sex differences in the 3rd and Barrett et al. in the 1st trimester of pregnancy regarding maternal E1, E2 and E3 levels. (177, 211)

Also, in umbilical artery and vein E1, E2 and E3, Antonipillai et al. observed no difference. (177, 205) No relationship between fetal sex and plasma E2 (197, 212) and E3 (200, 213) values was also found in individual studies. Warne et al. measured, among other factors, E2 and E3 values in amniotic fluid at different stages of pregnancy and did not determine any gender-specific differences. (214)

3.5.4 HPL

Houghton et al. analyzed increased hPL mean levels in maternal and umbilical cord blood in female compared to male pregnancies, however levels were only considered significantly different in cord blood. (215)

3.5.5 PRL

A study by Yuen et al. investigated whether fetal sex influences umbilical cord hormone levels and did not identify differences in boys and girls regarding PRL. (200) Gol et al. revealed no difference in maternal serum and umbilical cord blood PRL levels between pregnancies with male and female neonates. (197)

3.5.6 APN

De Leon-Luis et al. measured APN levels in amniotic fluid of women who were in their second trimester of pregnancy. Among other factors, they investigated a potential relation between amniotic fluid APN levels and fetal sex but did not detect any significant association. (216) This is in line with the findings of a study by Fazeli Daryasari et al. who also observed similar APN levels in maternal and umbilical cord blood of pregnancies with boys vs girls. (217)

3.5.7 Leptin

Al Atawi et al. investigated whether fetal sex influences maternal serum pregnancy hormone levels. Leptin levels were measured in the first, second and third trimester and after delivery. Results revealed that maternal serum leptin values were significantly higher in pregnancies with female compared to male fetuses. After delivery this difference disappeared. (196) Also, two further studies identified a relation between the offspring's sex and cord blood leptin levels at the time of delivery if the baby was female. (164, 218) Schubring et al. detected that significantly elevated leptin levels can be found in amniotic fluid of female fetuses, after performing an amniocentesis in the second trimester of pregnancy, compared to those of male fetuses. (219) By contrast, Marek et al. were not able to show any significant gender-related differences in amniotic fluid leptin levels, which was collected between the 14^{th} and 18^{th} week of pregnancy in the during amniocentesis. (220)

3.5.8 TNF-α

Ramiro-Cortijo et al. investigated whether maternal plasma cytokine levels, including TNF-α, differ according to fetal sex. Therefore, blood was analyzed in the 10^{th} week of gestation but there was no difference. (221) Similar observation was made by Mitchell et al. who did not detect a difference in maternal TNF-α depending on fetal sex. Interestingly, after LPS-stimulated cytokine production, TNF-α values were measured higher in mothers carrying

female fetuses. (222) However, stimulating placental trophoblast cells after delivery with LPS resulted in higher TNF-α release from male placentae in another study. (223) In two studies TNF-α and various other cytokine concentrations were determined in second trimester amniotic fluid to identify whether there is an association between cytokine levels and the sex of the baby but both authors did not describe any significant correlation. (224, 225)

4 Discussion

Recently it has been recognized that the sex of the fetus can influence the course of pregnancy and, among others, may contribute to GDM development. (165)

At present, various large retrospective and prospective studies exist in which was detected that carrying a male fetus independently increases the risk of developing GDM (162, 165-170) but there also is contradictory data in literature. (163, 171-174) However, due to inconsistent data, 2 meta-analyses, both including over 2 million participants, were conducted which revealed that male sex is in fact associated with higher risk for the mother to develop GDM. (160, 175) Moreover, some studies observed associations between fetal sex and maternal insulin resistance. (164, 173, 174) In two smaller studies was detected that women pregnant with a girl were more insulin resistant. (173, 174) It seems reasonable to conclude that if insulin resistance is higher in pregnancy with a female fetus, the likelihood of GDM is also higher. Thus, as an explanation for their study results one author discussed the possibility that the male fetus may somehow suppress maternal insulin secretion and the female fetus may eventually affect maternal insulin sensitivity. (174) However, these findings that female babies are associated with greater insulin resistance (173, 174) stay contradictory, since Walsh et al. by contrast revealed that women carrying females were less insulin resistant in the first and second trimester. (164) In another study was established that women carrying a female baby in their first GDM pregnancy were at higher risk of developing T2DM before their second pregnancy. (169) They speculated that women bearing a girl have poorer ß-cell function outside of pregnancy compared to those bearing a boy leading to higher likelihood of earlier development of T2DM in the female gender group. GDM in boy pregnancy may be partly created through the direct effect of the male gender on ß-cell compensation, however developing GDM in a girl pregnancy may be indicative of general worse ß-cell function. However, this hypothesis could not be confirmed in their current study. (169)

There are several possible explanations for this male sex-dependant GDM risk: One study suggests that a male fetus may promote additional insulin resistance due to higher levels of testosterone in the maternal circulatory system. (226) Furthermore, it was revealed that the placental weight is higher when the baby is male. (164, 176) Placental hormones are suggested to play an important role in altered glucose metabolism and insulin sensitivity during pregnancy. (1) It is believed that placental hormones can induce insulin resistance and this effect may be amplified at higher placental weights, as discussed in the paper by

Walsh and colleagues. (164) Additionally, GDM occurs due to the fact that the elevated insulin resistance in pregnancy cannot be compensated for by increased ß-cell mass and thus increased insulin secretion. Placental hormones are also thought to play a role in this compensatory process. Retnakaran and colleagues discussed in their paper that the male fetal sex might eventually interfere with the factors secreted by the placenta and thus this compensatory process is disrupted. (170)

Several studies investigated whether placental hormone levels in the mother are dysregulated in GDM. Studies exist regarding the hormones estrogens (20), hGH (53, 62, 63), PRL (65, 68, 71) and hCG (34, 39-43, 45, 47), concerning which the most literature exists. Various studies analyzed maternal serum hCG at different stages of pregnancy, detected a negative association between maternal hCG and GDM. (39-41, 45) whereas two authors stated decreased risk for GDM when having higher hCG values in the first trimester. (34, 43) However, in a meta-analysis by Donovan et al., where nine different studies were compared, lower first trimester hCG levels were associated with GDM. (42) Interestingly, a plethora of studies exist showing higher maternal serum hCG levels in female compared to male pregnancies. (177, 179-191, 218) Data regarding fetal sex and maternal hormonal concentrations are available for progesterone (177, 179, 193, 195-197, 201-204) and estrogens (177, 193, 196, 203, 209-211), which remain inconsistent and there is one study regarding PRL, where no differences were detected. (197)

More recently, various adipokines were increasingly investigated regarding their role in the pathophysiology of GDM. There are also many studies investigating whether maternal adipokine serum levels are dysregulated in pregnancies complicated by GDM. (88) There are various papers available that studied whether there is a correlation between maternal serum levels of APN (78-87, 95), leptin (85, 88, 95, 98-106, 109, 110), TNF-α (88, 95), resistin (88, 127), visfatin (131-136, 138), apelin (143-148) and AFABP (149, 153-157) and GDM. Weighing larger studies more heavily, it can be concluded that maternal serum levels of APN are decreased in GDM (88, 95), leptin (95), TNF-α (88, 95) and AFABP (156) levels are increased in GDM, data on resistin (88) levels are inconsistent and there are no differences between GDM and non-GDM pregnancies in maternal visfatin (140, 141) and apelin (143) serum levels. However, the data regarding sex differences in maternal adipokine concentrations is only modest since, there is only one study concerning APN levels and fetal gender where no differences were detected (217), one study detected higher leptin levels in female pregnancies (196) and no differences regarding maternal TNF-α levels and fetal sex were detected in two studies. (221, 222)

In this thesis placenta-derived hormones (estrogens, progesterone, hCG, hPL, pGH, PRL) and other bioactive factors (APN, leptin, TNF-α, resistin, visfatin, apelin, AFABP) and their influence on glucose metabolism during pregnancy were summarized. The aim was also to identify whether concentrations of placental hormones and adipokines are dysregulated in GDM, whether there are fetal sex differences in maternal placental hormone concentrations and to figure out if the sex of the baby is linked to the development of GDM. In summary, it can be said that placental hormones and bioactive proteins play an important role in the development of the naturally occurring insulin resistance during pregnancy, but also in the compensatory process by enlarging ß-cell mass and thus increasing insulin secretion to counteract this metabolic change. If this regulatory process is disrupted, among others, possibly due to fluctuations in the levels of placenta-derived factors, GDM may result. At present the data on gender differences in glucose metabolism during pregnancy and placentally derived hormones in the circulatory system of the mother is mainly inconclusive. The relationship between GDM development due to the influence of placental hormones and fetal sex needs to be explored by parallel investigation in further studies.

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