Thesis

Metformin in pregnancy – long- and short-term consequences for the offspring

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, 19.06.2023 *Lena Harling m.p.*

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Abbreviations

AACE .... *American Association of Clinical Endocrinologists*
ACOG .... American College of Obstetricians and Gynecologists
ADA .... *American Diabetes Association*
ALT .... *Alanine Aminotransferase*
AMH .... *Anti-Müllerian Hormone*
AMP .... *Adenosinmonophosphate*
AMPK .... *AMP-activated Proteinkinase*
ApoB .... *Apolipoprotein B*
ASRM .... *American Society for Reproductive Medicine*
ATP .... *Adenosintriphosphate*
BIA .... *Bioelectrical impedance analysis*
BMI .... *Body Mass Index*
BPD .... *Biparietal Diameter*
CDA .... *Canadian Diabetes Association*
CRP .... *C-reactive protein*
DPP-4 .... Dipeptidylpeptidase 4
DXA .... Dual-energy X-ray absorptiometry
ESHRE .... *European Society of Human Reproduction and Embryology*
FDG .... Fluorodeoxyglucose
FSH .... *Follicle-stimulating hormone*
GFR .... Glomerular filtration rate
GI .... *Gastrointesinal*
GLP-1 .... *Glucagon-like-Peptide-1*
GLUT 4 .... *Glucose transporter type 4*
GnRH .... *Gonadotropin-releasing hormone*
HAPO .... *Hyperglycemia and Adverse Pregnancy Outcomes*
HbA_{1c} .... Glycated hemoglobin
HC .... *Head Circumference*
HDL .... High-density lipoprotein
HELLP .... *Hemolysis, Elevated Liver Enzymes, and Low Platelet Count*
hs-CRP .... *High Sensitive C-reactive protein*
IADPSG .... *International Association of the Diabetes and Pregnancy Study Groups*
ICSI .... *intracytoplasmic sperm injection*
ICTRP .... *International Clinical Trials Registry Platform*
ICU .... *Intensive Care Unit*
IFG .... *impaired fasting glucose*
IGT .... *Impaired glucose tolerance*
IQ .... *ntelligence quotient*
IUGR .... *intrauterine growth restriction*
IVF .... *in vitro fertilization*
LDL .... *Low-density lipoprotein*
LGA .... *Large for Gestational Age*
LH .... *Luteinizing hormone*
MAD .... *Mean Abdominal Diameter*
MRI .... *Magnetic resonance imaging*
NICE .... *National Institute for Health and Clinical Excellence*
NICU .... *Neonatal Intensive Care Unit*

NPH...*Neutral Protamine Hagedorn*
OGTT ....*Oral glucose tolerance test*
OHSS...*ovarian hyperstimulation syndrome*
PCOS...*Polycystic ovary syndrom*
PET ....*Positron emission tomography*
PlGF ....*Placental growth factor*
RCT ....*Randomized controlled trial*
SERT ....*Serotonin transporters*
sFlt-1 ....*Soluble fms-like tyrosine kinase-1*
SGA...*Small for Gestational Age*
SHBG... *Sex hormone-binding globulin*
SIRT1 ....*Hepatic Sirtuin*
T2DM ....*Type 2 Diabetes Mellitus*
TxA2... *Thromboxane A2*
USPSTF ....*U.S. Preventive Services Task Force*
WHO ....*World Health Organization*

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Zusammenfassung (German Abstract)

Ziel:

Der Einsatz von Metformin in der Geburtshilfe wird immer beliebter, da dieses orale Antidiabetikum in der Handhabung einfach und im Allgemeinen gut verträglich ist. Die zentrale Frage, die in dieser Arbeit geklärt werden soll, sind kurz- und langfristigen Folgen der Anwendung von Metformin in der Schwangerschaft für Mutter und Kind, welche noch intensiver zu erforschen sind.

Methoden:

Hier handelt es sich um eine Literaturrecherche zur Anwendung des oralen Antidiabetikums in der Schwangerschaft, in der mögliche nachteilige Folgen und Komplikationen der Behandlung des GDM im Vergleich mit Insulin, sowie der Behandlung des PCOS in der Schwangerschaft im Vergleich mit Placebo erforscht werden. Für die Recherche wurde die Datenbank Pubmed und Google Scholar, sowie randomisiert kontrollierte Studien (RCTs) und Metanalysen aus den Jahren 2000-2022 genutzt, welche zuvor auf Qualität, Relevanz und Aktualität überprüft wurden.

Ergebnisse:

Um eine zufriedenstellende Kontrolle des Blutzuckers zu erreichen, so benötigen Frauen, die Metformin einnehmen, fallweise zusätzliches Insulin, um eine Euglykämie zu erreichen, allerdings wurde bei Frauen, die Metformin einnehmen, eine geringere Rate an hypoglykämischen Ereignissen festgestellt. Im Vergleich zu Insulin wirkt es sich positiv auf die Geburtsparameter aus, indem es die Häufigkeit von Makrosomie und LGA-Babys verringert und es Insulin in Bezug auf neonatale Hypoglykämie überlegen ist. Hinsichtlich der Häufigkeit von Kaiserschnitten und Geburtstraumata wurden vergleichbare Ergebnisse zwischen Metformin und Insulin festgestellt. Im Zusammenhang mit PCOS beobachtet man bei Frauen, die Metformin erhielten, eine Vorbeugung von LGA- und SGA-Babys, sowie von Frühgeburten und Fehlgeburten.
Bei Frauen mit GDM und PCOS konnte Metformin auch die Wahrscheinlichkeit einer Präeklampsie verringern. Darüber hinaus wurde sowohl bei der Behandlung von GDM als auch von PCOS mit Metformin eine günstige Auswirkung auf die Gewichtszunahme während der Schwangerschaft festgestellt, allerdings könnte es bei Frauen mit PCOS auch zu einer wöchentlichen Gewichtszunahme während der Schwangerschaft führen, die unter den Empfehlungen liegt.
Während Tiermodelle darauf hindeuten, dass die Exposition gegenüber Metformin während der Schwangerschaft zu einer Gewichtszunahme im späteren Leben des Kindes führt, auch wenn diese bei der Geburt kleiner sind, scheinen die Körperzusammensetzung und die Stoffwechselergebnisse von Kleinkindern und Kindern, deren Mütter Metformin erhielten, mit denen vergleichbar zu sein, die mit Insulin behandelt wurden.
Es gibt Hinweise dahingehend, dass die Behandlung mit Metformin bei Frauen mit PCOS zu übergewichtigen Kindern im Alter von 1, 4 und 8 Jahren führen könnte, allerdings sind hier weitere Untersuchungen erforderlich. In Bezug auf die motorische, soziale, sprachliche und neurologische Entwicklung der Kinder wurden keine auf die Metformin-Behandlung zurückzuführenden Einschränkungen festgestellt.
Was die mütterlichen Langzeitfolgen der Behandlung mit Metformin oder Insulin wegen GDM betrifft, so konnte keines der beiden Medikamente das Auftreten von Diabetes

mellitus Typ 2 verhindern. Außerdem führten beide Medikamente zu vergleichbaren Ergebnissen hinsichtlich Glukosetoleranz, HbA1C, Gewichtsverlust nach der Schwangerschaft, sowie Lipidlevel und Entzündungsparametern.

Schlussfolgerung:

Während Metformin kurzfristig als sicher angesehen werden kann, sind langfristige Folgestudien weiterhin erforderlich, um endgültige Schlussfolgerungen über die Sicherheit und Wirksamkeit des Medikaments und seine Auswirkungen auf die fetale Programmierung zu ziehen.

Abstract

Purpose:

The usage of Metformin in obstetrics is on the rise, as the oral antidiabetic drug is simple to use and generally well tolerated. The central question, that should be resolved in this thesis is the short- and long-term consequences of the use of metformin in pregnancy for mother and child, which still need to be researched more intensively.

Methods:

This is a literature research on Metformin in pregnancy, investigating the adverse effects and complications of treatment with Metformin in the context of GDM in comparison with insulin, as well as treatment of PCOS in pregnancy compared with placebo. To conduct the research, the database Pubmed and Google Scholar, as well as randomized controlled trials (RCTs) and meta-analysis from 2000-2022 were used and reviewed concerning quality, relevance, and actuality to find appropriate results.

Results:

When euglycemic blood sugar control in pregnant women taking Metformin requires additional insulin in some cases lower rates of hypoglycaemic events were reported in women taking Metformin. Compared to insulin it positively influences birth parameters with reducing the incidence of macrosomia and LGA babies, it also seems to be superior to insulin when it comes to neonatal hypoglycaemia. Comparable outcomes between Metformin and insulin were found concerning the incidence of caesarean section and birth trauma. In the context of PCOS a prevention of LGA and SGA babies, as well as preterm birth and miscarriage was seen in women receiving Metformin.
Metformin was also seen to prevent preeclampsia in women with GDM and PCOS. Moreover, a favourable effect on gestational weight gain was seen in both GDM and PCOS treatment with Metformin, however it might also lead to a weekly gestational weight gain that is below the recommendations in women with PCOS.
While animal models suggest that the exposure to Metformin during pregnancy lead to increased weight of the offspring later in life, even though being smaller at birth, body composition and metabolic outcomes seem to be comparable between toddlers and children whose mothers obtained Metformin to those who were treated with insulin.
There is evidence that the treatment with Metformin in women with PCOS might lead to overweight children at the age of 1, 4 and 8 years, however further investigation is needed here. No restriction was seen attributable to Metformin treatment in terms of motor-, social-, linguistic- and neurodevelopment of the infants.
Regarding maternal long-term consequences of the treatment with Metformin or insulin because of GDM, neither of the drugs was seen to be able to prevent the onset of T2DM. Furthermore, both drugs led to comparable results in terms of glucose tolerance, HbA1C, weight loss after pregnancy, as well as lipid levels and inflammatory parameters.

Conclusion:

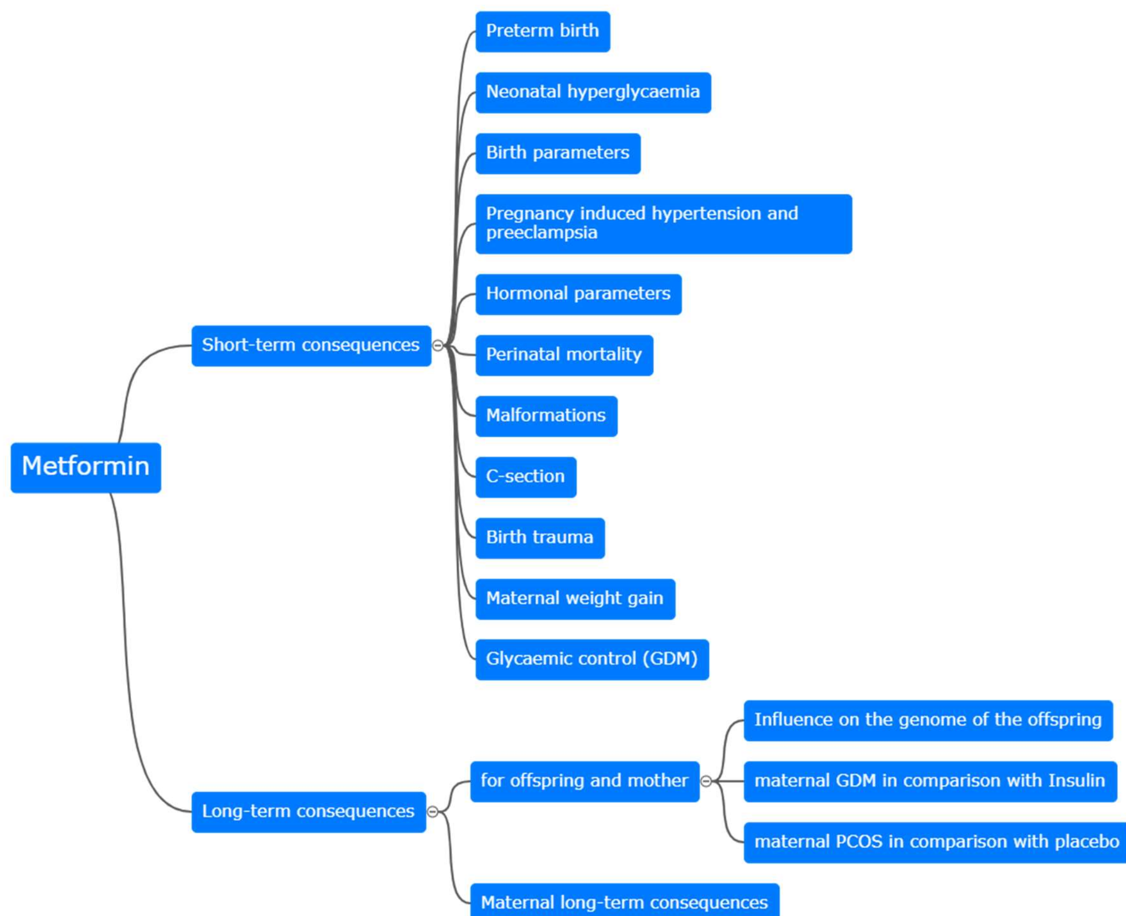
While Metformin can be considered safe in the short-term, long-term follow-up studies are still required to draw definite conclusions about the safety and effectiveness of the drug and its impact on fetal programming.

Introduction

The use of Metformin in obstetrics is becoming more and more common, as it is easy to handle and it needs less intensive patient care as a treatment with insulin. Even though Metformin readily crosses the placenta, recent studies suggest that it has no harmful effects on the offspring.

This thesis aims to review literature on short- and long-term effects of Metformin on the offspring and for the mother. Important topics that are covered are i) Comparison of Metformin and insulin in the treatment of gestational diabetes (GDM), and ii) Metformin for in treatment of polycystic ovary syndrome (PCOS) in comparison with to placebo.

Following topics covered in this thesis:



1 Metformin

As a constituent of many herbal remedies, the origin of the use of Metformin reaches back to the time of the Pharaohs in 1500 B.C.E. where it was described on an Ebers papyrus that was found between the legs of a mummy. Furthermore, in Europe the use of Metformin goes back to the middle age where Galega officinalis, a plant that contains Metformin, was used to treat polyuria and other symptoms of diabetes. (1)

1.1 Metformin and its usage

Metformin belongs to the biguanide category of antidiabetic drugs and has been extracted from the French lilac Galega officinalis. Being one of the three biguanides developed for diabetic therapy, Metformin has turned out as superior in terms of safety, effectiveness and it has been well tolerated and therefore oust the other two from the market. (2)
Even though its mechanisms remain largely elusive, it is the most prescribed antidiabetic worldwide. The main benefit of Metformin is its glucose lowering effect and its augmentation of insulin sensitivity. But beside that it has many other favourable side-effects: Metformin decreases food intake and body weight, positively influencies cardiovascular risk markers, such as lipid profile and fatty liver, it modifies inflammation, and possibly reduces cancer risk. (3) Metformin is not only used as first-line treatment for Diabetes Mellitus Type 2 (T2D), but is also more and more used to treat PCOS, Diabetes Mellitus during pregnancy, GDM, and pre-diabetes. Studies also suggest that it might have a beneficial effect on preventing pre-eclampsia. (1)

1.2 Pharmacokinetics

After an oral dose of immediate-release Metformin, 70% of the drug is absorbed in the small intestine while the remaining 30% are passed into the colon and remains are excreted in faeces. (4) The peak concentration in plasma after an oral intake occurs after 3 hours and the mean plasma half-life period is 20 hours. (2)
Metformin is excreted uncharged in urine. Bio-distribution studies using positron

emission tomography (PET) have shown that Metformin highly concentrates in the intestine, liver, kidneys, and the bladder that shows its route of elimination, as well as it shows that it slowly accumulates in muscle. These studies also revealed that the activity of the drug is much higher in the liver than in the plasma, but also an activity that is almost half as high as the liver concentration was found in the pancreas and adipose tissue. (4)

1.3 Mechanism of action

The antihyperglycemic mechanisms of Metformin are mostly based on lowering the hepatic gluconeogenesis, but it also affects glycogenolysis, ameliorates insulin resistance in liver and muscle, and, with to a lower amount, in adipose tissue. (5) Furthermore, Metformin has an impact on glucose absorption through the gastrointestinal tract and seems to interact with the incretin GLP-1 which enhances the secretion of insulin and inhibits the secretion of glucagon. (3)

Metformin has its highest concentration in the portal vein of the liver, where it has its most important antidiabetic effect, i.e., the regulation of gluconeogenesis, glycolysis, and glycogen synthesis. (3)
Gluconeogenesis is mediated by mitochondria that provide the required adenosintriphosphate (ATP), as gluconeogenesis is an energy-intensive process. Metformin is transported into the cell, into the plasma membrane and inner membrane of the mitochondria because of its positive charge. The cannel that transports Metformin into the mitochondria is organic cation transporter 1 (OCT1). Here, studies have shown that it inhibits the Complex I of the respiratory chain and hence the ATP production. (4) By compromising the ability of ATP-production of the mitochondria, Metformin activates AMP-activated proteinkinase (AMPK), which is switched on by increasing AMP: ATP and ADP: ATP ratios that are indicators for low cellular energy. (4) AMPK hinders lipogenesis and increases insulin sensitivity.

By inducing AMPK, Metformin not only reduces lipogenesis, but also stimulates fatty acid oxidation. This is acomplished by It phosphorylation of two isoforms of the enzyme acetyl-CoA carboxylase (ACC1/ACC2). In knock-in mice where ACC1/ACC2 cannot be phosphorylated, mice had higher diglyceride and

triglyceride levels in liver and muscle. Moreover, mice had steatosis, insulin resistance, hyperglycaemia, hyperinsulinemia, and glucose intolerance. (4)

The activation of AMPK might not be the only effect of Metformin on gluconeogenesis, another effect is the increase of AMP, which is a potent allosteric inhibitor of fructose 1,6-bisphosphatase, a key enzyme in gluconeogenesis. Moreover, an increase of AMP leads to an inhibition of the adenylate cyclase that produces cAMP and consequently the protein kinase A (PKA) is inhibited as well. (6)

There are many alleged ways how Metformin might affect the glucose metabolism through the gut. Investigating glucose utilisation with PET-imaging that visualizes fluorodeoxyglucose (FDG) uptake, revealed an enhanced uptake of FDG in colonic enterocytes. The FDG uptake goes hand in hand with AMPK phosphorylation, which suggests that Metformin increases glucose uptake and metabolism of systemic glucose. (4) An increase of the intestinal glucose uptake seems to appear functionally significant, because it is accompanied by an increased anaerobic glucose metabolism. This anaerobic glucose metabolism creates relatively little ATP and therefore, might contribute to weight loss during Metformin treatment. (7)

Another effect of Metformin on the intestine is the enhanced production of GLP-1 which stimulates the insulin release and inhibits glucagon secretion. Furthermore, lower levels of Dipeptidyl peptidase-4 (DPP-4) are reported in peopled treated with Metformin. DPP-4 is responsible for degrading GLP-1 and therefore, it reduces the release of insulin. This is why Metformin seems not only to increase insulin secretion but also to prevent its down-regulation. (3)

Finally, Metformin also affects the intestinal microbiome but the relation of altered microbiome with and glycaemic control is still unknown. (4)

1.4 Dosage

The standard dose of metformin is 500-1000 mg 1-2 times a day, with the maximum dose being 2000-3000 mg per day. (8) In obstetrics, a total daily dose of

2000 mg metformin should not be exceeded. (9) Since gastrointestinal intolerance often occurs if the dose is increased too fast, the dose should be increased only slowly. (5)

1.5 Adverse drug effects

The most common adverse effect of Metformin is gastrointestinal intolerance (GI) and it affects 20-30% of patients. Approximately 5% suffer from severe side effects resulting in discontinuation of the drug. Intolerance is often associated with rapid titration and initially high concentrations of Metformin in the upper gastrointestinal tract. A usage of slow-release formulas of Metformin can reduce GI intolerance because it diminished the inundation of high doses on enterocytes. The mechanisms underlying GI remain unclear, but there are several proposed mechanisms. (4) i) Changes in glucose metabolism of enterocytes might lead to local irritation. ii) More distally, Metformin might cause a bile salt malabsorption, which leads to a fluid retention in the large bowels and consequently causes loose stools and diarrhoea. (5) iii) Another mechanism that explains the GI involves serotonin that is either increasingly released by enterochromaffin cells, or its transport through serotonin transporters (SERT) is impaired which both lead to higher luminal serotonin levels. iv) Another putative mechanism is the impact that Metformin has on the microbiota, which is still uncertain. (4)

The most dreaded side effect of Metformin is the lactic acidosis which is a very rare but dangerous condition that mostly only occurs when contraindications are flouted. In the prodromal stage, symptoms of the lactic acidosis are nausea and emesis, as well as diarrhoea and stomach pain. The full clinical manifestations are muscle cramps, hyperventilation, apathy confusion and coma. Endangered are patients with a high frailty who might also have an undiagnosed kidney disease or are vulnerable for heart failure. (8) However, according to a systemic review that analysed 194 studies involving >60.000 patient years of treatment with Metformin, it has been concluded that the risk of lactic acidosis was not greater than that with other oral antidiabetics, when the contraindications had been respected. (5)

The risk of getting hypoglycaemia during Metformin treatment alone is relatively low as Metformin does not stimulate insulin secretion and does not influence the glucose counter-regulatory mechanism. (5)

1.6 Contraindications and pre-cautions

To prevent lactic acidosis, the list of contraindications for Metformin has been largely expanded. Nevertheless, there is enough clinical evidence that Metformin can also be used for mild renal impairment and other co-morbidities. (5) Metformin must not be prescribed in the event of hypersensitivity to any of the active ingredients or excipients. In addition to that, it must not be given in the case of diabetic ketoacidosis or diabetic coma. Another contraindication is severe renal insufficiency with a glomerular filtration rate (GFR) of less than 30 ml / min, whereas it may be given with a GFR of 60 ml / min, although the dose must be adjusted here. Conditions that impair kidney function should also be considered - these include shock, dehydration, high fever, and severe infections. Metformin should be discontinued 48 hours prior to surgical intervention to minimize the risk of lactic acidosis. The oral antidiabetic is contraindicated in conditions such as liver failure, heart failure, respiratory failure, chronic pancreatitis, and alcohol intoxication. As potential interactions, it should be mentioned that Metformin with sulfonylureas could possibly have a higher cardiovascular mortality. However, this has not yet been written down in the contraindications. (8)

2 Gestational diabetes

The term “Gestational diabetes” was coined by Carrington et al. in 1957 and became more popular after publications of John O´Sullivan in the year 1961 and 1964 . Nevertheless, the phenomenon of an increased blood glucose of women in the late second trimester or early third trimester of pregnancy were noted before and has challenged the medical profession with the intricacy of the diagnosis of GDM ever since. However, the diagnosis of GDM is of prime importance since GDM increases the risk of pregnancy complications and in addition to that, outlines a group of women and their offspring who are at higher risk to develop long term consequences such as T2D, obesity, and premature cardiovascular disease. (10)

2.1 Definition

The definition of gestational diabetes is an impaired glucose tolerance that is diagnosed for the first time during pregnancy with the help of an oral glucose tolerance test (OGTT). The OGTT reveals the response of the body to a standardised mount of glucose. To meet the criteria of the definition the measurement of the blood glucose must be taken out of venous plasma. (9)
The consequence of the very vague definition is that the term “Gestational Diabetes” allows a very degree of severity of impaired blood glucose tolerance from mild impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) which is detected in late pregnancy to overt diabetes. (10) The limit values for the diagnosis of GDM can be seen in upcoming chapter of the diagnostic criteria.

2.2 Epidemiology

As there is a lack of consensus concerning the diagnostic criteria of GDM it is difficult to compare the prevalence of GDM across countries and regions. Generally spoken, the prevalence of GDM varies between 1% and >30%. (10)
Observed in recent years the prevalence of GDM is increasing worldwide. This can be explained by a chance of the screening method and new diagnostic limits on the one hand and emerging risk factors such as obesity and increasing maternal age on the other hand. All in all, the more screening is carried out, the higher the is the risk of the population, and the lower the diagnostic criteria, the more often GDM will be diagnosed. (9)

The worldwide prevalence of GDM is shown in the illustration below (Figure 1). The prevalence is highest in the Middle East and some parts of North Africa (median 15,2%), closely followed with a median of 15% by South-East Asia and lowest in Europe with a median of 6,1% even though Europe also has the widest variation in different countries (range 1,8-31%). (10)

As already mentioned, the screening method critical to how often the diagnosis is made. Therefore the prevalence of GDM in Italy has increased by 25% after the introduction of the IADPSG-criteria (The International Association of the Diabetes and Pregnancy Study Groups) in 2010. But not only Italy marked a change in

GDM prevalence, also China noticed a prevalence that is 3,5 times higher in the year 2013 after switching to the recommendations of IADPSG-criteria than in the year 1999. (9)

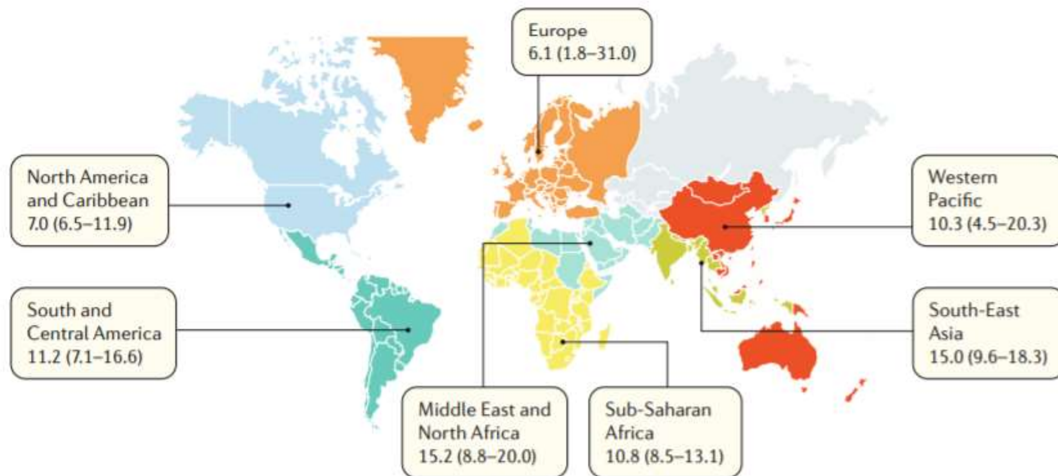


Figure 1 Prevalence of Gestational Diabetes worldwide by McIntyre et al. (10)

2.3 Pathophysiology

During a healthy pregnancy, the woman's body undergoes many physiological changes in different aspects, including cardiovascular, renal, hematologic, metabolic, and respiratory adaptations, to cover the needs of the fetus. One important feature that occurs during early pregnancy is an increased sensitivity to insulin, in order to be able to store energy for late pregnancy. However, later in the pregnancy an increase of hormones including progesterone, oestrogens, cortisol, leptin, placental growth hormone and placental lactogen, trigger a decreased insulin sensitivity and create higher levels of blood glucose which is transferred to the fetus and important for its growth. (11) The primarily hepatic endogenous glucose production is increased by 30% to compensate the higher demands of fasting energy in late healthy pregnancy. In contrast there is a 2-3-fold secretion of insulin to compensate insulin resistance and to maintain euglycemia. In fact, insulin sensitivity (which is defined as the ability of Insulin to increase the uptake of glucose in skeletal muscle and adipose tissue) decreases in late pregnancy to only 50%. (10) This is accomplished by pancreatic hypertrophy and hyperplasia. However, if this cannot be achieved, GDM develops. (11)

Women who develop GDM often have a decreased insulin sensitivity already before pregnancy . But as pregnancy proceeds and and insulin sensitivity of the first trimester diminishes, the β-cells cannot cope with the high demand of insulin, and this results in GDM. In this case, the defect in β-cell function has already existed before conception and pregnancy has unveiled the dysfunction. (10)
But there are many factors that contribute to the development of GDM, including s genetic, epigenetic, and environmental factors, but also adipose expandability, low-grade chronic inflammation, gluconeogenesis, oxidative stress, and placental factors which may contribute to an impaired glucose tolerance in late pregnancy. (11)

2.4 Diagnosis

Due to a lack of global consensus, there are different methods of screening for GDM. The American Diabetes Association (ADA) states that women at low risk who are less than 25 years old, do not belong to an ethnic group at high risk, have a BMI 25kg/m^2 or less, have no previous history of abnormal glucose tolerance or adverse obstetrics outcomes and who have no first-degree family members with a history of diabetes, do not benefit from a screening at all.
The IADPSG on the other hand recommends an early screening for women at high risk. (12) That means that at the first visit to a gynaecologist, pregnant woman with high risk should be screened to prevent complications concerning the pregnancy, the maternal and the fetal outcome. The focus here is on patients with an anamnesis of GDM in previous pregnancies, habitual abortion, impaired glucose tolerance, impaired fasting glucose, birth of a child with birthweight higher than 4500g, stillbirth, congenital malformations, women with metabolic syndrome or obesity, diabetic symptoms, vascular disease, age older than 35 or family history of a first-grade relative. If any of these risk factors is noticed very early even in the first trimester, a determination of the HbA_{1c} should be done and either the fasting blood glucose or any blood glucose should be measured, even an oral glucose tolerance test can be considered. A HbA_{1c} of more than 6,5% or a fasting glucose of 126 mg/dl could be evidence for a manifest diabetes in pregnancy. (13)

In case there is no evidence for a high risk for GDM or the examination of the high-risk group was not conspicuous, a screening test for GDM should be performed according to the IADPSG-criteria, and according to the ADA-recommendations who adopted the IADPSG-criteria, in any pregnant woman between the 24th and 28th weeks of gestation. In the oral glucose tolerance test 75g glucose dissolved in 100 ml water and needs to be taken by the fasting patient. According to the consensus-building of experts of the IADPSG the limit values of blood glucose taken from venous blood plasma are the following: fasting: 92 mg/dl (5,1 mmol/l), 1-hour after the consumption: 180 mg/dl (10 mmol/l) 2-hours: 153 mg/dl (8,5 mmol/l). The IADPSG based their findings on the HAPO study that outlined the effects of hyperglycaemia on maternal and fetal outcome. (9)

In contrast to the IADPSG-criteria the American Association of Clinical Endocrinologists (AACE) and the Canadian Diabetes Association (CDA) recommend a two-step approach in women at high risk which consists of a 50-g oral glucose challenge test between 24th and 28th week gestation, followed by the 2-h 75-g OGTT only if the threshold of 130-140mg/dl blood glucose after one hour has been surpassed. Some also prefer a 100g-OGTT after the 50g glucose challenge test. That method is most common in the United States. (14) Because of its simplicity in execution, more patient adherence, high accuracy in diagnosis of GDM and its closeness to international consensus the one-step approach should be preferred. (15)

2.5 Complications and risks for mother and offspring

GDM is associated with many complications for both the mother and the child. Maternal complications include preeclampsia, preterm birth and due to the larger size of neonates, a surgical delivery of the baby via caesarean section is more frequently necessary. (11) Moreover, mothers with GDM often have other risk factors that cause poor outcome for the mother like maternal overweight and obesity, belonging to an ethnic minority, high age or reduced physical activity – that is why for many years it used to be discussed if the complications of GDM were triggered by maternal risk factors or the hyperglycaemia per se. (10)

Regarding the long-term consequences of the mother, it is shown that 6 out of 10 women with medical history of GDM develop a T2DM later in life. Evidence also shows that GDM worsens the vascular health in women later in life, so that the patient is predisposed to cardiovascular disease with a probability of 63%, which can also partly be explained by the higher BMI. (11)

As there is constantly a higher blood glucose in women with GDM, the transplacental transport of glucose, fatty acids and amino acids is increased which leads to a stimulation of the production of insulin and insulin-like growth factor 1in the fetus. Insulin acts also as a growth hormone and therefore the increase of insulin production in the fetus can lead to macrosomia which causes problems like shoulder-dystocia and often causes the need to deliver via caesarean section. (11) Thus, the risk that the neonate has a bodyfat mass greater than the 90th percentile is 16,6% in women with GDM compared to 8,8% in non-diabetic women. (10) Moreover, the enhanced production of insulin can stress the fetal pancreatic β-cells which results in an insulin resistance of the baby even after birth. Therefore, and because the neonate is used to a higher glucose level in utero, it is more likely to suffer from neonatal hypoglycaemia, which is associated with a risk for brain damage. (11)

Concerning the long-term effects, children exposed to GDM in utero are twice as likely to develop childhood obesity than children of non-diabetic mothers. Furthermore, an impaired glucose tolerance in early age and a higher risk of developing T2D and cardio-vascular disease has been reported. (11)

Studies that used animal models have shown that GDM offspring have a higher risk of hyperglycaemia, diabetes, obesity, cardiovascular disease, and structural hypothalamic changes during their pregnancies, however these abnormal effects can be prevented by normalizing the blood glucose during pregnancy. (16, 17) These adverse outcomes were seen also in studies observing children of women who had different kinds of diabetes during pregnancy. Also in this study, offspring had a higher risk risk of diabetes and obesity. (10)

2.6 Therapy and management

There are three different methods of treatment for GDM: i) lifestyle modification, ii) insulin treatment and iii) oral antidiabetics. The basic therapy for all patients with GDM is lifestyle modification, if euglycaemia still cannot be achieved, further therapy with an oral antidiabetic, insulin or both is required.

2.6.1 Lifestyle modification

Studies show that 70-85% of women diagnosed with GDM using the Carpenter and Choustan diagnostic threshold (95mg/dl fasting plasma glucose, 180mg/dl 1-hour, 155mg/dl 2-hour and 140mg/dl 3-hour after a 100g OGTT) can be controlled by lifestyle modification and it is suggested that this proportion might even be higher using the IADPSG-threshold. (18)

Therefore, after diagnosing GDM, an education programme about medical nutrition therapy, glucose goals based on glucose monitoring and the importance of physical activity should be started. The aim of the dietary restrictions is to reduce postprandial hyperglycaemia. To achieve this goal a diet with 40% carbohydrate - that should mainly consist of complex carbohydrates - with 20% protein, and 40% fat has been recommended. Distributing the calories between three meals and two to three snacks to avoid postprandial glucose fluctuations, can also be helpful. Moreover, a glucose monitoring is necessary that focuses on fasting, 1 hour or 2 hours after the meal. The thresholds from the 5th International Workshop Conference on Gestational Diabetes Mellitus are fasting blood glucose below 95 mg/dl, 1 hour postprandial below 140 mg/dl or below 120 mg/dl 2 hours after the meal. (14) If there are no contraindications, women with GDM will also benefit from physical exercise especially 20-30 minutes after a meal to prevent a rapid increase of blood glucose. However, physical exercise has not influenced the fasting plasma glucose significantly, still physical exercise such as aerobic, fast walks, or dancing 3 times a week for 30-60 minutes can help to prevent or reduce the usage of insulin and besides, reduce the risk for a LGA baby and caesarean section. (9)

2.6.2 Insulin

Globally insulin is still the number one medical treatment of GDM. If glycaemic control cannot be achieved 1-2 weeks after lifestyle modification (c.f. above) it is

recommended to begin a therapy with insulin. Insulin is well studied and is considered as safe during pregnancy as it does not cross the placenta. (10) However, the oral antidiabetic drug Metformin has found its place in many international guidelines as it is as equal in its efficacy.

The intensified conventional therapy is based on a basal-bolus regime which consists of a long-acting basal insulin that helps to reduce the fasting glucose and of boluses of short acting insulins after the meal. It is important to adapt the therapy individually because a basal insulin alone or just short acting insulins postprandial might also help achieving glycaemic control. Furthermore, the dose of the injection needs to be adapted regularly to the weight of the mother and the progression of pregnancy. A fixed combination of insulins is not advised because of the poor controllability and should only be considered in patients with low adherence or in those patients who do not understand how to use the intensified conventional therapy. (9)
As long-acting basal injection of insulin Detemir or Glargin should be considered, as it does not cause hypoglycaemia as likely as neutral protamine Hagedorn (NPH) which is cheaper but has peak levels 6 to 7 hours after the first dose. (14)
Recommended short-acting Insulins are the short-acting human Insulins but also the Insulin analogues insulin Aspart and Lispro. The correct setting of the medication should be done on an outpatient basis. (9)
The downside of insulin therapy is that it is time consuming for caregivers and requires training and education of the pregnant women. Moreover, patient contact is frequently needed to adjust the insulin doses. (10)

2.6.3 Metformin

The oral antidiabetic agent is mostly used off-label during pregnancy. Moreover, it crosses the placenta which means that it might affect the fetus as well. However, studies show that the neonatal outcome is comparable to that of insulin. (10) While countries like Germany still act with reserve concerning the usage of Metformin, the National Institute for Health and Clinical Excellence (NICE), the American College of Obstetricians and Gynecologists (ACOG) and the guidelines in Austria (österreichische Gesellschaft für Gynäkologie und Geburtshilfe) rate Metformin as equivalent to insulin and act liberally regarding its usage. The ADA (American

Diabetes Association) recommends insulin as first-line medication although it is also very liberal-minded concerning Metformin in pregnancy. (9)
The comparison of Metformin and insulin as a treatment of GDM will be intensively discussed later in this thesis.

2.6.4 Sulfonylureas

During pregnancy and breastfeeding Sulfonylureas like Glibenclamid (also known as Glyburide in the USA) are contraindicated in the USA and Germany as they have significantly worse outcomes than insulin or Metformin. (9) Glibenclamid increases insulin secretion by binding to pancreatic β-cell adenosine triphosphate calcium channel receptors and therefore, declines plasma glucose and leads to a lower HbA_{1C}. (10, 14)
Similar to Metformin, Glibenclamid crosses the placenta. A meta-analysis of clinical trials using Glibenclamid and insulin has shown that Glibenclamid more likely caused neonatal hypoglycaemia, higher birthweight, and higher rates of macrosomia. In comparison with Metformin, it is also associated with higher birthweight and higher rates of macrosomia. Hence, insulin and Metformin treatment is considered superior to Glibenclamid treatment. Moreover, women who were treated with Glyburide were reported to have a higher risk of birth injury and their babies were more likely to have higher rates of LGA, neonatal hypoglycaemia, and neonatal admission to the ICU. To sum up, these outcomes do not speak for Glibenclamid as first-line treatment for women with GDM and therefore, Glibenclamid will not be discussed in this thesis. (10)

3 Polycystic ovary syndrome

Polycystic ovary syndrome is a collection of symptoms and results from an imbalance in female sex hormones. (19) The polycystic ovary syndrome is the most common endocrine disorder of women worldwide and it mainly affects women of childbearing age. It is not only a reproductive impairment syndrome, but it is also associated with cardiovascular and metabolic risk factors. Therefore, it can have a massive impact on the life expectancy and quality of life. Unfortunately, PCOS is still underdiagnosed and poorly understood, especially outside of gynaecology and obstetrics, although these women are predestined for diseases

such as diabetes mellitus, metabolic disorders, and cardiovascular diseases, and should be managed on an interdisciplinary basis. (20) Furthermore, PCOS can cause pregnancy related complications such as GDM, pregnancy-induced hypertension, and thus women with PCOS have an increased risk for abortion and sill-birth. (19)

3.1 Definition

To meet definition of the “European Society of Human Reproduction and Embryology“ (ESHRE) and the “American Society for Reproductive Medicine“ (ASRM), 2 out of 3 of the following criteria must be met to diagnose PCOS: clinical and / or biochemical signs of hyperandrogenaemia, oligo- or anovulation, and polycystic ovaries. (21)
Based in the Rotterdam criteria, there are 4 different subtypes of PCOS, which can be seen in the table below. The first type is the classic PCOS, which manifests itself through hyperandrogenism and oligo- / anovulation with polycystic ovaries (66% prevalence). The second type is called non-polycystic ovary PCOS and shows signs of hyperandrogenism and oligo- / anovulatory cycles without morphological polycystic ovaries being in the focus (11% prevalence). The third type is called ovulatory PCOS, here the women have a regular menstrual cycle, but they have polycystic ovaries and hyperandrogenism (13% prevalence). In mild or normo-androgenic PCOS (4th type), slightly increased androgen levels are found in those affected, but polycystic ovaries and oligo-anovulation can be found in concerned women (9% prevalence). (20)

Table 1Phenotypes of PCOS (20)

Phenotypes of polycystic ovarian syndrome				
criteria	**classic**	**2nd**	**3rd**	**4th**
Signs that show hyperandrogenism (hirsutism, alopecia, acne) Elevated testosterone	+	+	+	-
Oligoovulation or anovulation with less than 6-9 menstrual cycles per year or a cycle length >35d	+	+	-	+
Polycystic ovaries with 12+ antral follicles in one ovary or a total ovarian volume of >10ml	+	-	+	+

3.2 Epidemiology

Depending on what definition is used to characterise PCOS, the prevalence of the syndrome varies between 5 and 20% of women in childbearing age. According to the definition of the 2006 Androgen Excess & PCOS Society it ranges from 10-15% while regarding the 2003 Rotterdam criteria, the prevalence of PCOS ranges from 5-20%. (22) Nevertheless, higher frequencies of PCOS have been found in some ethnical communities. Those communities include the Han Chinese, and the Australian aborigine population among others. All in all, the prevalence of the syndrome is higher in populations where obesity and higher levels of insulin resistance are prevalent. (19) Comparing Black and White women with PCOS, it has been observed that there is no difference in reproductive features but there are mild differences in metabolic features. (23)

3.3 Pathophysiology

The pathophysiology of PCOS is complex and consists of genetic, metabolic, and environmental factors. Among these factors, impaired gonadotropin secretion, hyperandrogenaemia, insulin resistance and hyperinsulinemia, ovarian dysfunction, and follicular arrest play an important role of the pathophysiology. (24)

Affected women have a disorder of the hypothalamus-pituitary axis, so that there is an increased pulsatile release of GnRH and, as a result, an increased release of luteinizing hormone (LH). (22) Women with PCOS have an insufficient release of FSH, which leads to excessive androgen production in the ovary and ovarian dysfunction. Normally, progesterone would be the counter-regulator of GnRH, but a resistance to this negative feedback mechanism has been observed in women with PCOS. At the ovarian level, polycystic ovaries show a relative resistance to FSH. However, this effect might be the result of elevated levels of intraovarian Anti-Müllerian Hormone (AMH). (25) AMH, a potential PCOS marker, is produced by an increased number of preantral and antral follicles and it could enhance the androgen activity of theca cells by inhibiting FSH. (24)
As there is a relatively lack of FSH, testosterone is incompletely aromatized in adjoining granulosa cells and there is an androgen excess in the periphery. This condition leads to clinical signs of hyperandrogenism such as hirsutism, acne, and

alopecia. However, testosterone can be aromatized extragonadally which besides leads to unimpeded oestrogen levels that have its effect on the endometrium and increase the risk of endometrial cancer. (23)

As aforementioned, insulin resistance contributes to the pathophysiology of PCOS. Elevated insulin levels act synergistically with LH, stimulate androgen production , cause androgen excess, and moreover, supress the hepatic production of sex hormone-binding globulin and therefore insulin can increase bioavailable androgens. (22) In an animal study using rats, insulin was shown to enhance ovarian growth and follicular cyst formation. (24)

The mechanism of decreased Insulin sensitivity in PCOS still remains unclear, however evidence shows that there is an epigenetic and genetic caused dysfunction of the glucose transporter GLUT4. This leads to defects in insulin mediated glucose uptake and malfunctioning of insulin mediated lipolysis that might lead to insulin resistance. What also contributes to the insulin resistance is the abnormal adipocytokine production and action that has been observed in women with PCOS, leading to subacute chronic inflammation and free fatty acid dysregulation. (22)

3.4 Clinical presentation

The syndrome of PCOS has a collection of many signs and symptoms. The focus is on hyperandrogenic sequelae, oligo- to anovulation and polycystic ovaries. (22) The clinical presentation of PCOS varies from no clinical features to classical signs of the syndrome as aforementioned, depending on the subtype. (20)

Clinically, hyperandrogenism usually presents as hirsutism, with affected women showing a male-like hair growth pattern with the dominance of terminal hair over vellus hair. (22) Women with hirsutism show an increased hair growth over the chin, neck, lower face, and sideburns. Excessive hair growth is also often observed in areas like the lower back, abdomen, buttock, peri-areolar, perineal, and as well as inner thighs. (20) To objectify the hair growth pattern, the Ferrin-Gallway score was developed, which represents a visual scale in nine different

body regions. Within this system, points are awarded for the respective body regions from 0 (no visible terminal hair) to 4 (terminal hair as in a male individual) and added up at the end. To define hirsutism, Azziz R, et al. suggested a score of 6 or more in the modified Ferrin-Gallway score. (22)
Alopecia in women with PCOS typically represents as male-like hair loss like developing a vertex, crown, or diffuse hair loss. But in severe cases of hyperandrogenaemia affected women might suffer from bitemporal and frontline hair loss. (20)

3.5 Consequences

Due to the insulin resistance, metabolic changes are observed in women with PCOS. These include a higher body mass index, mostly 30 kg/m^2 or higher, hypertension, dyslipidaemia, and a higher risk for developing T2D, making those women more vulnerable for cardiovascular diseases. Increased insulin levels also contribute to an unhealthy fat distribution around the abdomen or central adiposity. PCOS also increases the risk for uterine cancer as the levels of unopposed oestrogen are higher and progesterone levels are lower due to ovarian malfunction, endometrial growth is favoured. (19) Moreover, the increased risk of developing a metabolic syndrome in PCOS leads to an increased occurrence of non-alcoholic fatty liver disease which can result in abnormal liver function, steatohepatitis, cirrhosis, and very unlikely but possible, hepatocellular carcinoma. (22)

At the ovarian level, most women with PCOS suffer from oligo- to anovulation which causes subfertility and makes reproduction complicated. Additionally, when conceived, there are more pregnancy complications like pregnancy-induced hypertension, GDM, and macrosomia. (22) When women report to have oligomenorrhea, there is an 80-95% chance for them to have PCOS. (20)

3.6 Diagnosis

The diagnostic workup for patients with supposed PCOS should always start with an anamnesis and a physical examination and lays its focus on the menstrual

cycle, signs and symptoms of hyperandrogenaemia, and weight. To make a diagnosis of PCOS according to the Rotterdam criteria, 2 out of 3 findings (hyperandrogenism, ovarian dysfunction and polycystic ovaries) are necessary, so the diagnosis of PCOS can often be made with a physical examination and laboratory testing alone. Nevertheless, an ultrasound is useful for risk stratification and is therefore recommended. (20)
Indicators of PCOS are anthropometric factors such as blood pressure and cycle duration, as well as an abnormal ultrasound (regarding ovarian volume and follicle count). Endocrine parameters such as SHBG, testosterone, free androgen index (FAI), FSH, AMH and the function of the thyroid should also be evaluated. Furthermore, the lipid profile, a glucose tolerance test (GTT) and a prolactin test can also determine whether a PCOS is present. Usually, normal prolactin levels are less than 500 mIU/L, but they are elevated in women with PCOS. Transvaginal as well as transabdominal ultrasound can show the typical morphology of the polycystic ovaries if present. (19)

3.7 Therapy

When treating women with PCOS the treatment options should be made individually and based on the subtype of the disease and on the woman's needs. Desired clinical effects could be alleviating hyperandrogenism, regulating the menstrual cycle, or treating infertility. (20)

3.7.1 Treatment of androgen excess in PCOS

Drug therapy against hyperandrogenism should only be given to women who are not planning to become pregnant. Frequently, local therapeutic agents against hirsutism are not sufficient and oral medication has to be used. Oral contraceptives with neutral or anti-androgenic progesterone and androgen receptor blockers are used here. Recommended drugs against hirsutism, alopecia, and acne are spironolactone (50-200 mg/day), flutamide (125-500 mg/day), 5α-reductase inhibitors (finasteride 5 mg/day) and the antiandrogenic progesterone cyproterone acetate (available as oral contraceptive in a 2 mg dose but has its best effects in doses of 10-20 mg daily). (22, 26)

The advantage of oral contraceptives is that they improve hyperandrogenism, balance the menstrual cycle, suppress ovulation, and prevent the formation of cysts. On the other hand, they promote thrombosis and can have a negative effect on bone growth. (19) However, studies have shown that oral contraceptives with a dosage of 20-35 µg of ethinylestradiole will supress androgen production due to three different pathways: first, they increase SHBG levels which leads to a decreased ovarian androgen production; second, they reduce the androgen production in the adrenal gland and third, they prevent conversion of testosterone in the more potent dihydrotestosterone. (20)
On the other hand, Metformin has also shown beneficial effects on hyperandrogenism as well, as it increases SHBG and therefore lowers androgens and circulation free testosterone. (19) For topical application against mild to moderate hirsutism,13.9% solution of eflornithine hydrochloride may be of value to eliminate undesirable facial hair. (22)

3.7.2 Targeting metabolic control

To begin with, it has been shown that regular moderate physical activity can reduce symptoms of PCOS and is therefore recommended as it can also improve the cardiometabolic profile in affected women. Furthermore, loss of abdominal fat lowers inflammation, reduces androgen levels, induces ovulation, and restores metabolic functions. (19)
Especially in metabolically challenged patients, the insulin-sensitizer Metformin (2000-2500 mg/day) should be considered as women will experience weight loss, improvements in menstrual cycle and ovarian function. Particularly women with an impaired glucose tolerance and elevated baseline insulin levels will benefit from a treatment with Metformin. (22) Also, it has been seen that women with PCOS treated with Metformin before and during in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) were at lower risk to suffer from ovarian hyperstimulation syndrome (OHSS). (19)

Finally, to prevent long-term consequences, it is important to monitor women with PCOS when it comes to other metabolic disorders such as hypertension and dyslipidaemia and treat them as soon as possible. (26)

3.7.3 Fertility care

Weight loss of 5-10% can increase ovulation and thus fertility and is therefore recommended for patients who wish to conceive. Also, bariatric surgery can be considered as it increases cycle regularity as well. (20)

For patients who desire immediate conception, the ovulation induction agent clomiphene or alternatively, letrozole should be used to induce ovulation. Usually, after three to five cycles, 50% of the patients conceive successfully. (22)

In clomiphene resistant PCOS cases most women benefited from an additional use of Metformin, even though Metformin can ameliorate ovarian function, it should not be used as first line therapy alone, as clomiphene is usually more effective. To treat clomiphene resistant PCOS, gonadotrophins stimulation might be rewarding. In addition to that, laparoscopic ovarian drilling is a method that can result in spontaneous pregnancy or improved response to an oral ovulation induction agent. (19, 22)

4 Materials and methods

The investigates the use, advantages and disadvantages of Metformin for the treatment of pregnant women suffering from GDM or PCOS. Therefore, terms such as "metformin in gestational diabetes" and "metformin in PCOS pregnancies" were mainly used for literature search. Furthermore, publications that were released in the year 2000 and later were considered and specific large RCTs, as well as meta-analysis were preferred.
Mainly the medical database PubMed was used as a source of information, but also Google Scholar was used to find appropriate reviews. To ensure that all relevant publications are included, a wide range of terms were included in the search. In addition, the reference list of the most important articles on PubMed were additionally evaluated.
In order to explore the short- and long-term effects of Metformin on mother and child in more detail, the selection of search terms in the search for reviews was adapted to the respective subchapter. Therefore, the search term “Metformin” was

combined with either “GDM” or “PCOS” and the respective term of the target parameter listed below.

Inclusion criteria: all studies were included that had Metformin administration as an intervention, with no limit set on the timing of Metformin administration. Preference was given to randomised controlled trials, although retrospective studies were also included. The studies that were included compared either Metformin with insulin in the context of GDM treatment. In the context of PCOS treatment, Metformin was compared with placebo. Occasionally, reviews comparing Metformin with pregnancies without interventions or dietary measures were also included. No restrictions were made to the diagnostic criteria for GDM, as these were not specified in many studies and do not significantly affect the outcome of the trials. Additionally, there was no difference made between the subtypes of PCOS when considered for the thesis.

Exclusion criteria: Studies comparing glyburide with metformin were excluded from the literature-research, as the former drug is not considered in this thesis due to the worse outcome of Glyburide compared to Metformin.

Target parameters: The effect of Metformin should be evaluated in both the short and long term.
The parameters sought were:

- Influence on the genome
- Preterm birth
- Neonatal hypoglycaemia
- Birth parameters
- Pregnancy induced hypertension and preeclampsia
- hormonal parameters
- perinatal mortality
- malformations
- caesarean section
- birth trauma
- maternal weight gain

- glycaemic control of GDM mothers
- Long-term effects in the context of GDM and PCOS treatment for the offspring
- Maternal long-term consequences

5 Results

The aim of this part is to evaluate the effects of Metformin during pregnancy on women with PCOS or GDM and their children in comparison with placebo or insulin. Here, the impact of the drug on the epigenome (fetal programming) is studied, as well as the short- and long-term consequences on the fetus and the mother.

5.1 Impacts of Metformin on fetal programming

Before birth, the development of the fetus can be easily influenced by its environment, so its structure and body function can be altered permanently. This phenomenon is called fetal programming and it can increase the risk for non-communicable diseases like overweight, metabolic syndrome and diabetes. Fetal programming is influenced by hormonal changes like androgen excess, which is associated with hyperinsulinemia, insulin resistance and metabolic changes, a non-favourable lipid profile and metabolic syndrome. Several studies demonstrated an impact of Metformin on the AMP-activated protein kinase (AMPK) pathway, an important pathway that affects hepatic glucose production, glucose uptake, and downregulation of lipogenic genes, suggesting that Metformin might have a serious impact on fetal programming. (27)

5.1.1 Metformin and epigenetics

Metformin acts by inducing a kind of cellular nutrient restriction as it reduces ATP production through the mitochondria. It works though histone acetylation and increases deacetylation via AMPK as the enzyme has the potential to increase the activity of hepatic sirtuin (SIRT1) a histone deacetylase through an activation of nicotinamide phosphoribosyltransferase (NAMPT). The activation leads to an inhibition of hepatic gluconeogenesis. (28, 29) The change in fetal hepatic

imprinting also leads to an alteration of several genes involved in the production of cholesterol, lipids, fatty acids, and steroids, so a rodent model suggests. Therefore, an observation of the exposed fetus until adulthood is necessary to draw conclusions on the effect of Metformin on the fat metabolism and the development of children of mothers receiving Metformin. (30)

Furthermore, an effect on the one carbon metabolism has been identified. The one-carbon metabolism is responsible for cell differentiation, growth, and proliferation and is therefore important for fetal growth and development. Also, methylation, biosynthesis of DNA, RNA, lipids, amino acids, and neurotransmitters are affected by the one-carbon metabolism. Two essential factors for a flawless one-carbon metabolism are Vitamin B12 and folate. It has been seen that the consumption of Metformin might lead to a malabsorption of Vitamin B12 in 10-30% of people, while 30% of people might have Vitamin B12 deficiency. Still, the pathophysiology behind that phenomenon is still uncertain. The most reasonable theory is that the calcium depended uptake of the intrinsic factor might be impaired by the drug as Metformin displaces calcium on the ileal surface. (31)
Studies of Adaikalakoteswari et al. have pointed out that a decreased level of Vitamin B12 during pregnancy leads to adverse lipid profiles, obesity, and insulin resistance. (32) However, a recent prospective study of 87 women could not confirm the connection between low Vitamin B12 levels in the second and third trimester and insulin resistance, infant weight, and placental weight. (31)
The observation of breast cancer cells that were exposed to Metformin suggested that it might have a tumour supressing effect by simulating antifolate activity. That is the reason why it has been supposed that Metformin might impair fetal growth by reducing folate levels. Nevertheless, a prospect cohort study of 336 pregnancies exposed to Metformin in the third trimester showed no increased risk of abortions or major birth defects. (31)

In mice, Metformin exposed offspring gained more weight and had a higher BMI later in life, even though they were rather small at birth, and their body composition was similar to the offspring of malnourished mothers. This could be explained by the “Baker Hypothesis”, a theory that suggests that the fetal environment influences the health of the offspring by changes in epigenetic programming. As

Metformin simulates a state of nutrient restriction via AMPK the offspring might resemble those of malnourished mothers and that might lead to an increase of weight later in life mainly caused by an increasing amount of fatty tissue when obtaining a normal diet. (33)

On the other hand, Salomäki et al. observed that offspring of mice, that obtained Metformin during pregnancy and were fed a high fat diet, were less susceptible to diet induced overweight/obesity later in life. Moreover, Metformin helped to prevent an impaired glucose tolerance in those mice. Transcriptome analysis revealed that Metformin altered expression of genes related to metabolic pathways. (34)

5.2 Short-term consequences for mother and offspring

This chapter discusses the short-term consequences for mother and child in the context of PCOS and GDM treatment. Birth events, the risk for preeclampsia, as well as intrauterine development is assessed in this part of the thesis.

5.2.1 Preterm delivery and early pregnancy loss

Both, GDM and PCOS are associated with a higher risk of having a pregnancy complication such as preterm delivery. In the case of GDM, women who do not obtain a treatment are 1.5 times more likely to give birth before the 37th week of gestation than healthy women. (35) The prevalence of preterm birth in women with PCOS is 2.2 times higher than in healthy women. (36)

5.2.1.1 Preterm delivery in women with PCOS

An epi-analysis performed by Vanky et al. pointed out the effect of Metformin on preterm delivery in women with PCOS. Two large randomised clinical trials were included; a single centre pilot study and a large multicentre study - the so-called “PregMet” (Metformin in pregnancy) study. 313 women were included in this study and they were randomised to Metformin (n=153) or placebo (n=160). Five (3%) women in the Metformin group and 18 (11%) women in the placebo group had a

late miscarriage or a preterm delivery (delivery between 22 and 37 weeks of gestation). However, no women of the Metformin compliant women gave birth before week 35 of gestation. Therefore the researchers concluded that Metformin was able to decrease the risk of preterm delivery (p=0.008) in women with PCOS. (36)

5.2.1.2 Preterm delivery and early pregnancy loss in women with impaired glucose tolerance

In contrast to the studies of women with PCOS, studies about preterm birth during Metformin treatment in GDM with women are controversial. While Bao et al. suggest in a meta-analysis of 2828 participants that Metformin does not decrease the risk for preterm delivery in women with GDM compared to insulin treatment (p = 0.11), a RCT by Mesdaghinia et al. including 200 women with GDM revealed that there was a lower incidence of preterm delivery in the Metformin group. (37, 38) Moreover, observational studies also support the findings of Mesdaghinia and researchers. (39) Controversially, Rowan et al. reported a higher incidence of iatrogenic indicated preterm birth and spontaneous delivery before 37 weeks of gestation in their RCT in Metformin treated women (751 women of which 363 were assigned to Metformin, p=0.04). (40)

The outcome of early pregnancy loss was examined in a study that was performed to assess the teratogenic effect of Metformin during gestation of 458 women taking Metformin due to diabetes or other indications. When all indications for Metformin prescription are considered, 21% of women suffered a miscarriage in early pregnancy, while only 10% of women in the reference group experienced a miscarriage. Those who received Metformin for pre-diabetes had a 24% risk for a miscarriage, whereas the risk of early pregnancy loss was 17% considering the other indications for prescribing Metformin. This suggests that Metformin exposure might increase the risk of early pregnancy loss. (41)

5.2.2 Neonatal Hypoglycaemia

Neonatal hypoglycaemia is an important factor to observe when prescribing a medication that affects the maternal blood glucose levels in pregnancy. Hypoglycaemia often is a reason for a stay at the intensive care unit and therefore a factor for prolongation of hospitalization.

5.2.2.1 Neonatal Hypoglycaemia in babies of GDM mothers

Considering if Metformin can lower the risk of neonatal hypoglycaemia, all studies agree that it does not increase the risk of it at all. (39) Rowan et al. investigated blood glucose levels of 733 newborns by measuring their glucose levels within 2 hours after delivery and before each feeding. Levels below 2.6 mmol/l were regarded as hypoglycaemic and treated as so. The study revealed a lower incidence of severe neonatal hypoglycaemia (<1.6 mmol/l of glucose per litre [28.8 mg/dl]) in the Metformin group (n= 363) compared to the insulin group (n= 370), while the incidence of hypoglycaemia in general (<2.6 mmol/l) did not differ significantly. (40)
In addition to that, a retrospective cohort study performed in New Zealand also reveale a reduced risk of neonatal hypoglycaemia in women with GDM who received Metformin (n=3818) when compared to insulin treatment (n=3450) with a RR (95% CI) of 0.65. Furthermore, the risk of NICU admissions after birth was lower in the Metformin exposed infants and it resulted in a lower risk of prolonged hospitalization for the baby. (42)
A meta-analysis performed by Bao et al. assessed the risk of neonatal hypoglycaemia of GDM babies in 15 studies. The use of Metformin turned out to reduce the risk for hypoglycaemia of the newborn (p=0.001) and the admission to the NICU (p=0.01), which was evaluated in 13 studies. (37)

5.2.2.2 Neonatal Hypoglycaemia in babies of PCOS mothers

Although data about neonatal hypoglycaemia is lacking in PCOS pregnancies, there is no evidence of Metformin increasing the risk of hypoglycaemia in newborns of PCOS mothers. (43) In the RCT by Glueck et al. no case of neonatal

hypoglycaemia was reported in a group of 72 women with PCOS treated with Metformin. (44)

5.2.3 Large for gestational Age and macrosomia in GDM neonates

Especially women with GDM have an increased risk of giving birth to LGA babies. Furthermore, LGA neonates show signs of insulin resistance and have lower insulin secretion at a very early age compared to babies of normal weight for their gestational age. This highlights the importance of normoglycaemia during pregnancy. (45)

A meta-analysis of seven trials that observed the intrauterine exposure of Metformin compared to insulin evaluated the incidence of having LGA babies. The analysis revealed that the risk for LGA babies was lowered by obtaining Metformin. In addition to that, Metformin did not increase the risk for macrosomia, i.e., excessive neonatal fat accretion. (46)
Another meta-analysis performed in 2019 showed that in a total of 3723 Metformin or insulin exposed offspring the Metformin exposed neonates weighed on average 107.7 g less than those whose mothers were randomised to insulin ($p = 0.005$). The same study also showed a decrease in macrosomia of 40% in Metformin exposed neonates. Furthermore, the Metformin exposed neonates had a significantly lower neonatal ponderal index, as well as a lesser head circumference and smaller chest circumferences. (47) By contrast, in a study by Rowan et al. (n= 733) the abdominal circumference between Metformin and insulin exposed neonates did not differ (32.8 +/- 2.7 cm vs. 32.8 +/- 2.8 cm). (40) However, Silver et al. performed a retrospective study of 705 women treated with either Metformin, insulin or both. Women who received both insulin and Metformin were 3.5 times more likely to deliver a LGA baby. (48)

5.2.3.1 Metformin in preventing LGA neonates in non-diabetic obese women

Maternal body weight in pregnancy has a significant impact on the bodyweight of the fetus and therefore, on impact on delivering a LGA baby. Furthermore,

maternal weight affects the rates of infants with macrosomia. (49) As Metformin has the ability to ameliorate metabolic health in obese women by improving the glucose uptake in muscles and by reducing gluconeogenesis in the liver, researchers hoped that it might also improve the perinatal outcome of obese non-diabetic women regarding the delivery of LGA babies. However, a Cochrane Database meta-analysis of 831 infants of obese women who did not have diabetes but received either Metformin or placebo did not able detect an improvement. (50)
In a clinical trial performed between October 2014 and December 2017 in Brazil, 357 women who were diagnosed as obese were randomised to either 1000 mg Metformin or placebo. The outcome was not different between the groups. Nevertheless, regarding the rate of caesarean section in this study 39,8% of the Metformin group compared to 62,9% of the control group had to undergo a caesarean section. Therefore, women of the control group were at higher risk to undergo a C-section (p=<0.01). (49)

5.2.4 Small for gestational age in GDM neonates

While most studies on Metformin and LGA neonates agree that there is a beneficial effect of the drug, researchers are still concerned about the outcome of SGA neonates. (51)

From 2016 to 2019, Picon et al carried out a large randomised multi-centre study in Spain, and randomised 200 women in the second and third trimester to insulin or Metformin. However, there was no difference in the SGA prevalence between the groups. Also the neonatal birth weight was unchanged. (52)
In a meta-analysis carried out in 2019, 7 studies assessed the risk of delivering a SGA infant when treated with either insulin or Metformin in the context of GDM. The finding pointed out that there was no difference between insulin and Metformin treatment. (47)

5.2.5 Fetal Growth in PCOS neonates

Women with PCOS are at higher risk to deliver a SGA baby. Furthermore, Metformin has its effects by creating a sort of cellular nutrient restriction and therefore, there are concerns that Metformin might impair embryonic growth as

well. Thus, it is important to study the effect of Metformin on fetal growth in PCOS pregnancies. (53)

A multi-centre study in Norway observed fetal growth parameters of 258 were pregnant women with PCOS who were randomised to either Metformin or placebo. The biparietal diameter (BPD) and the mean abdominal diameter (MAD) were calculated via ultrasound at gestational week 19 and 32. Furthermore, the head circumference (HC), birth weight and the birth length of the newborns were analyzed. There was no difference was seen in BPD in week 19, however offspring exposed to Metformin had a larger BPD at week 32 (∆BPD, 0.9 mm; p= 0.027). Moreover, the HC at birth was larger in the Metformin group than in the placebo group (∆HC,0.5 cm; p= 0.007). A lager head size is associated with a good brain development, which might implicate a favourable effect on cognitive function. Nevertheless, there was no difference in MAD at week 19 and 32 between the two groups. Birthweight and birth length were similar in the Metformin and the placebo group. (54)

Compared to the reference cohort of women without PCOS, not taking Metformin, a study of 14,929 women with PCOS showed that those who did not receive Metformin during their pregnancy were at higher risk of giving birth to an SGA baby by 29%. Furthermore the LGA risk was increased by 11%. (55) In addition to that, a metaanalysis of Zeng et al. showed that receiving Metformin in the first trimester did not have an adverse impact on growth, head circumference and birthweight of the baby. Furthermore, the researchers reported a lower rate of intrauterine growth restriction (IUGR) (21 of 155 in the Metformin group vs. 35 of 75 infants in the control group). (56)

5.2.6 Pregnancy-induced hypertension and preeclampsia

According to the German, Austrian and Swiss Guidelines of hypertensive pregnancy disorders, preeclampsia is defined as any (even pre-existing) blood pressure value of over 140/90 mmHg during pregnancy with at least one organ involvement, to which no causal cause can be assigned. The organ manifestation usually involves the kidney, where a proteinuria of over 300 mg / day or a protein /

creatinine quotient of over 30 mg / mmol can be detected. If there is no proteinuria, preeclampsia is still likely if there are hypertension and pathological patterns or functional restrictions in the following organs: kidney, liver, respiratory system, placenta (IUGR, SGA), haematological system, or central nervous system. If hypertension is present and preeclampsia markers (sFlt-1 / PlGF) are positive, preeclampsia can be assumed even without organ manifestations. (57)

Risk factors for the development of pregnancy induced hypertension and preeclampsia have been intensively studied, as those morbidities have the potential to put mothers and their fetuses at disproportionate risk for further complications and can lead to lifelong consequences for the mother, like increasing the risk for cardiovascular diseases and cerebrovascular diseases in long term. The major risk factors include pre-gestational diabetes, chronic hypertension, multiple gestation, pre-gestational BMI >30 kg/m^2 and Antiphospholipid-Syndrome. (58)

At the moment, the only recommended medication to prevent preeclampsia is Aspirin. Both the U.S. Preventive Services Task Force (USPSTF) and the American College of Obstetricians and Gynaecologists (ACOG) suggest the use of Aspirin in those women who are at high risk to develop the disease and it should be started between the 12th and 28th week of gestation and continued until delivery. (59) In contrast, the recommendations in German-speaking countries are for starting low dose Aspirin (150mg/day) before the 16th week of pregnancy up to 34th to 36th week of gestation. According to current studies, heparin, magnesium, selenium, vitamin D, calcium and fish oil have unfortunately not shown any preventive effects. (57)

Recently, there is growing interest in statins preventing preeclampsia, as they might have the ability to interfere in some pathophysiological pathways that are associated with preeclampsia. In animal models statins increase levels of PlGF and decrease sFlt-1 and TxA2. Furthermore, statins are reported to ameliorate trophoblastic invasion, placental blood flow, anti-inflammatory and antioxidant effects, protection of the endometrium, inhibition of platelet adhesion, and

anticoagulant actions. However, study outcomes, especially those who used pravastatin, are mixed and teratogenic effects cannot be ruled out yet. (59)

However, great promises in the prevention of preeclampsia have been seen when using Metformin. Metformin might improve endothelial dysfunction and enhance angiogenesis as it reduces the activity of complex I in mitochondria, consequently sFlt-1 and soluble endoglin secretion is reduced which leads to a favourable condition on endothelial cells. (1)

5.2.6.1 Preeclampsia in women with GDM

Preeclampsia is one of the adverse outcomes of. (37) In a meta-analysis of 26 randomised controlled trials, a total of 4921 pregnancies affected by GDM were studied and the impact of insulin compared to Metformin was assessed. Women treated with insulin had a significantly higher risk (OR 1.61) of developing pre-eclampsia than women treated with Metformin. (60) A network meta-analysis of Yu et al. comparing insulin, Metformin and Glyburide even revealed that Metformin has the highest probability of being the most successful treatment for the prevention of preeclampsia in women with GDM. (61)

5.2.6.2 Preeclampsia in women with PCOS taking Metformin

Women with PCOS have a higher risk of developing preeclampsia during their pregnancy with a threefold higher risk than that of the reference population. However, Metformin might have an impact on the regulation of sympathetic nerve activity and angiogenesis, which are linked to the development of diabetes, obesity, PCOS, metabolic syndrome as well as preeclampsia. Though, according to a meta-analysis, which includes 18 studies conducted in Norway, China, Egypt, Bangladesh, Finland and a multi-national study, metformin can reduce the likelihood of pre-eclampsia by 63% and pregnancy-induced hypertension by 55% in women with PCOS compared to women with PCOS not taking Metformin. (55, 62)

5.2.6.3 Preeclampsia in non-diabetic obese women taking Metformin

Metformin may also be promising for overweight women who do not have diabetes. After all, it reduces glucose production in the liver and increases uptake in smooth muscle cells, so it should improve metabolic health and reduce unwanted pregnancy complications. A research team has set itself the task of pulling out all the findings from Cochrane Pregnancy and Childbirth's Trials Register, ClinicalTrials.gov, the World Health Organization (WHO) and the International Clinical Trials Registry Platform (ICTRP) to search and identify randomised controlled trials comparing Metformin with placebo and evaluating the effects on pregnancy. However, there was only a subtle difference in the risk of preeclampsia when women obtained Metformin. (50)

Nevertheless, a recent study published in 2020 compared 357 overweight, non-diabetic women who were randomised to Metformin or placebo. Women were included before the 20th week of gestation. A significant improvement by Metformin treatment on preeclampisa development was identified. (49)

5.2.6.4 Hypertensive disorders in pregnant women with T2D taking Metformin

As Metformin has been shown to play a role in vascular remodelling and angiogenesis, it might have a beneficial effect on preventing preeclampsia and hypertensive disorders.
In a retrospective cohort study 254 pregnancies complicated by T2D were compared. One group received Metformin while the other one not. The primary outcome of the study was gestational hypertension, preeclampsia, eclampsia and HELLP Syndrome. The researchers found a significant difference in hypertension outcome to the benefit of Metformin (22.7% vs. 33.1%). Moreover, preeclampsia with severe features was less common in the Metformin group as well. Therefore, these data suggest that the administration of Metformin in women with T2D may be of benefit beyond the regulation of glycaemic control. (63) However, a large prospective multicentre study performed in Canada and Australia investigated the effects on pregnancy of T2D women taking insulin when given 1000 mg metformin

in addition compared to placebo. In this context, there was no benefit in relation to preeclampsia. (64)

5.2.7 Hormonal balance of PCOS mothers and their offspring

Evidence shows that Metformin can decrease androgen levels of women with PCOS. However, its impact on androgen levels during pregnancy is not very clear yet. Vanky et al. suggested in their RCT of 18 Metformin exposed women that the decrease of androgens in the first and second trimester is lead back to the pregnancy and not to the usage of Metformin, as it was observed in the placebo group as well. (55, 65)
Interestingly, researchers observed a lowering effect of Metformin on androstenedione and testosterone in non-obese women carrying a male fetus, a phenomenon which was not seen in women pregnant with a female fetus. Nevertheless, the interpretation must be done with caution, as the sample size of the subgroups was small. (66)

A potentially relevant effect of Metforming was observed when reducing the hormone level of antimüllerian hormone. Since increased AMH levels occur during a PCOS pregnancy, PCOS can be transmitted transgenerational via programming. Thus, Metformin could stop this transmission across generations. (55)

In an animal model Tartarin and colleagues observed a decrease of Leydig cell count and testosterone in embryos, which was no longer seen at birth. This could lead to the assumption that Metformin has a negative impact on testis development. Nevertheless, further research is needed here. (30)

5.2.8 Perinatal mortality

The outcome of perinatal death in GDM women was observed in a meta-analysis of Zhao et al. and Martis et al. Both meta-analyses suggested that there was no conclusive context between increased perinatal mortality and Metformin intake compared to insulin treatment. (67, 68) In the study performed by Dodd and researchers observing 527 (261 Metformin and 263 placebo) obese non-diabetic women (BMI >25kg/m^2) there were no maternal deaths, however, two stillborn

infants were reported in the placebo group. One was caused by acute chorioamnionitis, and the other was due to early onset fetal growth restriction and preeclampsia. In the Metformin group, there was one neonatal death following an extremely preterm birth. (69)

5.2.9 Congenital malformations

Regarding the outcome of congenital malformations, researchers were concerned that Metformin might have an adverse effect due to the activation of AMPK, as a higher AMPK activity in mouse embryos was seen in diabetic mice leading to a higher rate of congenital abnormalities. However, the researchers could not demonstrate an increased probability of congenital malformation at that dose where maternal AMPK is activated by Metformin. (70)

In a large meta-analysis that observed over 50,000 children with congenital malformation, 168 of them were exposed to Metformin in the first trimester. It was seen that a consumption of Metformin in early pregnancy does not increase the risk of congenital abnormalities. (51) The only significant finding was that babies exposed to Metformin in utero had a higher rate of pulmonary valve atresia. This pathology, however, is associated with maternal diabetes which might have been confounding the study, as there was no hint that Metformin increases the risk of pulmonary valve atresia before. Nevertheless, further study is recommended in an independent dataset. (71)

5.2.10 Caesarean Section

It is known that women with GDM have an increased risk of delivering their baby via caesarean section, therefore there is a great interest to physicians and patients to find ways to prevent this outcome.
A metanalysis published in 2019 by Guo and researchers involving 15 studies with a total of 2611 women with GDM could not show a statistical difference between the treatment with insulin and Metformin concerning the frequency of caesarean section (RR, 1.00; P = 0.96). Regarding the outcome of elective C-section, 3 studies were included in the metanalysis that did not show a significant heterogeneity between the studies. In the pairwise metanalysis, it was seen that

there was a lower incidence of elective C-section in the Metformin group compared to the insulin group (RR, 0.73; P = 0.05). However, the researchers could not find a significant difference between the Metformin and the insulin group concerning the outcome of emergency caesarean section. (72)

The Swedish population-based cohort study performed by Fornes and researchers discussed the pregnancy results of over 14,929 women with PCOS with or without Metformin. Regarding caesarean section as an outcome, women with PCOS not taking Metformin were more likely to get a C-section (OR = 1.08). (55)
A meta-analysis of 4 studies showed that Metformin may reduce the rate of caesarean sections and increase the likelihood of vaginal birth in women with PCOS, still the researchers could not find a statistical difference (both p = 0.48). (56)

5.2.11 Neonatal birth trauma in GDM pregnancies

Concerning the outcome of birth trauma, no statistical significance was found in a randomised clinical trial performed in Iran which analysed 286 women who were divided into an insulin group (143 patients) and a Metformin group (also 143 patients). While 13 women of the Metformin group happened to have an injured baby, 12 women in the insulin group did so as well. (73)
In line with the results of the study from Iran, the clinical trial of 733 women (373 in the Metformin group and 370 in the insulin group) by Rowan et al. found 16 babies with mild birth trauma (bruises or abrasions, that were gone 6 weeks after delivery) in the Metformin group and no newborn with a severe injury, while 17 in the insulin group had a birth trauma of which 2 suffered from a severe one. Hence, the difference between the two groups was not significant either. (40)

5.2.12 Maternal weight gain

As Metformin leads to a modest weight reduction in non-pregnant people, researchers hoped to find a favourable effect on weight gain in pregnant women as well.
In the RCT by Rowan et al. 48% of the women taking Metformin required additional insulin to obtain glycaemic control. When the researchers compared the

weight gain from the enrolment to the study to 36th or 37th week of gestation of 329 women with GDM in the Metformin group and 300 in the insulin group the patients taking Metformin had a significantly lower weight gain (0.8 ± 3.2 kg) compared to the group taking insulin alone (2.0 ± 3.3 kg). Moreover, those women who required insulin to their Metformin treatment also gained less weight than those who received insulin alone. (40)

In overweight or obese women with a BMI above 30 kg/m² or 35 kg/m², the women receiving Metformin might have a lower gestational weight gain compared to placebo. This was the finding of a Cochran Database collection; however, the quality of evidence is low. (50) Another study revealed that there was not only a significantly lower weight gain in the obese, non-diabetic study population receiving Metformin compared to placebo (3.9 ±4.6 kg vs.7 ±4.5 kg), but the Metformin group experienced also a decrease in visceral fat mass after delivery. (70)

In the context of PCOS treatment, a study conducted by Molin and researchers investigated the weight gain of 73 women with PCOS of whom 36 were taking Metformin and 37 were receiving placebo. There was a significantly lower median gestational weight gain in the Metformin group compared to placebo (9.0 vs. 11.6 kg, p = 0.01). Moreover, women taking Metformin were less likely to gain more weight per week as recommended (28% in the Metformin group vs. 62% in the placebo group, p=0.01). Nevertheless, the women in the Metformin group were also more likely to gain less weight per week than generally recommended (33% in the Metformin group vs. 8% in the placebo group, p=0.01). (74)

5.2.13 Glycaemic control of mothers with GDM

Women diagnosed with GDM require strict glycemic control in order to prevent pregnancy complications caused by high blood sugar levels. Insulin has the ability to effectively prevent these complications for both, the mother and the fetus. However, Metformin is more patient-friendly, cheaper, and readily available, making it easier for non-specialized healthcare teams to manage. (52)

Comparing Metformin with insulin Ruholamin et al. could not find a difference in their meta-analysis regarding blood glucose control in women with GDM. However, one study found out, that in terms of fasting glucose and glycosylated haemoglobin Metformin had a more beneficial effect than insulin. (39) Moreover, a meta-analysis of 12 RCTs and 5 observational retrospective case-control studies revealed that maternal HbA1c values at week 36-37 of gestation as well as the maternal weight gain was significantly lower in the Metformin group. (75) In the MiG trial, a large RCT of 751 women randomised to Metformin or insulin, 92.6% of participants continued to take Metformin until delivery, but 46.3% of them had to receive insulin in addition to the 2500 mg Metformin dose to achieve glycaemic control. (40) Another RCT that observed the glycaemic control of 286 women with GDM who received Metformin or insulin showed that the two groups did not differ significantly in terms of fasting plasma glucose (89.16 ± 3.44 mg/dl vs. 88.03 ± 5.00 mg/dl), 2- hour (119.38 ± 4.03 mg/dl vs. 118.99 ± 6.24 mg/dl), and HbA1c (5.40 ± 0.54% in the Metformin group vs. 5.55 ± 0.62% in the insulin group). (73)

However, a recent multicentre, randomized clinical trial performed in Spain revealed that the 100 women randomised to Metformin had a greater post-prandial glucose control after lunch, 117± 14 mg/dL vs. 124 ± 16 mg/dL in the insulin group, that also consisted of 100 women. The glucose levels after dinner were also lower in the Metformin group with 121 ± 14 mg/dL vs 126 ± 15 mg/dL. Nevertheless, there was no difference between the two groups regarding mean fasting glycaemia and HbA1c at 35 and 37 weeks of gestation. Concerning hypoglycaemic events (blood glucose lower than 70 mg/dL), 55,9% of the women in the insulin group had at least one incident compared to 17,7% in the Metformin group. (52)

5.3 Long-term consequences of Metformin exposed children

Metformin instantly passes the placenta and the concentration of the drug in the fetus is similar to the mothers. While there is currently no evidence of harmful short-term effects from the exposure to Metformin for the offspring during pregnancy, there is limited information on its long-term effects.

5.3.1 Long-term effects in the context of GDM treatment, compared to insulin

When evaluating the long-term impacts of Metformin treatment during pregnancy, follow-up studies have shed light on the safety and effectiveness for the management of GDM and its effects on the health and development of the offspring.
In a study by Rowan et al. that investigated 318 children at the age of 2 years born to GDM mothers who were either randomised to Metformin (n=154) or insulin (n=164). The study revealed that their total fat mass did not differ. However, children in the Metformin group had more subcutaneous fat than the children in the insulin group. Another significant difference was that children exposed to Metformin in utero had a higher upper arm circumference (P = 0.002) and the subscapular skinfolds and biceps skinfolds were bigger (P=0.02 and P=0.04). (76)

Ijäs et al. studied height, weight, and head circumferences of 6-, 12-, and 18-months old children whose mothers were either treated with Metformin or insulin. The table below shows the differences between the two groups. At the age of 6 months there was no difference concerning the mean weight and height. However, infants at the age of 12 months exposed to Metformin were significantly heavier (10.47 vs. 9.85 kg, p=0.038), and at the age of 18 months, heavier (12.05 vs. 11.32 kg, p=0.04) and taller (83.9 vs. 82.2 cm, p=0.023). Nevertheless, the Ponderal-Index did not differ between the groups, neither did the head circumference. Furthermore, the motor, social, and linguistic development of the infants was examined by the doctors at the ages of 6-, 12-, and 18- months and the rates of developmental delay were rare and there was no difference between the two groups. (77)

Table 2 Anthropometrics of Metformin and insulin exposed offspring (77)

	Metformin n=45	**Insulin n=48**	**P**
6 month			
Height (cm)	68.1 ± 3.8	67.4 ± 2.7	0.286
Weight (kg)	8.281 ± 0.99	7.925 ± 0.99	0.071
Head circumf. (cm)	43.8 ± 1.3	43.8 ± 1.5	0.865
12 months			
Height (cm)	76.9 ± 3.3	75.6 ± 3.1	0.062
Weight (kg)	10.466 ± 1.49	9.847 ± 1.26	0.038
Head circumf. (cm)	46.9 ± 1.6	46.8 ± 1.7	0.979
18 months			
Height (cm)	83.9 ± 3.6	82.2 ± 3.1	0.023
Weight (kg)	12.051 ±1.87	11.318 ± 1.45	0.040
Head circumf. (cm)	48.3 ± 1.5	48.4 ± 1.7	0.856

Other studies that were performed to show the differences between Metformin and insulin exposed infants regarding neurodevelopment, using mental development and psychomotor development indices or the Bayley Scales of Infant and Toddler Development, could not find any differences between the groups. (78)

5.3.1.1 Metabolic profile

Studies have aimed to identify possible long-term effect on metabolic health in children whose mothers received Metformin during pregnancy, by exploring the impact on various metabolic parameters such as glucose regulation and body composition.
A follow-up study by Rowan et al. investigated the body composition and metabolic outcomes of 7- to 9-year-olds exposed to Metformin or insulin in utero, performed in Auckland and Adelaide. 208 children (which is 28% of the original cohort) were assessed,103 were exposed to Metformin and 105 to insulin. In the Adelaide subgroup the mean age of assessment was 7 years, even though the infants in the Metformin group were 5 months younger at the time of consultation than the ones in the insulin group. In this subgroup there was no difference concerning body composition between the two groups. In contrast, at the 9-year assessment in the Auckland subgroup, Metformin exposed offspring were larger

on several measures. They had increased weight (37.0 ± 12.6 kg vs 32.7 ± 7.7 kg; p=0.049), mid-upper arm circumference (23.0 ± 4.3 cm vs 21.2±2.9 cm; p=0.02), waist circumference (69.1 ± 12.2 cm vs 64.2 ± 8.4 cm; p=0.04) and waist to height ratio (0.51 ± 0.08 vs 0.47 ± 0.05; p=0.02). Furthermore, the BMI was slightly higher (p=0.051) and the triceps skinfolds were slightly bigger (p=0.05). Even tough, Metformin exposed children had a significantly higher fat mass, the body fat percentage measured by DXA, and BIA was similar. In the MRI the Metformin group had higher abdominal fat volumes (subcutaneous fat: 3231 ± 2412 cm^3 vs 2398 ± 1566 cm^3; p=0.059, visceral fat: 941 ± 629 cm^3 vs 722 ± 365 cm^3; p=0.051) but the percentage of total abdominal fat (36.0% ± 14.4% vs 32.2%±10.9%; p=0.16), subcutaneous fat (27.6% ± 12.6% vs 24.4% ± 9.7%; p=0.18), and visceral fat (8.5% ± 3.1% vs 7.7% ± 1.9%; p=0.19) was similar between the groups and so was liver fat (2.5 ± (1.1–6.1)% vs 1.8 ± (1.3–2.6)%; p=0.10). (79)
As data about the metabolic effect of Metformin exposed offspring in utero is limited, a follow-up study on 9-year-olds was performed in Finland by Paavilainen et al. The aim of the study was to investigate anthropometrics, glucose intolerance, blood pressure and lipoproteins of Metformin (n=82) and insulin (n=90) exposed children, whereas 27% of the mothers of the Metformin group had to receive additional insulin during pregnancy. The maternal and parental baseline characteristics in both groups were similar, as well as the neonatal outcomes. (80)
While Rowan et al. found no difference in HDL cholesterol, LDL cholesterol levels and triglycerides in their follow-up 9-year-olds, the authors of the finish study reported higher HDL (1.72 mmol/L vs. 1.54 mmol/L, p=0.039) and lower LDL levels (2.39 mmol/L vs. 2.58 mmol/L, p=0.046) in their Metformin study cohort, as well as higher apolipoprotein B levels in the insulin group (0.63 ratio in the Metformin group vs. 0.67 ratio in the insulin group, p=0.043). The effect of higher HDL levels in the Metformin group was particularly conspicuous in boys (1.85 vs 1.54 mmol/L, p=0.003). (79, 80)
In addition to that, the Finnish authors reported a significantly lower 2-hour glucose level in boys during an OGTT in the Metformin group (5.3 mmol/L vs. 5.9 mmol/L, p=0.015). Taking these outcomes into account, Metformin might have a sex-associated influence on the metabolic profile of the offspring. (80) This might be explained by the role of the non-coding RNA 866 (nc866) epiallel methylation status. Marttila and researchers found that non-methylated nc866 is associated

with higher HDL levels in children and adolescents, but only in the boys and not in the girls. The researchers suggest that periconceptional environment and glucose metabolism have an influence on the methylation status of nc866. (81)
Concerning the blood pressure of children exposed to either Metformin or insulin, two studies, one by Battin et al., and one by Paavilainen et al. revealed that there is no difference between the blood pressure neither at the age of 2-years, nor at the age of 9 years. (80, 82)

5.3.1.2 Testicular development

Investigations into the effects of Metformin use during pregnancy have highlighted the possible influence on testicular development and future reproductive health of male offspring. Rodent models suggested a harmful effect of Metformin treatment during pregnancy on testicular development. However, a follow-up study that compared the prepubertal testicular size of boys whose mothers participated in a study that compared the effect of Metformin and insulin treatment in GDM could not prove that. The mean age at the presentation was 60 months and there were no significant differences concerning the testicular size, neither when determined via ultrasound, nor measured with a ruler, between the groups. In addition to that, height, weight, and BMI of the boys did not differ significantly. (83)

5.3.2 Long-term effects in the context of PCOS treatment, compared to placebo

Anthropometric measurements like weight, height, and body mass index, are important predictors for the development of children, and have been important outcomes in studies that explore the long-term effects of Metformin in offspring of pregnant women with PCOS in comparison with placebo.

A study investigated the long-term effects in offspring of 109 women with PCOS who received Metformin was carried out in the US by Glueck et al. The growth and motor–social development 3, 6, 9, 12, and 18 months after birth of children whose mothers were exposed to 1,5-2,5 g Metformin per day during their pregnancy, but observed no difference between the Metformin exposed group and the control group. (84, 85) Carlsen et al. performed a study in Norway and observed 256

women and their offspring, who received 2 g Metformin per day from the first trimester until delivery, one year after the child’s birth. Women lost less weight, and their children were heavier than those in the placebo group one year after they gave birth (10.2 ± 1.2 kg vs 9.7 ± 1.1 kg). The same investigators observed that female infants exposed to Metformin had larger head circumferences than the placebo group at the age of 1 year. Nevertheless, this phenomenon was not observed in male babies. However, the female as well as the male children at the age of 4 had an odds ratio of 2.17 (1.04–4.61) to be overweight or obese. (85)

Another study that observed anthropometrics in 182 children at the age of 4 years exposed to Metformin also revealed a higher BMI, apparent from 6 months of age. This could be explained by the higher maternal BMI of the PCOS cohort (the BMI of the reference cohort is unknown). However, the babies had no statistically higher head circumference at 1 year of age. (33) Another study assessed the anthropometrics of 12 children and pointed out that 8-year-olds exposed to Metformin (850 mg per day because of maternal PCOS) in utero had a higher risk of having higher glucose levels and tended to have higher systolic blood pressure and lower LDL-cholesterol levels. (85). The study did not show any other significant differences between Metformin and placebo in 8-year-olds concerning bodyweight, height, and body composition. (86)
A nationwide population-based cohort-study of Fornes et al. with a median follow up of 5.8 years could not show a higher probability of children of PCOS mothers exposed to Metformin of becoming obese. By contrast, women with PCOS not receiving Metformin during pregnancy were more likely to have obese infants. (55)

5.3.2.1 Cognitive function

Studies examining the long-term effects of in utero exposure to Metformin in children of mothers with PCOS suggest that there may not be a significant difference in cognitive function or IQ. Another follow-up study carried out in Norway performed an IQ test in 52 children (mean age 7.7 years) in the Metformin group and 41 in the placebo group. There was no significant difference in IQ

between the two groups. Furthermore, no significant between-group difference was found in the subscales of verbal comprehension, working memory, perceptual organization, or processing speed. Even after adjusting for parental education level the results did not change. The mean IQ in the Metformin group was 100.0 and 100.9 in the placebo group which corresponds with the IQ of the standard population. (87)

5.4 Maternal long-term consequences of mothers with GDM

GDM during pregnancy is a known risk factor for the onset of both T2D and metabolic syndrome later in life of the women. Even before GDM is diagnosed a reduced beta-cell function and increased insulin resistance can be found in affected women, as well as post-delivery. Therefore, researchers speculate if the treatment of GDM can influence the likelihood of long-term metabolic health issues. (88)

5.4.1 Impaired glucose tolerance

For the expectant mother, the diagnosis of GDM during pregnancy means to have a greater risk of developing T2D in the future, with a risk being at least seven times higher than undergoing a healthy pregnancy. Therefore, it is of central importance to evaluate whether a treatment with Metformin or insulin may decrease this risk.
Pellonperä et al. studied the effects of Metformin, insulin, and diet interventions during pregnancy on the occurrence of insulin resistance 6-8 weeks and 1 year after delivery. The study included 221 women with singleton pregnancies who showed at least two pathological results in a 2-hour 75-g oral glucose tolerance test and were treated with diet and lifestyle interventions or needed medical treatment because the target glucose levels could not be reached with the lifestyle intervention only. The researchers found no significant differences in glucose tolerance and HbA1c either 6-8 weeks or 1 year after delivery among women who were treated with Metformin (n=110) or insulin (n=107). Women who received diet-

only treatment had a lower level of impaired glucose tolerance 1 year after giving birth compared to those who received a medical treatment. (89)
Along with the results of Pellonperä et al. a Finnish research team by Huhtala et al. found in a 9-year-follow-up study of 165 women treated with Metformin or insulin (77 Metformin, 88 insulin) comparable results between the two groups for the outcome of the occurrence of T2D (14.3% vs. 15.9%) and pre-diabetes (26.0% vs. 30.7%). (88)
To sum up, both studies agree that the usage of either Metformin or insulin have no positive impact on the development of impaired glucose tolerance and therefore cannot prevent the probability of the development of T2D.

5.4.2 Metabolic Health and weight loss

While short-term studies of the influence of Metformin of maternal metabolic health show promising results, the long-term effects of Metformin on maternal weight loss and metabolic health, including lipid levels and other markers of metabolic syndrome, remain to be fully understood.
In the MiG trial, women in the Metformin group (n= 363) had a greater weight loss than those in the insulin group (n= 370, p=0.006) from the time of enrolment to 6-8 weeks after delivery, as well as from early pregnancy to 6-8 weeks postpartum. However, in the study performed by Pellonperä et al., the weight loss of the 221 mothers from their first prenatal visit to 6-8 weeks postpartum decreased with no significant difference between the Metformin and insulin group. A potential explanation might be that the women in the MiG trial were significantly more overweight that the women in the study by Pellonperä et al. (40, 89)
Pellonperä and researchers found no significant weight loss in their three groups (Metformin, insulin and diet-only) after adjusting for age with a mean weight loss of 1,17 kg between the first antenatal visit and the visit 6–8 weeks postpartum. (89)

According to a 9-year follow-up study conducted by Huhtala and his team, over half of the 165 women were obese, while only 13.4% had a normal weight. No significant differences were noted between the Metformin and insulin groups in terms of maternal anthropometry, glucose metabolism, insulin resistance, alanine

aminotransferase (ALT), inflammation, serum lipids, and adiponectin. However, the lipid profile was slightly better in the Metformin group, with lower levels of ApoB, LDL and non-HDL cholesterol, and higher levels of HDL cholesterol (p-values 0.057 to 0.099). Low-grade inflammation markers like CRP and interleukin 6 are associated with an increased risk of T2D, while adiponectin has a protective effect. Moreover, Metformin treatment was associated with lower levels of hs-CRP. However, this was one of the exploratory secondary outcomes and thus has a high risk of being a false-positive result. (88)

Overall, the studies found that neither Metformin nor insulin treatment showed a significant advantage in reducing the incidence of metabolic disorders or increasing favourable weight loss of the mothers in the long-term. However, there were small differences in hs-CRP levels, with lower rates found in the Metformin group, and in ApoB and HDL cholesterol, with lower ApoB and higher HDL cholesterol in the Metformin group. (88, 89)

6 Conclusion

The findings of this thesis allow important insights in the efficacy and safety of Metformin in pregnancy, especially in terms of short- and long-term consequences for both, mother and offspring.

Fetal programming

The impact of Metformin on fetal programming remains still uncertain, as there is limited evidence available. Even though, there are several studies but no significant conclusions can be made. Limitations are that the sample sizes in the studies are relatively small and there is a need for more follow-up studies to draw definitive conclusions.
However, the animal study of Salomäki and co-researchers has pointed out that offspring of mice fed a high-calorie diet during pregnancy and given, were healthier after birth despite being fed a high-calorie diet, which suggests a

promising influence of Metformin on fetal programming and in preventing impaired glucose tolerance.

Short-term effects

Especially when the short-term effects of Metformin on the perinatal outcomes are considered, Metformin can be as effective as insulin for the treatment of GDM. When examining the anthropometric measurement of babies born to mothers with GDM, it seems that Metformin might have favourable effects compared to insulin, as it has shown to reduce the incidence of LGA babies and reduce the risk for macrosomia. However, it might have no influence on the incidence of SGA babies. In the context of PCOS, Metformin seems to have a favourable effect on the anthropometric measurements of babies intrauterine and after birth, women not receiving Metformin had a higher risk of giving birth to a SGA or a LGA baby. Moreover, offspring of mothers receiving Metformin had a higher head circumference in utero which is associated with a better cognitive development. Drawing a definitive conclusion about the relationship between Metformin therapy and early miscarriage in women with GDM seems to be challenging, as the prescription for Metformin therapy had heterogeneous indications, which means that women receiving Metformin might have a higher-risk pregnancy per se. Nevertheless, promising results have been obtained in women with PCOS regarding preterm birth and late miscarriage, as Metformin reduced these outcomes compared to placebo.
When it comes to the outcome of neonatal hypoglycemia in babies born to women with GDM, Metformin appears to be significantly superior to insulin. All studies that were assessed agree on that finding and moreover, there was a lower probability for a stay in the intensive care unit, and a shortened hospitalization.
Both GDM and PCOS are associated with an increased risk of developing preeclampsia, so it was important to investigate this phenomenon in women receiving Metformin. The results of the assessment revealed that women with GDM who were treated with Metformin had a significantly lower probability of developing preeclampsia compared to women receiving insulin or Glyburide. Moreover, Metformin treatment of women with PCOS reduced the risk of developing preeclampsia or hypertension during pregnancy compared to women

who did not receive Metformin. Nevertheless, no effect on preeclampsia risk was observed in obese women taking Metformin. In T2D women, Metformin reduced the risk for preeclampsia.
The influence of Metformin on hormone levels in offspring and mothers with PCOS needs further investigation. While some researchers agree that androgen levels normalize during pregnancy, others speculate a correlation with Metformin. There might be a difference between fetal sex, as different results were found in women carrying a male vs female fetus. Moreover, there is a positive effect of Metformin on the Anti-Mullerian Hormone, which plays a role in the transgenerational transmission of PCOS. Thus, a reduction of AMH caused by Metformin could be promising for preventing such transmission, but further studies are needed.
Metformin exposure might slightly increase the outcome of congenital malformations like pulmonary valve atresia, but this requires further studies to confirm. Nevertheless, there is no conclusive context for the occurrence of perinatal death and the intake of Metformin.
Even though Metformin seemed to decrease the probability of giving birth to a LGA or macrosomic baby in comparison with insulin, both seem to have comparable outcomes regarding the incidence of C-section. Nevertheless, there might be a positive influence of Metformin on increasing the probability of vaginal delivery in the context of PCOS treatment.
There seems to be no difference between the Metformin and the insulin group regarding the outcome of birth trauma, as had been comparable results concerning the occurrence of mild injuries.
Known that Metformin can influence the bodyweight of patients with diabetes in a positive way, there was hope that it might favourably affect the maternal gestational weight gain as well. All studies that were investigated in this thesis showed that Metformin did indeed have a positive influence on gestational weight gain, both, in the context of GDM treatment as well as in the context of PCOS therapy. Moreover, overweight or obese women also experienced a lesser weight gain when taking Metformin.
There are still heterogeneities when comparing different studies regarding the glycaemic control of women with GDM. While some studies showed no differences between the Metformin and the insulin treatment, others suggested a favourable effect of Metformin on glycosylated haemoglobin and postprandial glucose levels.

However, there seems to be a significant benefit of Metformin when it comes to hypoglycaemic events.
Yet, a downside of Metformin was observed regarding the achievement of glycaemic control in the MiG trial, as almost half of the women receiving 2500 mg of Metformin required supplemental insulin even though they needed less than the group who received insulin alone.

Long-term effects

While short-term effects on the offspring are generally well studied and the drug can be considered safe, long-term studies are still very limited and further trials with representative numbers are necessary. In order to study the impact of the drug in the long term it would be necessary to follow the test persons of the study into adulthood. However, the children included in the trial were followed up to a maximum of 9 years. Thus, it is difficult to speculate whether the drug has relevant long-term influences on the exposed offspring or not.
Regarding the anthropometrics children of mothers with GDM exposed to Metformin at the age of 2 years it was seen that those children had a similar total fat mass to toddlers exposed to insulin during pregnancy. However, the subcutaneous fat mass was larger in the Metformin group, which is known to have a higher insulin sensitivity, suggesting that Metformin exposed offspring could have a favourable fat distribution compared to insulin exposed children. Moreover, children at the age of 18 months might be heavier and taller than toddlers whose mothers received insulin during pregnancy, however the body composition might be similar.
No difference in motor-, social-, linguistic- and neurodevelopment of the infants between the two groups could be observed.
At the age of 9 years Metformin exposed children have a similar body fat percentage than those whose mothers received insulin, nevertheless the children in the Metformin group were larger than those of the insulin group.
Concerning metabolic parameters, the investigated trials differed. While Rowan et. al. suggested no differences regarding HDL and LDL levels of the 9-year olds, Paavilainen found higher HDL and lower LDL levels in the Metformin group in their follow-up GDM study. The higher HDL level was especially found in boys,

moreover favourable 2-hour postprandial glucose level were conspicuous in male subjects, which might suggest that there is a sex difference regarding the metabolic influence of Metformin, however, further investigations are needed here. Though, there seem to be no difference concerning blood pressure and testicular development between the two groups.
Regarding the long-term effects of Metformin in children whose mothers had PCOS and were exposed to Metformin, data is limited as well. Furthermore, there is a heterogeneity between the studies. While some researchers found no difference between the Metformin and the placebo group, some suggest that infants exposed to Metformin were more likely to become overweight at the age of 1 and 4 years. Moreover, it might also cause increased glucose levels, higher systolic blood pressure and lower LDL-cholesterol levels in 8-years. On the contrary, a nation-wide population-based cohort study showed that women with PCOS not receiving Metformin were more likely to have obese children. Nevertheless, children of mothers with PCOS receiving Metformin have not shown any deficiencies regarding cognitive function, IQ, verbal comprehension, working memory, perceptual organization, or processing speed.

Long-term consequences for mothers with GDM

As there is a relevant association between GDM and maternal impaired glucose tolerance even after delivery, it is important to evaluate whether Metformin treatment or insulin treatment during pregnancy can reduce the likelihood of developing T2D and prediabetes. However, there is no evidence that neither of the drug can influence that outcome in a positive way.
Concerning metabolic long-term outcomes of mothers with previous GDM, Metformin seems to have a positive influence on weight loss after the pregnancy in women who are overweight, which was seen in the MiG trial, however, this outcome was not found in other studies. Thus, there seems to be no difference between insulin and Metformin treatment concerning weight loss postpartum. Furthermore, Metformin and insulin had comparable results in anthropometry of the mother, glucose metabolism, insulin resistance, ALT, inflammation, serum lipids, and adiponectin. Still, there seems to be a favourable effect of Metformin on hs-CRP levels, a marker that is associated with an increased risk of T2D, so

therefore Metformin might still prevent the incidence of T2D, however, further research is needed here.

Limitations

In this thesis there is a heterogeneity regarding the initiation of Metformin since no restrictions were made depending on when the therapy with Metformin started. While most studies on GDM began the treatment after the diagnosis of GDM (24th to 28th week of gestation), the researchers studying the outcomes of Metformin on obese pregnant women started their treatment between the 12th and 16th week of gestation, in addition to that, the start of treatment in studies about the influence of Metformin on PCOS usually was before conception.
Furthermore, the daily treatment dosage of the drug is varies between 500 mg and 3000 mg between the studies, therefore no correlations can be made between the dosage of Metformin and the effect of the drug. Moreover, some women required additional insulin to achieve glycaemic control, however, those women were still counted as "Metformin group" and were only considered separately when it was obviously relevant.
Glibenclamid was not considered in this thesis, as it is not regularly prescribed in Europe for the treatment of GDM and is considered to be inferior to Metformin.

Future perspectives

While there is enough evidence to say that Metformin can be considered safe during pregnancy in terms of its short-term effects and therefore is at least equal to insulin, the long-term effects are still insufficiently researched, however the existing studies about long-term effects of Metformin in the context of GDM treatment show promising results, as no negative impact on the development of the children was seen. Nevertheless, long-term outcomes of children of women with PCOS require further investigation. While no deficiencies were found concerning cognitive development, it might contribute to the emerge of overweight and obese children. Thus, long-term studies that follow children into adulthood are required.

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