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METFORMIN IN GESTATIONAL DIABETES MELLITUS

by

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ABSTRACT

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METFORMIN IN GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) is a state of impaired glucose tolerance with onset or first recognized during pregnancy. Treatment of GDM is important, since adequate treatment reduces maternal and neonatal adverse effects. GDM is associated with an elevated risk of maternal blood pressure problems during pregnancy, cesarean deliveries and it raises the risk of type 2 diabetes later in life. The fetus has an increased risk of macrosomia, delivery complications and neonatal hypoglycemia. Medication is needed if adequate glycemic control is not achieved by diet. Insulin is the traditional medication for GDM but metformin as an oral drug has been suggested to be an alternative. Metformin crosses the placenta, but the transfer mechanism is not clear.

The main aim of this study was to compare the efficacy and safety of metformin and insulin in the treatment of GDM patients by evaluating the influence of medication on maternal and fetal outcomes in a retrospective and a randomized controlled trial (RCT). Predictors of the need for additional insulin with metformin to meet good glycemic control were evaluated.

The impact of metformin exposure on maternal and fetal outcomes was studied by assessment of metformin concentrations in maternal serum and umbilical cord serum. The mechanism of metformin placental transfer and the role of active organic cationic transporters (OCT) in metformin transfer were studied by *ex vivo* placental perfusion.

Measurements of metformin concentrations at birth indicated that there is a high degree of placental transfer of metformin from the mother to the fetus (96%). Metformin does not seem to accumulate in the fetus. The *ex vivo* placental perfusion study indicated that OCTs may not have a significant role on the placental transfer of metformin.

Metformin concentration levels were not related to fetal outcome. Higher metformin concentrations and a maximum clinical dose of metformin had a favorable effect on retarding maternal weight gain during pregnancy.

Compared to insulin, metformin did not increase the maternal, fetal or neonatal risks of adverse events, and the delivery modes were unaffected. Glycemic control evaluated by HbA1c and serum fructosamine levels was similar during metformin and insulin therapies. However, 21% of the metformin-treated patients needed additional insulin to obtain good glycemic control. High maternal age, performing the oral glucose tolerance test and initiation of medication early during pregnancy and high HbA1c and fructosamine values are associated with a need of additional insulin.

Key words: gestational diabetes, insulin, metformin, placental transfer, OCT

TIIVISTELMÄ

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METFORMIININ KÄYTTÖ RASKAUSDIABETEKSESSA

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Raskausdiabeteksella tarkoitetaan sokeriaineenvaihdunnan häiriötä, joka todetaan ensimmäisen kerran raskauden aikana. Hoidolla voidaan vähentää raskausdiabetekseen liittyviä äidin ja vastasyntyneen haittoja. Lääkitystä tarvitaan, jos ruokavaliohoidolla ei saavuteta hyvää sokeritasapainoa. Perinteisesti lääkityksenä on käytetty insuliinia, mutta metformiinin käyttöä insuliinin vaihtoehtona on ehdotettu. Metformiini läpäisee istukan, mutta sen läpäisymekanismi ei ole selvillä.

Tämän tutkimuskokonaisuuden pääasiallisin tarkoitus oli verrata metformiinin tehokkuutta ja turvallisuutta insuliiniin raskausdiabeteksen hoidossa selvittämällä lääkkeen vaikutusta äitiin ja vastasyntyneeseen. Lisäksi haluttiin tutkia, mitkä tekijät ennustavat insuliinin tarvetta metformiinin lisänä, jotta saavutettaisiin hyvä sokeritasapaino. Metformiinin annoksen vaikutus äitiin ja vastasyntyneeseen arvioitiin mittaamalla metformiinin pitoisuus äidistä, ja sikiön puolelta napanuoran veressä. Tässä tutkimuksessa selvitettiin myös aktiivisen kuljetusproteiinin (OCT) merkitystä metformiinin kulkeutumiseen istukan läpi perfusiomalla istukkaa *ex vivo* .

Ex vivo istukkaperfuusiotutkimuksen tulokset viittasivat siihen, että OCT-kuljetusproteiinilla ei ollut todennäköisesti merkittävää osuutta metformiinin kulkeutumisessa istukan läpi.

Metformiinin pitoisuusmittaukset synnytyksen yhteydessä osoittivat metformiinin siirtyvän sikiöön istukan läpi suuressa määrin (96 %) kertymättä kuitenkaan sikiön verenkiertoon. Metformiinin pitoisuudella ei ollut vaikutusta vastasyntyneen hyvinvointiin. Maksimaalisella metformiinin annostuksella ja korkealla metformiinipitoisuudella todettiin olevan suotuisa vaikutus äidin painon nousuun raskauden aikana.

Insuliiniin verrattuna metformiini ei lisännyt äidin, sikiön tai vastasyntyneen haittatapahtumia, eikä sillä ollut vaikutusta synnytystapaan. Sokeritasapaino insuliini- ja metformiinilääkityksen aikana oli yhtäläinen arvioitaessa sitä HbA1c- ja fruktosamiinimittauksilla, mutta 21 % metformiinin käyttäjistä tarvitsi lisäksi insuliinia hyvän sokeritasapainon saavuttamiseksi. Tutkimuksesssa todettiin, että mitä iäkkäämpi äiti oli, mitä varhaisemmassa raskauden vaiheessa sokerirasitus oli tehty ja lääkitys aloitettu, ja mitä korkeammat HbA1c ja fruktosamiinipitoisuudet olivat, sitä suuremmalla todennäköisyydellä metformiinin lisänä tarvittiin insuliinia.

Avainsanat: raskausdiabetes, metformiini, insuliini, istukan läpäisy, OCT

CONTENTS

ABBREVIATIONS

ABC	ATP-binding cassette
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BMI	body mass index
BRCP	breast cancer resistance protein
CI	confidence interval
CL	clearance
C_{fa}	concentration in fetal arterial inflow
C_{ma}	concentration in maternal arterial inflow
C_{ss}	concentration at steady state
$C_{ss,fv}$	steady state concentration in fetal venous outflow
$C_{ss,mv}$	steady state concentration in maternal venous outflow
FFR	fetal flow rate
fP	fasting plasma
GDM	gestational diabetes mellitus
GFR	glomerular filtration rate
Gw	gestational weeks
HbA1c	glycosylated hemoglobin
HPLC	high performance liquid chromatography
LGA	large for gestational age
MATE	multidrug and toxin extrusion proteins
MFR	maternal flow rate
NICU	neonatal intensive care unit
OCT	organic cation transporter
OGTT	oral glucose tolerance test
PCOS	polycystic ovary syndrome
P-gp	P-glycoprotein
pp	postprandial
RCT	randomized clinical trial
RDS	respiratory distress syndrome
SD	standard deviation
SGA	small for gestational age
SLC	solute carrier
TI	transplacental transfer index
TPT%	transplacental transfer percentages
t_{ss}	time to reach steady state concentration

LIST OF ORIGINAL PUBLICATIONS

1. Tertti K, Ekblad U, Vahlberg T, Rönnemaa T. Comparison of metformin and insulin in the treatment of gestational diabetes: a retrospective, case-control study. Rev Diabet Stud 2008;5:95-101.

2. Tertti K, Ekblad U, Heikkinen T, Rahi M, Rönnemaa T, Laine K. The role of organic cation transporters (OCTs) in the transfer of metformin in the dually perfused human placenta. Eur J Pharm Sci 2010;39:76-81.

3. Tertti K, Ekblad U, Koskinen P, Vahlberg T, Rönnemaa T. Metformin vs. insulin in gestational diabetes. A randomized study characterizing metformin patients needing additional insulin. Diabetes Obes Metab 2013;15:246-251.

4. Tertti K, Laine K, Ekblad U, Rinne V, Rönnemaa T. The degree of fetal metformin exposure does not influence fetal outcome in gestational diabetes mellitus. Submitted.

1. INTRODUCTION

Gestational diabetes mellitus (GDM) is a state of impaired glucose tolerance recognized during pregnancy in women not known to have had impaired glucose tolerance before pregnancy. It is common and affects globally approximately every tenth pregnancy. The prevalence of GDM is increasing as the occurrence of obesity, one of the risk factors predisposing to impaired glucose tolerance, is increasing. This creates a growing global challenge. GDM is associated with several health problems of the mother and child. Glucose passes freely through the placenta. The maternal state of hyperglycemia leads to the hyperglycemic state of the fetus which causes excessive fetal growth, i.e. macrosomia, which in turn increases the risk of neonatal and maternal injuries at birth. The avoidance of birth injuries leads to a high incidence of labor inductions and elective cesarean sections. When the fetus is exposed to hyperglycemia *in utero*, neonatal hypoglycemia due to hyperinsulinemia often needs treatment with intravenous glucose after birth. GDM is associated with an elevated risk of maternal blood pressure problems during pregnancy and raises the risk of type 2 diabetes later in life of the mother.

GDM is diagnosed by the oral glucose tolerance test (OGTT), which is usually performed in the second trimester of pregnancy. The risks for mother and child increase linearly with rising OGTT glucose values (Metzger et al. 2008). There are several international recommendations for testing GDM and for OGTT cut-off values to assess GDM. Treating GDM is clearly beneficial because it results in better maternal and neonatal outcomes (Crowther et al. 2005, Landon et al. 2009, Horvath et al. 2010).

Treatment of GDM is always based on diet modifications. If fasting and postprandial glucose target values are not met with diet alone, medication is needed. Historically, insulin has been used most. It is effective and does not affect the fetus, since it does not usually cross the placenta (Menon et al. 1990). However, the subcutaneous administration route, the risk of hypoglycemia and the tendency to increase appetite and weight gain (Norman et al. 2004) are disadvantages of insulin. There is growing evidence favoring the use of the oral agents glibenclamide (sulfonylurea) (Langer et al. 2000, Ecker and Greene 2008) and particularly metformin (Moore et al. 2007, Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Mesdaghinia et al. 2013, Spaulonci et al. 2013) as an alternative to insulin in GDM patients.

Metformin crosses the placenta in late pregnancy according to *ex vivo* human term placental perfusion studies (Nanovskaya et al. 2006, Kovo et al. 2008a) and *in vivo* studies (Hague et al. 2003a, Vanky et al. 2005, Charles et al. 2006, Eyal et al. 2010) where maternal and cord blood metformin concentrations have been measured and compared. However, the exact mechanism and the degree of placental metformin transfer are unclear.

Although metformin crosses the placenta, maternal, fetal or neonatal risks have not increased in GDM patients on metformin compared to insulin according to the results of randomized studies (Moore et al. 2007, Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Mesdaghinia et al. 2013, Spaulonci et al. 2013). Metformin alone is not always sufficient medication for good glycemic control, but the factors predicting the need for additional insulin are not clear.

In the present study the mechanism and degree of placental transfer of metformin was studied by human term placental perfusion studies and by measurements of metformin concentrations in maternal and cord serum *in vivo*. The effectiveness and safety of metformin treatment compared to insulin treatment of GDM patients was evaluated in a randomized controlled trial (RCT) and prospective study, and the factors predicting the need for additional insulin were studied. The association between the metformin concentration in maternal serum at 36 gestational weeks (gw), in maternal and umbilical cord serum at birth, and maternal and neonatal outcomes were investigated.

2. REVIEW OF THE LITERATURE

2.1. Gestational diabetes mellitus

2.1.1. Definition

Gestational diabetes mellitus (GDM) is classically defined as "any degree of glucose intolerance with onset or first recognition during pregnancy" (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 2003). The definition includes unrecognized impaired glucose tolerance before pregnancy and a persistent situation after the pregnancy. O'Sullivan and Mahan (1964) introduced the original criteria for GDM which were set to predict future maternal diabetes. In 1949, White introduced the classification for diabetes during pregnancy (White A to F). This classification intended to predict how well the pregnancy of an individual diabetic proceeds and the prognosis of the neonate with respect to survival (Sacks and Metzger 2013). White A was considered as biochemical diabetes (i.e. gestational diabetes) and the only one not diagnosed before the onset of pregnancy. Later on, in 1986, White A diabetes was subdivided by the American College of Obstetricians and Gynecologists into class A_1 and A_2 diabetes (or A and A/B): type A_1 (A) indicates diet therapy and type A_2 (A/B) insulin therapy during pregnancy (Sacks and Metzger 2013).

2.1.2. Glucose metabolism in normal pregnancy

Basal and postprandial glucose metabolism is altered in pregnancy. During pregnancy eating causes stronger insulin secretion, but postprandial glucose concentrations are still higher than in non-pregnant individuals (Cousins et al. 1980). Although fasting glucose is decreased, basal hepatic glucose production is increased, because hepatic insulin sensitivity and glucose suppression are reduced. This, in turn, leads to increased insulin production (Lain and Catalano 2007). Insulin production is also increased because estrogen and progesterone secreted by the placenta induce enlargement of the islets of Langerhans and hyperplasia of pancreatic β-cells (van Assche et al. 1978). Other reasons for changes in glucose metabolism may include a dilution effect, increased glucose utilization from the placenta to the fetus and inadequate production of glucose during pregnancy (Catalano et al. 1992, Lain and Catalano 2007).

Insulin sensitivity decreases as pregnancy advances, and by the third trimester it is 33-78% of that of non-pregnant women (Catalano et al. 1991, Lain and Catalano 2007). Insulin resistance is caused by increased maternal adiposity and insulin-desensitizing effects of the placental hormones, progesterone and placental growth hormone (PGH) (Ryan and Ennes 1988, McIntyre et al. 2009). Progressive insulin resistance is compensated by increased insulin production during the normal pregnancy (Lain and Catalano 2007).

Glycosylated hemoglobin (HbA1c) is decreased in pregnancy (Mills et al. 1998) since the mean blood glucose concentration is reduced and the red blood cell count increased.

HbA1c value during pregnancy declines 0.6 %-units compared to in the non-pregnant state (Nielsen et al. 2004). The erythrocyte life span is reduced from ~ 120 days to ~ 90 days in pregnancy and thus the HbA1c-value reflects the average glycemia over a shorter time than in non-pregnant subjects (Lurie and Mamet 2000).

2.1.3. Glucose metabolism and pathogenesis of GDM

Fasting glucose concentrations are higher in pregnancies complicated by GDM than in normal pregnancies (Lain and Catalano 2007), while basal hepatic glucose production is similar. Insulin sensitivity is lower in pregnancies of lean and obese GDM patients compared with normal pregnancies (Catalano et al. 1993 and 1999). Insulin resistance is increased by 40% in late pregnancy in patients with severe GDM compared with normal pregnancies (Lain and Catalano 2007).

GDM occurs when the pancreatic β-cells do not produce enough insulin to combat the increased insulin resistance (Buchanan and Xiang 2005). Obesity and chronic insulin resistance are the most common factors that predispose to β-cell dysfunction during pregnancy (Buchanan et al. 2012). Probably the same genes that cause deficient insulin secretion and predispose to type 2 diabetes operate also in GDM (Mao et al. 2012). Some GDM patients (< 10%) have autoimmunity towards β-cells (glutamic acid decarboxylase antibodies, insulin autoantibodies and/or anti-islet cell antibodies) (Catalano et al. 1990, Damm et al. 1994), some (1-5%) have maturity-onset diabetes of the young (MODY) with autosomal dominant heredity (Weng et al. 2002), and some GDM patients have type 2 diabetes that has not been diagnosed previously.

Of the levels of biochemical mediators associated with insulin resistance in GDM patients leptin (Kautzky-Willer et al. 2001) and tumor necrosis factor (TNF-α) are increased (Coughlan et al. 2001) and adiponectin decreased (Retnakaran et al. 2004) compared with healthy pregnant non-GDM-patients. High serum C-reactive protein (CRP) concentrations are associated with GDM, especially in late pregnancy (Leipold et al. 2005).

2.1.4. Prevalence

In Finland, 12.7% of all pregnancies in 2012 were associated with GDM and 1.8% of the pregnant women required insulin to be started (National Institute for Health and Welfare 2013). The prevalence of GDM is generally approximately 10%, but varies from 1% to 14%, depending on the diagnostic test, criteria, ethnicity of the population and environmental factors (American Diabetes Association 2007). African, Indian and Asian women have a higher incidence of GDM than Caucasian women (Dornhorst et al. 1992, Chawla et al. 2006). In addition to ethnicity, the risk of GDM rises with a family history of type 2 diabetes or GDM, increased maternal age, parity, previous GDM or macrosomic child, polycystic ovary syndrome (PCOS) and especially obesity with increased insulin resistance (Torloni et al. 2009, Reece 2010).

In a meta-analysis of over 670 000 pregnant women, the risk of GDM was assessed and quantified in relation to the maternal body mass index (BMI) before pregnancy (Torloni et al. 2009). It was shown that the risk of GDM was 2 times higher in overweight (BMI 25-29 kg/m^2), 3 times higher in obese (BMI >30 kg/m^2) and 6 times higher in severely obese (BMI >35 kg/m^2) women (Torloni et al. 2009) compared to women with normal prepregnancy BMI. Women with previous GDM have a risk of 30-84% of recurrent GDM (Kim et al. 2007).

2.1.5. Screening and diagnosis

Globally, there are variety of screening and diagnosing strategies for GDM (Table I.) GDM is diagnosed by OGTT, which can be performed by a one-step approach (single glucose tolerance test) or by a two-step approach with a diagnostic OGTT for those with positive screening test (two glucose tolerance tests). OGTT is usually performed at 24-28 gw since insulin sensitivity decreases as pregnancy advances (Lain and Catalano 2007).

Table I. International recommendations for testing GDM, and OGTT cut-off values for GDM diagnosis.

Recommendation	**Screening**	**Diagnostic OGTT and cut-off values**				
	Universal 50g glucose screen, cut-off value (mmol/l)	Glucose load (g)	Fasting (mmol/l)	1-hour (mmol/l)	2-hour (mmol/l)	3-hour (mmol/l)
ADA:one- or two- step (ADA 2013)	≥7.2 or 7.8	100	≥5.1	≥10.0	≥8.5	≥7.8
CDA:two-step (CDA 2008)	≥7.8	75	≥5.3	≥10.6	≥8.9	-
WHO:one-step (Alberti and Zimmet 1998)	-	75	≥5.8	none	≥7.8	-
ADIPS: one-step (Nankervis et al. 2013)	-	75	≥5.1	≥10.0	≥8.5	-
NICE:one- or two- step (NICE 2008)	≥7.8	75	≥7.0		≥7.8	-
Finland:one-step (Gestational diabetes: Current Care Summary, 2013)	-	75	≥5.3	≥10.0	≥8.6	-
IADPSG:one-step (Metzger et al. 2010)	-	75	≥5.1	≥10.0	≥8.5	-

ADA, American diabetes Association; CDA, Canadian Diabetes Association; WHO, World Health Organization; ADIPS, Australian Diabetes in Pregnancy Society; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; NICE, National Institute for Health and Clinical Excellence (England and Wales). Modified from Meltzer et al. 2010.

Screening can be universal or selective. In selective screening very low-risk women are not screened, and this excludes some patients from screening (Evensen et al. 2012). Universal screening is routinely performed widely because treating diagnosed GDM is beneficial (Hillier et al. 2008, Horvath et al. 2010). Although international screening programs vary, it is recommended that pregnant women at high risk of pre-existing but undiagnosed diabetes should be screened for GMD early in pregnancy (Evensen et al.

2012). In the diagnostic 1-step procedure either all pregnant women, or women with risk factors for GDM (ethnicity, maternal age, obesity, parity, family history of type 2 diabetes, PCOS) undergo the OGTT (Torloni et al. 2009, Reece 2010).

The results of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (Metzger et al. 2008) gave cause for the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) to propose new criteria for detecting hyperglycemia in pregnancy (Metzger et al. 2010). IADPSG recommends diagnostic OGTT for all pregnant women without a screening test. The OGTT cut off values (Table I) rely on the results of the HAPO study (Metzger et al. 2008). A diagnosis of GDM is set, if one or more values are out of range. When this procedure is used with these cut off-values, the incidence of GDM is approximately 18% (Metzger et al. 2010).

Glycosylated hemoglobin (HbA1c) does not usually have clinical value for the diagnosis of GDM, but it is useful for diagnosing pre-existing diabetes in early pregnancy (Sacks et al. 2011). Fructosamines are glycosylated proteins in the serum and they reflect the glycemic balance during the previous 2-3 weeks i.e. a shorter period than HbA1c (Li and Yang 2006). Li and Yang (2006) have studied the value of measuring fructosamine during pregnancy in patients with abnormal glucose tolerance. They found that the mean level of fructosamine decreases with gestational age, and that the level of fructosamine is similar in GDM and non-GDM patients in gw 16-20. Thus, fructosamine does not have clinical value for diagnosing GDM.

Before 2008, pregnant women with predisposing risk factors (O'Sullivan and Mahan 1964, Hyvönen 1991) for GDM underwent OGTT in Finland, and the OGTT cut off-values varied within different regions of the country. It was, however, shown that over 50% of the Finnish GDM patients did not have risk factors (Pöyhönen-Alho 2005) and therefore the recommendation was settled 2008 (Gestational diabetes: Current Care Guideline, 2013) as shown in Table 1. A diagnostic OGTT is now performed for most pregnant women in Finland [excluded are 1) primiparas with a BMI < 25 kg/m^2 and with no first degree relatives with type 2 diabetes and 2) pregnant women under 40 years of age with a BMI < 25kg/m^2 and no previous GDM or macrosomia of newborns] (Gestational diabetes: Current Care Guideline, 2013). OGTT is performed in gw 24-28, and during the first trimester of pregnancy if the risk of GDM is high. A diagnosis of GDM is set, if there are one or more pathologic OGTT values as defined by the recommendations of the American Diabetes Association in 2007.

2.1.6. Maternal risks of GDM

2.1.6.1. Short-term risks

In GDM patients the risk of pregnancy induced hypertension and pre-eclampsia is increased 2-3 fold (Suhonen et al. 1993, Schmidt et al. 2001), and for cesarean deliveries 2-fold (Tan et al. 2009) compared to non-GDM patients. In Finland the incidence of hypertensive problems is observed to be 20% in GDM patients (Suhonen et al. 1993).

In HAPO study a linear association with no obvious threshold value for elevated plasma glucose and increased risk of cesarean delivery was found (Metzger et al. 2008).

2.1.6.2. Long-term risks

GDM is a strong risk factor for type 2 diabetes (Kim et al. 2002, Malcolm 2012). A large meta-analysis of over 10 000 women with type 2 diabetes reported a 7-fold risk of type 2 diabetes among GDM patients compared with women without GDM (Bellamy et al. 2009). The risk of diabetes in GDM patients over 9 years is over 9 fold compared to women without GDM (Feig et al. 2008). The prevalence of type 2 diabetes, within 10 years after a diagnosis of GDM in a Danish cohort (Lauenborg et al. 2004) was 41%, and in the USA it is estimated that no less than 30% of all GDM patients will have diabetes or impaired glucose metabolism postpartum (England et al. 2009).

The risk of the metabolic syndrome in GDM patients is over 3-fold higher 10-11 years after delivery compared to subjects with no previous GDM diagnosis (Verma et al. 2002, Lauenborg et al. 2004). The prevalence increases over 4-fold if the GDM mother is obese (Lauenborg et al. 2004). In another study the relative risk of the metabolic syndrome was 2.4 in GDM patients independently of obesity (Gunderson et al. 2009).

A history of GDM raises the risk of cardiovascular diseases (CVD) but the major underlying risk factor is diabetes, which emerges after delivery (Shah et al. 2008). The risk of CVD rose 13% over 11 years after GDM when adjusted for diabetes (Shah et al. 2008). In another study the risk of CVD was some 1.7-fold after 12 years among patients with GDM compared to patients without a history of GDM (Retnakaran et al. 2009).

2.1.7. Fetal and neonatal risks of GDM

The study of hyperglycemia and adverse pregnancy outcomes (HAPO) was planned to "clarify the risks of adverse outcomes associated with various degrees of maternal glucose intolerance less severe than that in overt diabetes mellitus" (Metzger et al. 2008). The primary endpoints in the HAPO study were birth weight >90th percentile, primary cesarean delivery, neonatal hypoglycemia and cord serum C –peptide level >90th percentile. Significant changes in the occurrence of these endpoints did not relate to any clear OGTT-cut-off values. Instead of specific threshold glucose values, there was a linear relationship between maternal glycemia and these adverse outcomes (Metzger et al. 2008). Thus, even mild hyperglycemia during pregnancy can increase maternal and fetal risks (Metzger et al. 2008, Reece 2010).

2.1.7.1. Short-term risks

GDM increases the risk of fetal macrosomia, shoulder dystocia, birth injuries (brachial plexus palsy and bone fractures), hypoglycemia, respiratory distress syndrome (RDS) and hyperbilirubinemia (Reece 2010) (Table II).

Table II. Fetal and neonatal short-term risks.

Outcome	Incidence	Study
Macrosomia* or LGA**	14-40 % 4-5 x more frequent in insulin treated GDM patients than in diet treated patients (Suhonen et al. 2008)	Ehrenberg et al. 2004, Jensen et al. 2000 and 2003, Langer et al. 1994, Surkan et al. 2004, Metzger et al. 2008 (HAPO)
Shoulder dystocia	2-11%	Esakoff et al. 2009
Brachial plexus palsy	2.4-2.7 %	Suhonen et al. 2008
Hypoglycemia	3-24 % 3 x higher in insulin treated GDM patients and 10 x higher in GDM patients without treatment compared with non-GDM patients (Langer et al. 2005b).	Jensen et al. 2000, Metzger et al. 2008, Esakoff et al. 2009
RDS	1.5-4 %	Esakoff et al. 2009
Hyperbilirubinemia	2-13%	Metzger et al. 2008, Esakoff et al. 2009

*birth weight ≥ 4000g or ≥4500g
** birth weight > 90th percentile or >2SD

Macrosomia is the main factor linked to other fetal complications (Nold and Georgieff 2004). Glucose passing through the placenta to the fetus induces excessive fetal insulin production due to the hyperglycemic state of the mother. Already in the 1920s Jorgen Pedersen formulated the hyperglycemia-hyperinsulinemia hypothesis (the Pedersen hypothesis), which is commonly used to explain fetal macrosomia (Catalano and Hauguel-De Mouzon 2011). Insulin has anabolic effects and it acts as a growth factor for the fetus (Schwartz et al. 1994). Fetal hyperinsulinemia together with an increased energy supply in the form of glucose leads to macrosomia. Macrosomia is mainly seen as a disproportion between the head and body of the fetus: there is increased regional adiposity in the shoulder and abdominal areas and, possibly, hepatomegaly, splenomegaly and cardiomegaly (Nold and Georgieff 2004).

The incidence of macrosomia in non-diabetic pregnancies is around 16-28%, depending on the maternal BMI (Owens et al. 2010). Maternal overweight and obesity or excessive weight gain during pregnancy are independent risk factors for fetal macrosomia (Ehrenberg et al. 2004, Langer et al. 2005a, Cheng et al. 2008, Ouzounian et al. 2011). In the HAPO study, the frequency of LGA (birth weight >90thpercentile) in non-GDM pregnancies was 8% and in mild GDM pregnancies 16% (Metzger et al. 2008). GDM patients with hyperglycemia requiring insulin treatment have also an increased risk of fetal asphyxia caused by fetal hyperglycemia and hyperinsulinemia (Teramo 2010).

2.1.7.2. Long-term risks

The association between GDM of the mother and disturbances in glucose metabolism and obesity of the young child is controversial (Hillier et al. 2007, Catalano et al. 2009, Dabelea et al. 2009, Pirkola et al. 2010).

The study of Pirkola et al. (2010) showed that GDM was not an independent risk factor for childhood obesity, while the prepregnancy BMI was. GDM raises the incidence of the metabolic syndrome of children 3.5-4 fold compared to children of non-GDM mothers (Boney et al. 2005, Clausen et al. 2009, Vääräsmäki et al. 2009). The study of Boney et al. (2005) showed that GDM is not an independent risk factor for the metabolic syndrome in childhood but in association with fetal macrosomia it constitutes a significant risk for the newborn of the metabolic syndrome in childhood. Based on a meta-analysis of 9 prospective and 15 retrospective cohorts (Burguet et al. 2010), the incidence of type 2 diabetes among children to GDM mothers was 1.6-7.8 fold compared to the children of non-GDM mothers.

The association could be explained by heredity and environment (Buchanan et al. 2012). Maybe epigenetic mechanisms in the hyperglycemic prenatal environment have some effect on metabolic dysregulation in children born to GDM patients (Fernandez-Morera et al. 2010).

2.1.8. Treatment

The primary goals of the management of GDM are to prevent macrosomia and to detect and prevent pregnancy complications (Evensen 2012). There are two large RCTs (Crowther et al. 2005, Landon et al. 2009) and one systematic review and meta-analysis of RCTs comparing usual care with specific treatment of GDM patients (Horvath et al. 2010). These studies have demonstrated that treatment of GDM patients has significant beneficial effects.

In the study of Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) (Crowther et al. 2005), 1000 GDM patients were randomized to routine care (control group) or to dietary advice and insulin if needed (intervention group). The intervention group had a lower rate of serious perinatal complications (death, shoulder dystocia, bone fractures or nerve palsy), but a higher rate of admission to neonatal nursery and labor induction than the control group, which may have been related to the fact that physicians were aware of the diagnosis of the participants. In the trial of Maternal-Fetal Medicine Units Network (MFMU) (Landon et al. 2009) there was no difference in a composite perinatal outcome of stillbirth, neonatal death, birth trauma, jaundice, hypoglycemia and elevated cord-blood C-peptide between intensified treatment and usual care. The participants had mild GDM, and the intervention was associated with favorable changes in birth weight, neonatal fat mass, shoulder dystocia, and cesarean delivery. It has also been demonstrated that treatment of even mild GDM reduces maternal weight gain and the incidence of blood pressure problems during pregnancy (Landon et al. 2009). Horvath et al. (2010) concluded that the incidence of shoulder dystocia and LGA-infants is reduced in women on intensive treatment.

Both preprandial and postprandial glycemia seem to be of importance. de Veciana et al. (1995) showed that there is a connection between perinatal complications and high

postprandial glucose values. Several studies have established a connection between complications and fasting glucose values (Naylor et al. 1996, Suhonen et al. 2008, HAPO 2009, Durnwald et al 2011) and the study of Rowan et al. (2010) demonstrated that fasting and postprandial glucose values carry predictive information.

2.1.8.1. Nutritional treatment

Nutritional therapy is accepted and recommended as a primary treatment for GDM. There is, unfortunately, only little specific information from controlled trials to give guidance for nutritional recommendations (Buchanan et al. 2012). The nutritional recommendations are similar in pregnancy and in non-pregnancy. Ideally, weight gain during pregnancy should be lower for obese GDM mothers, and limiting the carbohydrate content to 35-40% of total calories may be advisable for overweight and obese women (Peterson and Jovanovic-Peterson 1991).

It is essential to identify GDM patients needing treatment in addition to nutritional treatment in an effort to minimize maternal and fetal complications. It is a common practice to ask GDM patients to measure their blood glucose values before breakfast and 1-2 hours after meals (Buchanan et al. 2012), although the optimal timing and frequency of measurements is not clear, and glucose targets have also varied in different studies (Buchanan et al. 2012). Common target recommendations are for fasting plasma glucose < 5.5 mmol/l, for 1-hour postprandial glucose < 7.8 mmol/l and for 2-hour postprandial glucose <6.7 mmol/l (Metzger et al. 2007). The risk of complications is lowest at fasting glucose values <4.9 mmol/l and 2-hour postprandial values 5.6-6.2 mmol/l (Kjos et al. 2001). Sonographic measurement of the abdominal circumference (AC) of the fetus may be helpful in detecting GDM patients needing intensified management (Buchanan et al. 2012). Among diet treated GDM patients with fasting plasma glucose level < 5.8 mmol/l the fetal AC-measurement > 75th percentile at 29-33 weeks of gestation increases the risk of LGA by 28% (Buchanan et al. 1994).

There are no clear guidelines on how to interpret and use HbA1c values in GDM patients. HbA1c values may, nevertheless, be useful for achieving better glycemic control (Jovanovic et al. 2011). Fructosamine can be used as a marker of short-term control of maternal glycemia, particularly in the third trimester (Cefalu et al. 1990).

2.1.8.2. Medical treatment

Traditionally, insulin has been the primary medical treatment if maternal glucose targets are not achieved by dietary therapy. Insulin is safe for the fetus, because it does not normally cross the placenta (Menon et al. 1990). Insulin treatment is planned individually to meet glycemic targets. There is no convincing evidence to avoid any available insulin from use during pregnancy (Buchanan et al. 2012). Often intermediate-acting NPH-insulin (neutral protamine Hagedorn) and/or rapid acting insulin are used. Insulin has several disadvantages since its use needs training, it is administrated by subcutaneous injections, it can cause hypoglycemia and it increases appetite and weight (Norman et al. 2004).

Of the oral antidiabetic agents, glibenclamide and metformin are the most studied agents to treat GDM patients. Glibenclamide is a sulfonylurea, also known as glyburide, and it binds to pancreatic β-cell ATP (adenosine triphosphate) sensitive potassium channels. This raises the intracellular calcium content in β-cells and stimulates insulin release (Berggren and Boggess 2013). US authors have claimed that glibenclamide can replace insulin as a first line treatment of GDM in many situations (Ecker and Greene 2008). The statement is mainly based on the randomized study of Langer et al. (2000), where 404 GDM patients were treated with glibenclamide or insulin. Patients on glibenclamide had a lower incidence of maternal hypoglycemia (2%vs 20%, p=0.03). The level of glycemic control and perinatal outcomes were the same in both groups. Only 4% of the glibenclamide treated patients needed additional insulin. A meta-analysis of 9 studies and 745 GDM patients on glibenclamide and 637 on insulin (Moretti et al. 2008) reported no differences between the groups in terms of birth weight, macrosomia, LGA or neonatal hypoglycemia. The failure of glibenclamide to achieve glycemic control is approximately 4-21% (Cheung et al. 2009).

A meta-analysis of 6 studies with 395 GDM patients on metformin, 291 on glibenclamide and 702 on insulin reported no differences between the groups in terms of maternal fasting and postprandial glycemic control (Dhulkotia et al. 2010). The use of metformin or glibenclamide compared to insulin did not increase the rate of neonatal hypoglycemia, birth weight, incidence of LGA-babies or cesarean deliveries (Dhulkotia et al. 2010).

Placental perfusion studies have shown that the placental transfer of glibenclamide is 1-9% (Elliott et al. 1991, Nanovskaya et al. 2008). However, the clinical study of Hebert et al. (2009) showed that the concentration of glibenclamide in the umbilical cord blood is around 70% of the concentration in maternal blood.

Acarbose was studied in a randomized trial in the treatment of GDM patients (Bertini et al. 2005). It was compared with insulin and glibenclamide. Glycemic targets were met more infrequently with acarbose than with other medications. There are no studies on the use of glitazones, meglitinides, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) agonists or sodium/glucose cotransporter 2 (SGLT2) inhibitors during pregnancy. Placental perfusion studies show a placental transfer of 33% of rosiglitazone (Nanovskaya et al. 2008,) and 1.5% of repaglinide (Tertti et al. 2011).

2.2. Human placenta, principles of action

2.2.1. Placental anatomy

The placenta is an organ between maternal and fetal circulations. The structure and function of the placenta change during pregnancy (Panigel 1986, Enders et al. 1999). After implantation of the embryo to the uterus in the beginning the pregnancy, the primary function of the placenta is to regulate nutrient transport from mother to fetus, waste elimination from fetus to mother and gas exchange between mother and fetus (Myren et al. 2007).

2.2.2. Placental circulation

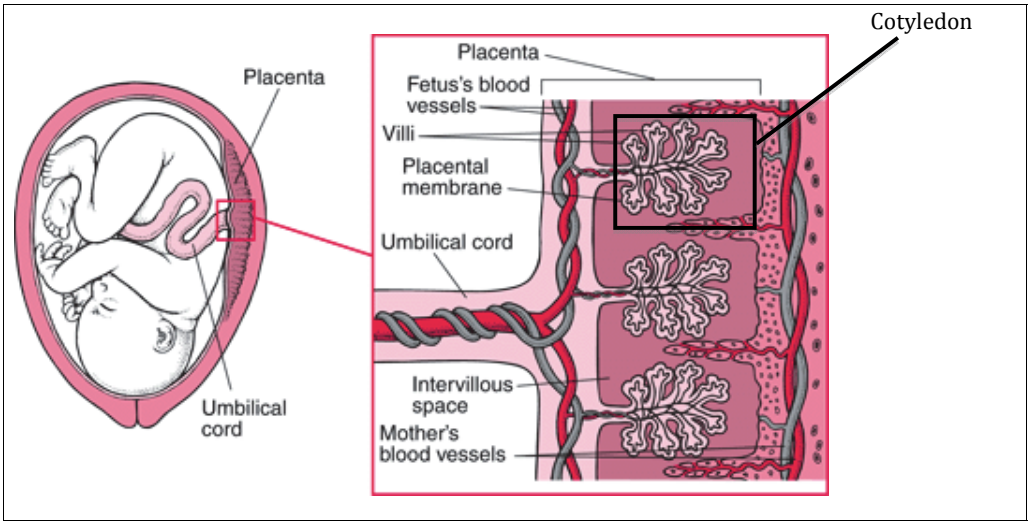


Figure 1. Placental circulation. Modified from Foley 2004, Merc Manual. Copyright©2010-2013 Merc Sharp & Dohme corp., reproduced with permission.

The placenta consists of chorionic villi, threadlike projections in which the fetal circulation ends. Each chorionic villous tree forms a vascular unit, a cotyledon. The full term placenta is composed of 10 to 40 cotyledons (Myren et al. 2007). The placenta develops from trophoblast, the outer layer of the blastocyst (Cross et al. 2006).

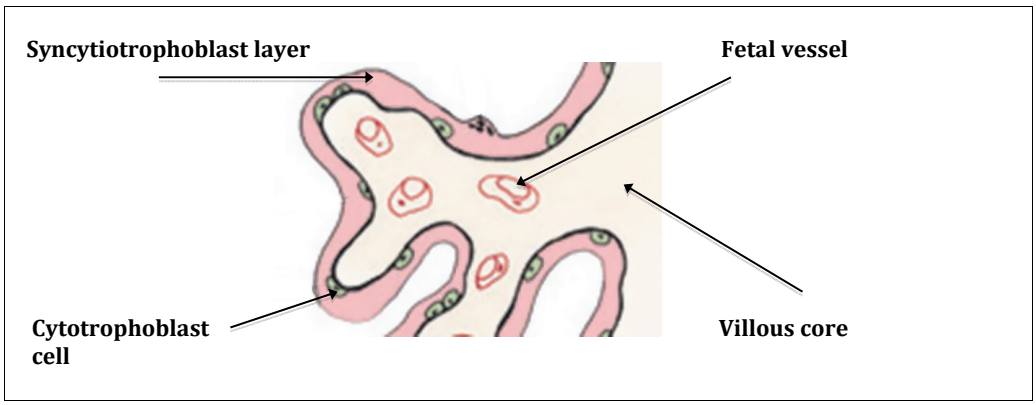


Figure 2. Trophoblast layer. Modified from Scifres et al. 2009. Copyright © 2013 by The Physiological Society, reproduced with permission.

The trophoblast layer is further divided into the syncytiotrophoblast layer and cytotrophoblast cells. In the beginning of pregnancy, fetal and maternal circulations are separated by five tissue layers, but at the end of pregnancy there is only the fetal

endothelium and the syncytiotrophoblast layer which separate the two circulations from each other (van der Aa et al. 1998). The syncytiotrophoblast layer is situated on maternal side and functions as a barrier between mother and fetus (Enders et al. 1999). The cytotrophoblast cells are situated on the fetal side of the villi. Maternal oxynated blood flows through the spiral arteries to the intervillous space and bathes the villi to allow oxygen change take place between maternal and fetal circulation (Bourget et al. 1995). Oxynated blood flows to the fetus through one umbilical vein. Fetal deoxynated blood flows to the placenta through two umbilical arteries, which branch into smaller vessels in the villi.

2.2.3. Placental glucose transfer in normal and GDM pregnancy

The human fetus produces only minimal quantities of glucose itself, and over 95% of the glucose in the fetal plasma is transferred through the placenta from the maternal side (Kalhan et al. 2000, Staat et al. 2012). Glucose is transported by facilitated diffusion following the concentration gradient and is flow-limited (Desoye et al. 2011, Larque et al. 2013). Glucose is transported by the glucose transporter isoforms GLUT 1 and GLUT 3, which have been identified in the human placenta (Hahn et al. 2001). Glucose is also transported back from the fetus to the placenta and stored there as glycogen to serve fetal emergency demands (Desoye et al. 2011). It has been shown that the placental transfer of glucose is enhanced in term placentas of GDM patients treated with insulin compared with patients treated with diet only (Osmond et al. 2001). The placentas of GDM patients contain much glycogen (Leonce et al. 2006, Cross et al. 2006).

2.2.4. Placental drug transfer

The mechanisms involved in placental transfer are passive diffusion, facilitated diffusion, active transport, filtration and pinocytosis (Pollex et al. 2010). Molecules with low molecular weight, i.e. <500 Da, high lipophilicity, unionization and low protein binding cross easily the placenta by passive diffusion (Ala-Kokko et al. 1993, Syme et al. 2004). The rate of transfer depends on maternal and fetal blood flows and is referred to as flow-limited transfer (van der Aa et al. 1998). Membrane-limited transfer means that the rate of transfer depends on the ability of the molecule to permeate a membrane (van der Aa et al. 1998), as is the case for polar, ionized and hydrophilic compounds. Structural changes over the course of gestation influence placental permeation. Barriers get thinner, surface areas increases and the formation of a pH-gradient establishes a slightly acidic fetal environment (Audus 1999). The metabolism and elimination of drugs in the maternal and fetal organism affect placental transfer (Syme et al. 2004).

2.2.4.1. Active transport

The placenta expresses a variety of active transporters, which transfer compounds through biological membranes (Syme et al. 2004). These active transport mechanisms are located at the maternal-facing (apical brush border membrane) as well as at the fetal-facing (basolateral membrane) syncytiotrophoblast (Marzolini and Kim 2005, Mölsä et al. 2005, Staud et al. 2012). Active transporters can work against a concentration gradient (Syme et al. 2004) and molecules can be transported even back to the maternal circulation (Young et al. 2003, Ganapathy and Prasad 2005). The drugs can be pumped into (influx) or out from (efflux) the syncytiotrophoblast into the maternal or fetal circulation, or drugs can be transported bidirectionally (St-Pierre et al. 2002, Ganapathy and Prasad 2005). Efflux transporters situated at the maternal-facing surface can protect the fetus from exposure, and efflux transporters situated at fetal-facing membrane may have an opposite effect (Vähäkangas and Myllynen 2009).

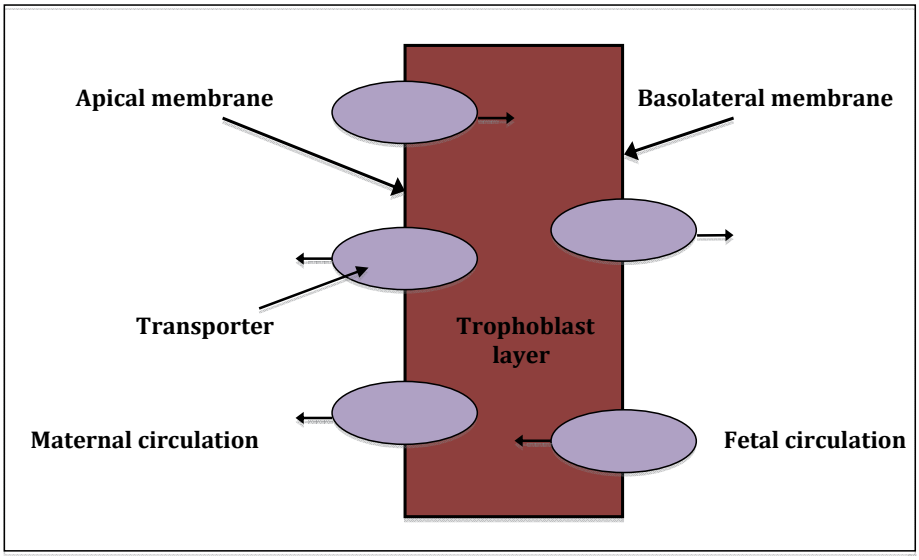


Figure 3. Schematic presentation of active transport mechanism in human placenta. Modified from Staud et al. 2012.

Several drug transporters of the ATP-binding cassette (ABC) and solute carrier (SLC) families have been discovered in the human placenta (Staud et al. 2012). Three proteins of the ABC family are particularly involved in drug transport: P-glycoprotein (P-gp/MDR1, ABCB1), breast cancer resistance protein (BCRP, ABCG2) and multidrug resistance-associated proteins (MRPs, ABCCs) (Staud et al. 2012). ABC transporters pump their substrates out of the trophoblast cells into the maternal (P-gp, BCRP, MRP2) or fetal (MRP1) circulation, and these transporters situated in the maternal-facing surface are considered to be the main active components of the human placental barrier (Staud et al. 2012).

SLC transporters facilitate energy-independent uptake of hydrophilic or charged molecules, e.g. metformin, by trophoblast cells (Staud et al. 2012). SLC transporters detected in human placenta belong to subfamilies of organic cation transporters (OCTs); OCT1, OCT2 and OCT3, organic anion transporters (OATs), carnitine transporters (OCTNs), nucleoside transporters (CNTs, ENTs), organic anion transporting polypeptides (OATPs) and multidrug and toxin extrusion proteins (MATEs) (Staud et al. 2012). OCTs and MATEs may act synchronously: OCTs transfer organic cations into trophoblast cells from the fetal circulation, and MATEs act as vectors to transport compounds into the maternal circulation (Staud et al. 2012) (Figure 4).

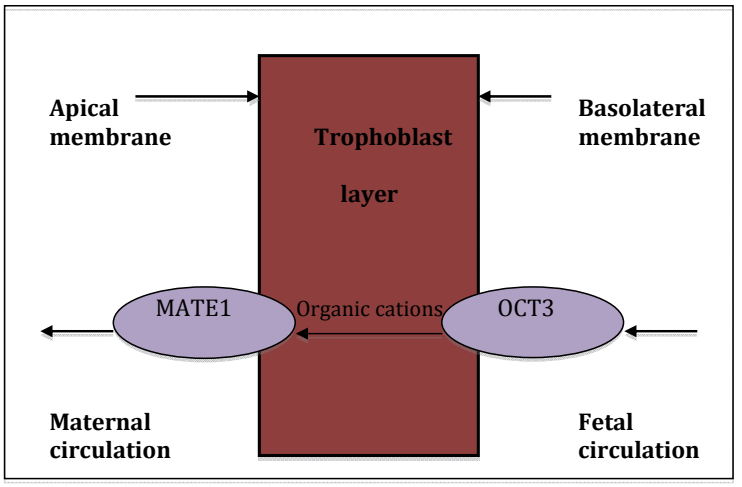


Figure 4. Vectorial transport by OCT3 and MATE1. Modified from Staud et al. 2012.

2.2.4.2. Methods to investigate placental drug transfer

It is often unethical to study placental drug transfer directly in humans, results from animal studies are not necessarily applicable to humans and the feto-placental unit is not available before delivery. To overcome these investigative hurdles different methods to determine placental drug transfer have been developed (Hutson 2011). Methods include *in vivo* (living organisms in their normal condition), *in vitro* (components of an organisms that have been isolated from their biological environment, "test tube experiments") and *ex vivo* (organism outside their normal condition but with minimum alterations) studies (Table III).

Table III. Methods of studying placental drug transfer.

Type	Example	Advantages	Disadvantages
In vitro	Trophoblast cultures and tissue preparations	Useful to determine the mechanism of transport.	Not useful to study the amount of drug transferred by the placenta.
Ex vivo	Placental perfusion	Resembles most closely *in vivo* situation usually in late pregnancy. Transfer over the time can be studied. Placental tissue sampling is available.	Does not give information about early pregnancy. No standardized methodology in different laboratories.
In vivo	Animal models	Drug transfer at different gestational ages and drug accumulation in fetal tissue can be studied.	Difficult to extrapolate to humans. Variability of placental structure in different species.
	Termination of pregnancy by social indication	Drug transfer can be studied in different gestational ages.	Ethical issues, usually used in first trimester. Generally no longer conducted.
	Umbilical cord blood and maternal blood at delivery	*In vivo* measurement in humans. Information of drug concentration in maternal and fetal circulations. No ethical issues.	Represents only one time point. Large individual variation. Usually restricted to advanced gestational age.

Adopted and modified from Hutson 2011.

2.2.4.2.1. Ex vivo human term placental perfusion method

A method of human placental perfusion was developed to study the kinetics of placental transfer. It gives also information on fetal exposure to compounds, on the potential role of transporters and on placental metabolism (Myren et al. 2007). Human placental cotyledon perfusion was introduced by Panigel in 1967. This method has subsequently been modified by several investigators, and the validity of the method is well documented (Schneider et al. 1972, Miller et al. 1985). Perfusions have separate maternal and fetal circulations (dual perfusion). Perfusions of term placental lobules can be performed in closed (perfusates are recirculated) and open (perfusates are collected after one passage through placenta) systems. Placental drug transfer can be studied in both system, but the closed system is a better simulator of the *in vivo* physiological conditions and allows study of the metabolism of a compound (Vähäkangas and Myllynen 2006). The open system is usually used to determine the transfer rates of drug under steady state conditions, and drug clearance values can be calculated (Bourget et al. 1995, Vähäkangas and Myllynen 2006).

2.3. Metformin

2.3.1. Mechanisms of antihyperglycemic action and pharmacokinetics

Metformin is a compound used for its antihyperglycemic effect. Metformin alleviates hyperglycemia by three different primary mechanisms. Its main effect is to decrease

liver glucose production by inhibiting hepatic gluconeogenesis and glucogenolysis (Zhou et al. 2001, Kirpichnikov et al. 2002, Natali and Ferrannini 2006, Viollet et al. 2012). Secondly, metformin functions as an insulin sensitizer in skeletal muscles (Zhou et al. 2001), although this action has been questioned (Hällsten et al. 2002). Thirdly, metformin delays intestinal absorption of glucose. Metformin also decreases appetite and may result in weight reduction (Cicero et al. 2012). It improves lipid metabolism by inhibiting lipolysis, and it lowers the concentration of free fatty acids and triglycerides in the plasma (Laakso 2006, Viollet et al. 2012). Metformin does not increase insulin production and thus does not induce hypoglycemia.

Metformin activates LKB1- AMPK (liver kinase B1 adenosine monophosphate activated protein kinase) in insulin sensitive tissues, which lowers glucose levels (Zhou et al. 2001). AMPK is a protein kinase, which regulates the energy balance in cells (Laakso 2006, Viollet et al. 2012) by transferring them from an anabolic to a catabolic state, and this is expressed as inhibition of glucose, protein and lipid synthesis (Viollet et al. 2012). AMPK is regulated by AMP (adenosine monophosphate) and by ATP-molecules (Laakso 2006, Viollet et al. 2009 and 2012). Metformin may activate AMPK without the influence of AMP and ATP ratio (Laakso 2006, Hardie 2006) but via mitochondria, which seems to be the primary target of metformin (Viollet et al. 2012). It has also been shown that metformin induces inhibition of mitochondrial respiratory chain complex I (Owen et al. 2000). Nowadays it is accepted that the primary antihyperglycemic effect of metformin is to decrease hepatic gluconeogenesis through mild inhibition of the mitochondrial respiratory chain complex (Viollet et al. 2012).

Metformin (1,1-dimethylbiguanide), a biguanide derivate, was originally described in 1921 (Werner and Bell 1921). It is a small-molecule weight (129 Da), water-soluble base, which at physiological pH-values exists as an organic cation. Although the molecular weight and protein binding of metformin are low, it diffuses passively through membranes rather poorly (Detaille et al. 2002). OCT1 and OCT2 are the main active transporters, which facilitate the transport of metformin across the liver, intestine and kidney tissues (Shu et al. 2007, Graham et al. 2011). OCT1 is considered to be a liver-specific and OCT2 a kidney-specific OCT (Staud et al. 2012).

Metformin is absorbed mainly from the small intestine and its oral bioavailability is 50-60%. The peak plasma concentration is reached within 1-3 hours after oral intake of immediate release tablets and within 4-5 hours after oral intake of extended release tablets (Tucker et al. 1981). The half-life of elimination is about 6.5 hours. Metformin is not metabolized, and 90% is excreted unchanged into the urine by tubular secretion. Steady state is reached within 24 to 48 hours and the plasma concentration is usually less than 1μg/ml. In controlled clinical studies the maximum concentration of metformin has not exceeded 4 μg/ml. The clinical maximum dose of metformin is 3 g daily. Metformin clearance is over 400ml/min in subjects with normal renal function (Kirpichnikov et al. 2002, Tucker et al. 1981).

Of the adverse effects of metformin, gastrointestinal side effects are common. The most serious potential side effect is lactic acidosis. The incidence of lactic acidosis is 5-9 per 100 000 person years (Misbin et al. 1998, Seidowsky et al. 2009), but the risk is much lower if metformin is not used in patients with kidney or liver failure, alcohol overdose, conditions associated with hypoxia (cardiac and pulmonary disease), septic infection or febrile gastroenteritis (Salpeter et al. 2003, Stades et al. 2004). Long-term (4 years) use of metformin may cause vitamin B_{12} and folate deficiency (de Jager et al. 2010). Results from studies on the effects of short-term (6-28 weeks) metformin use on vitamin levels are inconsistent (Wulffele et al. 2003, Carlsen et al. 2007, Sahin et al. 2007).

2.3.2. Use in patients with type 2 diabetes

Metformin is generally recommended as first-line treatment of patients with type 2 diabetes (Kahn et al. 2006, Cicero et al. 2012). This recommendation is mainly based on the results of the UK Prospective Diabetes Study (UKPDS) study of 4075 patients, which showed that obese patients treated with metformin had a lower risk of myocardial infarction than patients treated with glibenclamide or insulin (UKPDS 1998). Metformin is safe and efficacious as monotherapy and also in combination with other oral antidiabetic agents or insulins (Cicero et al. 2012). Metformin treatment has been also evaluated in a Cochrane review: the effect of metformin on glycemic control was comparable to that of other oral antihyperglycemic agents (Saenz et al. 2005). In addition to effectiveness, metformin monotherapy improves weight maintenance, plasma lipid levels and blood pressure (Saenz et al. 2005). According to a systematic review of 216 controlled trials and cohort studies (Bolen et al. 2007) and on a meta-analysis of 194 studies of over 35 000 patient-years (Salpeter et al. 2003) metformin-treated patients do not have more often lactic acidosis than patients on other oral antidiabetic medication. In addition, patients on sulfonylureas and repaglinide have a greater risk of hypoglycemia than patients on metformin (Bolen et al. 2007).

2.3.3. Use in patients with polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is a condition of ovarian dysfunction characterized by two or more of the following: irregular or absent ovulation, high levels of androgenic hormones and polycystic ovaries (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. 2004). Hyperinsulinemia, insulin resistance and impaired glucose tolerance are associated with PCOS (Ehrmann et al.1999), and obesity is common (Boomsma et al. 2006). The use of metformin to treat PCOS was first reported in 1994 (Velazquez et al. 1994), and it is currently used to correct metabolic abnormalities and menstrual cyclicity, ovulation and serum androgen levels (Costello et al. 2003, Palomba et al. 2009, Tang et al. 2010).

2.3.4. Use of metformin and cancer

Type 2 diabetes and obesity increase the risk of cancer, especially of the breast, colon, prostate, kidney and pancreas (Giovannucci at al. 2010). The risk has been linked to high plasma insulin levels and stimulation of the insulin-like growth factor 1 (IGF-1) receptor, which promote cell growth and survival (Jalving et al. 2010). The risk of cancer among patients on metformin may be reduced by almost 40% and metformin may improve cancer prognosis (Giovannucci at al. 2010, Johnson and Pollak 2010). Metformin controls cell proliferation and tumor growth (Viollet et al. 2012). The effect of metformin on cancer has been explained by improvement of blood glucose and insulin levels (Goodwin et al. 2008), by a positive impact on chronic inflammation (Grisouard et al. 2011), by direct, cellular action on tumor growth via activation of the LKB1-AMPK (liver kinase B1 adenosine monophosphate activated protein kinase) pathway (Gotlieb at al. 2008, Viollet et al. 2012), by activation of apoptotic pathways (Zhuang et al. 2011) and by decreased cellular energy (Buzzai et al. 2007).

2.3.5. Metformin and pregnancy

2.3.5.1. Effect of pregnancy on metformin pharmacokinetics

Renal plasma flow and glomerular filtration rate are increased during pregnancy by 50-60% compared to the non-pregnant state (Sturgiss et al. 1994), and these physiological changes alter the pharmacokinetics of metformin in the second and third trimesters (Hughes et al. 2006, Eyal et al. 2010). Metformin renal clearance rises by 29%, metformin secretion clearance by 38% and creatinine clearance by 21% during pregnancy when compared to the situation postpartum (Eyal et al. 2010). A marked decrease (20%) in the plasma drug concentration-time curve (AUC, area under curve) takes place during pregnancy compared to the postpartum state (Hughes et al. 2006). These findings have, however, been contested and Charles et al. (2006) concluded that pregnancy does not change the pharmacokinetics of metformin.

2.3.5.2. Placental transfer

The exact mechanism of how metformin is transferred across the placenta is not known, but OCTs may play a significant role. Metformin is a substrate for MATE1, MATE2, OCT1, OCT2 and OCT3, and of these, OCT1, OCT2, OCT3 are expressed in the human placenta (Staud et al. 2012). There is controversial information on the expression of MATEs in the human placenta (Ahmadimoghaddam and Staud 2013). OCT3 is primarily expressed in the placenta, and it is localized in the basolateral, fetus-facing membrane of the trophoblast (Sata et al. 2005). In a study by Ahmadimoghaddam and Staud (2013), the transfer of metformin across the rat placenta was coordinated by the activity of OCT3 and MATE1 (Figure 4). This matter has not been studied in the human placenta. A study by Hemauer et al. (2010) suggested that also ABC proteins contribute to the transfer of metformin across the human placenta. They stated that metformin is transported by

P-gp and BRCP, which are situated in maternal-facing membrane of syncytiotrophoblast and thus pump a drug back to the maternal circulation (Hemauer et al. 2010).

2.3.5.2.1. **Ex vivo** *human term placental perfusion studies*

In *ex vivo* studies, the placental transfer of metformin at the time of term pregnancy from the mother to the fetus is 11-17% (Nanovskaya et al. 2006, Kovo et al. 2008a). These perfusion studies were done in open and closed systems at a metformin concentration of 1µg/ml using placentas from uncomplicated and GDM term pregnancies; there was no difference between the groups of placentas regarding transfer rates (Nanovskaya et al. 2006). In a closed placental perfusion study two different concentrations of metformin were used (10µg/ml and 1 mg/ml) to characterize the permeability of the human placenta (Kovo et al. 2008b): placental transfer was 11% and 17%, respectively. Thus, the placental transfer of metformin may depend on the dose and a transporter of cationic compounds (Kovo et al. 2008b).

2.3.5.2.2. **In vivo** *studies*

In clinical *in vivo* studies the concentration of metformin has been measured in umbilical cord and maternal blood at the time of delivery. In these studies the concentration of metformin in cord blood has been at least 50% of that in maternal blood (Hague et al. 2003a, Vanky et al. 2005, Charles et al. 2006, Eyal et al. 2010).

Two of these studies, both small (n=15 and n=12 mothers, respectively) (Vanky et al. 2005, Eyal et al. 2010), provided data on maternal and umbilical blood samples taken simultaneously after delivery. Metformin concentrations were almost the same in the umbilical artery and vein (Vanky et al. 2005, Eyal et al. 2010). Eighty five per cent of concentrations were higher in umbilical cord serum than in maternal serum except for the first hours after metformin intake (Vanky et al. 2005).

Two groups have reported 33-45 % lower umbilical cord metformin concentrations compared to maternal serum, but in these studies the blood samples were not collected simultaneously (Hague et al. 2003a, Charles et al. 2006).

2.3.5.3. Metformin in pregnant patients with type 2 diabetes

The earliest reports on pregnant women with type 2 diabetes using metformin and GDM patients originate from South Africa in the late 1970s (Coetzee and Jackson 1979 and 1984, Ekpebegh et al. 2007). These studies consisted of a heterogeneous population, but they illustrated the fact that metformin could be used in pregnancy.

In a retrospective study evaluating oral hypoglycemic agents to treat pregnant diabetic patients, patients treated with metformin had a higher incidence of pre-eclampsia and the perinatal mortality rate was higher than among those treated with sulfonylurea (Hellmuth et al. 2000). However, the patients on metformin were older, more obese and had been diagnosed and treated later in pregnancy than the patients on sulfonylurea

(Hellmuth et al. 2000). In a retrospective study pregnant patients with type 2 diabetes had been treated either with metformin or not with metformin in addition to other treatments. There were no differences between the groups in terms of maternal or fetal outcomes (Hughes and Rowan 2006). Women in the metformin group were more obese, and they had more chronic hypertension and higher levels of HbA1c. In an observational study of pregnant patients with type 2 diabetes on metformin or insulin, Rai et al. (2009) showed that glycemic control was better in metformin group throughout gestation. Hickman et al. (2013) conducted a randomized study comparing tolerability and effect on glycemic control of metformin and insulin during pregnancy in women with pre-existing type 2 diabetes and early gestational diabetes. In the metformin group women had fewer subjective episodes of hypoglycemia than in the insulin group. In the metformin group 43% of the patients needed additional insulin (Hickman et al. 2013).

2.3.5.4. Metformin in GDM patients

Metformin has been compared to insulin in randomized studies (Moore et al. 2007, Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Mesdaghinia et al. 2013, Spaulonci et al. 2013) and observational studies (Balani et al. 2009, Rai et al. 2009, Goh et al. 2011) in GDM patients. The characteristics of the studies and maternal and neonatal outcomes are presented in Tables IV and V.

In addition, in the study of Gandhi et al. (2011) metformin treatment was compared to dietary therapy in 592 GDM patients. Maternal baseline characters were similar in the two groups. Of the metformin-treated patients 21% and of the dietary treated patients 37% needed insulin. The incidence of fetal macrosomia and birth weight >90 percentile was lower in the metformin group than in the diet group (Gandhi et al. 2011).

Two RCTs comparing metformin and glibenclamide in GDM patients have been published (Moore et al. 2010, Silva et al. 2012). Thirty-five percent of the mothers in the metformin group (n=75) and 16% in the glibenclamide group (n=75) needed insulin therapy, and the failure rate among the patients on metformin was 2.1 times higher than among the patients on glibenclamide (p=0.01) (Moore et al. 2010). In the study of Silva et al. (2012) there was no difference in the degree of maternal glycemic control between metformin (n=104) and glibenclamide (n=96) groups; 21% of the patients on metformin and 29% on glibenclamide needed to change to insulin to maintain adequate glucose control. Birth weights were lower in the metformin group than in the glibenclamide group (p=0.02 and p=0.01, respectively)(Moore et al. 2010, Silva et al. 2012), and the patients on metformin gained less weight than the patients on glibenclamide (p=0.04) (Silva et al. 2012).

Table IV. Characteristics of the randomized and observational studies comparing metformin and insulin in GDM patients.

	Number of patients metf / ins	Dose of metformin (mg)	Mean dose of insulin (iu)	Additional insulin needed	Gestational weeks when medication started	OGTT cut-off values for GDM dg, (≥1 or ≥2 abnormal values needed depending on the study)					Cut-off glucose capillary values for starting medication fP-gluc, pp-gluc (mmol/l) (variable number of abnormal values)			Target glucose capillary values fP-gluc, pp-gluc (mmol/l)		
						loading	0-h	1-h	2-h	3-h	0-h	1.5-h	2-h	0-h	1.5-h	2-h
Moore et al. 2007* USA	32/31	1000-2000	Not reported	0%	24-30	100g	5.8	10.6	9.2	8.1	5.8		6.7	<5.0		<6.7
Rowan et al. 2008*(MiG study) New Zealand and Australia	363/370	500-2500	50	46.3%	20-33	75g	5.5		8.0		5.4		6.7	<5.5		<7.0
Ijäs et al. 2010* Finland	47/50	750-2250	30	31.9%	12-34	75g	5.3#	11.0#	9.6#		5.3	6.7		<5.3	<6.7	
Niromanesh et al. 2012* Iran	80/80	1000-2500	Not reported	13.8%	20-34	100g	5.3	10.0	8.6	7.8	5.3		6.7	≤5.3		≤6.7
Mesdaghinia et al. 2013* Iran	100/100	500-2500	Not reported	22.0%	24-34	100g	5.3	10.0	8.6		5.3		6.7	<5.3		<6.7
Spaulonci et al. 2013* Brazil	47/47	1700-2550	Not reported	26.1%	30 (mean)	100g or 75 g	5.3	10.0	8.6	7.8	5.3		6.7	≤5.3		≤6.7
Balani et al. 2009 UK	127/100	1000-2000	Not reported	13.0%	30-34 (metf) 28-33 (ins) (p<0.01)	75g			≥7.8		6.0	8.0 (1-h)	7.0	<6.0	<8.0 (1-h)	<7.0
Rai et al. 2009 † India	30/30	1500-2000	Not reported	6.7%	Not reported	100g	5.3	10	8.6	7.8	5.6		7.2	<5.6		<7.2
Goh et al. 2011†† New Zealand	465/399	-3000	Not reported	46.5%	Not reported	75g	5.5		9.0		5.0		6.0-6.5	<5.0		<6.5

*RCTs

Capillary plasma concentration; equals venous plasma concentrations 5.3-10-8.6 mmol/l (Current care guidelines, Duodecim, Finland)

† Study includes two patients with type 2 diabetes in the metformin group and six patients in the insulin group.

††Study consists of three groups of GDM patients: those treated with diet, metformin or insulin.

Table V. Maternal and neonatal outcomes in randomized and observational studies comparing metformin and insulin in GDM patients.

	Mean maternal weight gain (kg) metf/ins	Induction of labor % metf/ins	Cs % metf/ ins	Gestational weeks at delivery metf/ins	Preterm birth % metf/ins	Birth weight (g) metf/ins	LGA% metf/ins	SGA% metf/ins	Neonatal hypoglycemia % metf/ins	NICU admission % metf/ins
Moore et al. 2007*	Not reported	No differences	21.9/32.3	38.1/37.9	Not reported	3452/3500	Not reported	Not reported	0/6.5	6.3/12.9
Rowan et al. 2008*	0.4/2.0‡ (p<0.001)	54.0/56.2	36.1/38.4	38.3/38.5	12.1/7.6 (p=0.04)	3372/3413	19.3/18.6**	7.2/9.7	3.3/8.1*** (p<0.01)	18.7/21.1
Ijäs et al. 2010*	8.6/9.2	51.0/52.0	38.3/20.0 (p=0.047)	38.9/39.3	2.1/6.0	3712/3558	8.5/10.0#	Not reported	8.5/14.0# #	14.9/22.0
Niromanesh et al. 2012*	11.3/13.7† 3.3/4.5†† (p<0.001)	Not reported	42.5/46.3	37.9/38.0	11.3/5.0	3300/3400 (p<0.01)	17.5/35.0** (p=0.012)	3.8/2.5	3.8/2.5	6.3/2.5
Mesdaghinia et al. 2013*	Not reported	Not reported	Not reported	Not reported	0/8 (p<0.01)	3512/3528	16/24	0/0	10/15	14/33 (p<0.01)
Spaulonci et al. 2013*	Not reported	Not reported	71.7/65.2	38.3/38.2	10.9/10.9	3144/3238	4.3/6.5	13.0/8.7	6.5/22.2$ (p=0.03)	Not reported
Balani et al. 2009	0.9/2.7° (p<0.01)	26.0/24.0	48.0/52.0	Not reported	0/10 (p<0.01)	3372/3511	14/25	10/16	9/18°°	6/19 (p<0.01)
Rai et al. 2009	5.5/7.4 (p=0.02)	Not reported	No differences	38.1/37.6	6.5/3.3	2980/3100	None	6.5/23.3	0/6.7$	9.6/36.7 (p=0.02)§
Goh et al. 2011&	Not reported	Not reported	37.0/ 45.6	Not reported	12.5/19.2	3221/3176	12.5/18.5	8.7/11.8	11.7/14.3	12.7/18.7

*RCTs
‡Weight gain from enrollment to 36 or 37 wk of gestation
**Birth weight >90th percentile
***Blood glucose level <1.6 mmol/l
Birth weight > +2SD
Needed intravenous glucose
& Study analyses performed between three groups, GDM patients treated with diet, metformin or insulin.
† Total weight gain
†† Weight gain after randomization
$ Capillary glucose level < 2.2 mmol/l
°Weight gain from enrollment to term
°°Glucose <2.6 mmol/l within 2 h after birth
NICU = Neonatal intensive care unit
§ NICU stay >24 hours

In addition to the data given in Table IV, there is further heterogeneity in the studies. For example, in the study of Moore et al. (2007) pre-pregnancy BMI was higher in the insulin group than in the metformin group (p=0.045). Spaulonci et al. (2013) did not report the values of OGTT and Balani et al. (2009) did not report the parity. Otherwise there were no differences in the baseline maternal data (values of OGTT, maternal age, parity, pre-pregnancy BMI) (Moore et al. 2007, Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Spaulonci et al. 2013, Balani et al. 2009, Rai et al. 2009).

It is worth noting about the studies in Table V that Balani et al. (2009) did not report the incidence of birth injuries and Ijäs et al. (2010) did not report the incidence of RDS, otherwise there were no differences in other neonatal complications (birth injuries, RDS, hyperbilirubinemia) between the groups. Balani et al. (2009) reported more hyperbilirubinemia in the insulin group (p<0.01), Mesdaghinia et al. (2013) reported more hyperbilirubinemia (p=0.02) and RDS (p=0.04) in insulin group and Moore et al. (2007) reported one stillbirth in the metformin group. There were no reported differences in the incidence of maternal hypertensive complications between the groups (Moore et al. 2007, Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Spaulonci et al. 2013, Rai et al. 2009).

2.3.6. Fetal, neonatal and childhood safety

Because pregnancy is a condition where two individuals are exposed to a drug prescribed to the mother, fetal and neonatal risks must be carefully considered (Thomas and Yates 2012). The adverse drug effects on the fetus can vary over time as gestation proceeds following changes in fetal drug exposure or sensitivity to drug (Mihaly and Morgan1984). Glucose uptake and glucose transport by the human term placenta were not affected by metformin in a placental perfusion study (Elliott et al. 1997). It is essential to collect data on metformin use in pregnancy, because metformin crosses the placenta (Eyal et al. 2010) and could, in theory, cause potential harm to the fetus, neonate or later in childhood.

There are data to substantiate concerns of organogenesis, lactic acidosis and absorption of vitamin B_{12} and folate related to the use of metformin in pregnancy (Table VI). There are also data on metformin use during lactation (Table VI).

Table VI. Data on metformin use in pregnancy: effects on fetal and neonatal safety.

Variable	Findings	Study
Congenital malformations	No evidence of increased risk based on studies of PCOS-patients treated with metformin throughout pregnancy. Maybe positive effect on early pregnancy loss.	Glueck at al. 2001 and 2004a Jakubowicz et al. 2002 Gilbert et al. 2006 Ghazeeri et al. 2012
Lactic acidosis	Case report: Nondiabetic pregnant patient ingested single dose 80 tbl containing 500mg of metformin in gw 28 as a suicide attempt. Acidosis was corrected. Healthy newborn was delivered at 38 gw.	Hong et al. 2008
Absorption of vitamin B_{12} and folate	No change in B_{12} level in pregnant PCOS-patients treated with metformin for 26-28 weeks. No differences were detected in vitamin B_{12}, holotranscobalamin*, and homocysteine** concentrations measured in GDM patients on metformin or insulin at gw 20-34.	Carlsen et al. 2007 Gatford et al. 2013
Use during lactation	Metformin use during lactation has shown to be safe. The amount of metformin excreted in breast milk is less than 0.5% of weight-adjusted maternal dose. Metformin treatment during lactation has shown no adverse effects on breast milk fed infants' growth, motor or social development.	Gardiner et al. 2003 Glueck et al. 2006 Eyal et al. 2010

*marker of functional vitamin B_{12}
**Low level of vitamin B_{12} may raise the concentration of homocysteine in the blood (Hague 2003b). Impaired homocysteine metabolism during pregnancy may associate with fetal neural tube defects, pre-eclampsia and recurrent pregnancy loss (Hague 2003b).

Growth, motor and social development and the metabolic and hormonal profile of children exposed to metformin *in utero* has been analyzed in several studies (Table VII).

Table VII. Data on metformin use in pregnancy: effects on children.

Effect on child	Finding	Study
Growth, motor and social development	No adverse effect on growth or motor-social development by age 18 months in children exposed to metformin *in utero* from the first trimester of pregnancy in PCOS-mothers	Glueck et al. 2004b
Weight gain	Children of PCOS-mothers exposed to metformin *in utero* compared to placebo weighed more at the age of 1 year (p=0.003).	Carlsen et al. 2012
Metabolic profile	No differences in glucose, lipids and C-reactive protein measured from the cord plasma in GDM patients on metformin or insulin (MiG study).	Barrett et al. 2013
	Children exposed to metformin *in utero* had larger upper arm circumference, subscapular thickness and biceps skin fold at age 2 years than children exposed to insulin (MiG study). No differences in total fat mass and percentage body fat.	Rowan et al. 2011
Growth and metabolic profile	No differences in height, weight or fat composition of children aged 7-9 years born to PCOS-mothers on metformin from the first trimester of pregnancy compared to mothers on placebo. Higher fasting glucose level recorded in children exposed to metformin *in utero*.	Rø et al. 2012
Hormonal effects	Metformin exposition during pregnancy increases fetal sex hormone binding globulin (SHBG) levels in umbilical vein (p=0.005) compared to controls. Androgen and estrogen levels are unaffected.	Carlsen and Vanky 2010

After metformin exposure of animal ovarian cells in culture steroid production sank (Tosca et al. 2007). Tartarin et al. (2012) studied the effects of metformin on human and murine testicular cells *in vitro.* Metformin decreased testosterone secretion compared to controls in organotypic cultures of human testicular tissue, obtained from first trimester legally induced abortion, and of murine testicular tissue. In an *in vivo* study on mice, administration of metformin to pregnant mice reduced the size of the fetal and neonatal testes compared to controls. The number of germ cells was not affected but the number of Sertoli cells was reduced (Tartarin et al. 2012). There are no reports on the effects of antenatal metformin exposure on the development of testes in man.

3. AIMS OF THE STUDY

The present study was designed to investigate the placental transfer of metformin in late and term pregnancy and the effectiveness and safety of metformin in the treatment of GDM patients.

The specific aims of the study were:

1. To investigate the degree of placental transfer of metformin and the role of OCT transporters in the transfer using *ex vivo* perfused term human placentas (**Study II**).

2. To determine *in vivo* the placental transfer of metformin at birth in relation to fetal outcome, and to investigate the impact of the trough concentration of metformin in maternal serum at 36 gw on maternal glycemic control, maternal weight gain and fetal outcome (**Study IV**).

3. To compare retrospectively and prospectively metformin and insulin with regard to antihyperglycemic effectiveness and maternal outcome in GDM patients (**Studies I and III**), and to examine the predictors of additional insulin in patients treated with metformin (**Study III**).

4. To compare retrospectively and prospectively fetal and neonatal outcome and the safety of metformin and insulin in pregnant GDM patients and their newborns (**Studies I and III**).

4. SUBJECTS, MATERIALS AND METHODS

The clinical part of this study was carried out in the Department of Obstetrics and Gynecology, Turku University Hospital in 2005-2012. **Study I** was an observational, retrospective, case-control study on GDM patients treated at the unit between 2003-2006. **Study III** was a randomized controlled trial (RCT) conducted in 2006-2010. The study included investigation of *in vivo* placental transfer of metformin (**Study IV**). **Study II** was an *ex vivo* human placental perfusion study with metformin and it was performed in the laboratory of the Joint Clinical Biochemistry Laboratory of the University of Turku and the Turku University Hospital in 2005-2006.

The studies were approved by the Ethics Committee of the Intermunicipal Hospital District of Southwest Finland. The RCT was also approved by the Finnish National Agency of Medicines, and European Union Drug Regulatory Agency (EUDRA). The RCT was registered in Clinicaltrials.gov, NCT01240785;http://clinicaltrials. gov/ct2/show/NCT01240785. All participants in the studies gave written informed consent. The metformin concentrations were determined by the Department of Pharmacology, Drug Therapeutics, University of Turku, and the other laboratory assessments were made by the Department of Clinical Chemistry, University of Turku and TYKSLAB, Intermunicipal Hospital District of Southwest Finland.

4.1. Clinical studies and *in vivo* placental transfer of metformin (Studies I, III and IV)

4.1.1. Patients and study design

Table VIII. Patients and study design, **Study I and III.**

Study	Number of patients with singleton pregnancies metf/ins/diet	Criteria for GDM dg: at least two abnormal plasma glucose values in 75g OGTT (mmol/l)			Gw at OGTT / rand	Criteria for starting medication: at least two abnormal capillary glucose values, (mmol/l)			Target capillary glucose values, (mmol/l)		
		0-h	1-h	2-h		fP	1-h pp	1.5 h pp	fP	1-h pp	1.5 -h pp
I	45/45/83	≥4.8	≥10.0	≥8.7	11-32	≥5.5		≥7.8	<5.5		<7.8
III	110/107/0	≥4.8 ≥5.3*	≥10.0 ≥10.0	≥8.7 ≥8.6	22-34	≥5.5	≥7.8		<5.5	<7.8	

*Guidelines for diagnostic OGTT cut-off values changed in 2008 from 4.8–10.0-8.7mmol/l to 5.3-10.0 -8.6 mmol/l.

Patients and study design of **Study I** and **III** are shown in Table VIII. The allocation for **Study III** is shown in Figure 5. In **Study I** patients were excluded if fP-glucose was

>7.0mmol/l and/or pp–glucose was > 10.0mmol/l. The exclusion criteria in **Study III** were cardiac or renal failure, liver disease, metformin use within 3 months preceding pregnancy or during pregnancy before the OGTT, fP-glucose **≥**7.0 mmol/l or 60 min pp-glucose **>**11.0 mmol/l. In **Study III** the non-inferiority study design was applied to analyze the primary end point, birth weight (g and SD-units). Primary end points in **Study I** were birth weight (g) and incidence of neonatal hypoglycemia.

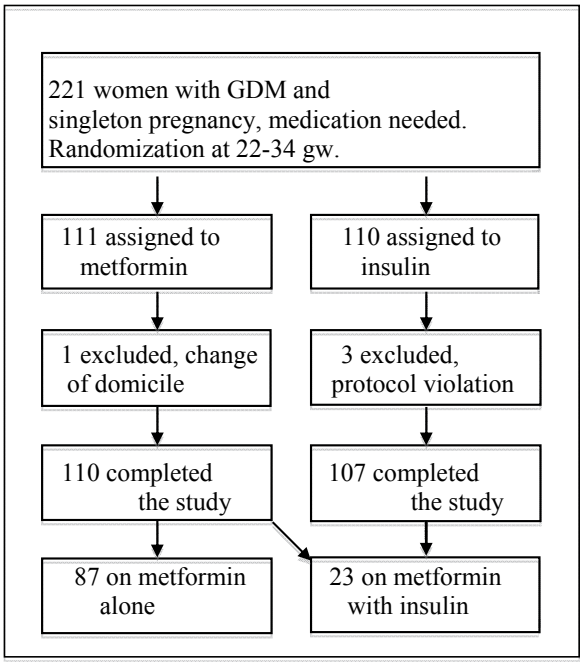


Figure 5. Allocation chart of **Study III**.

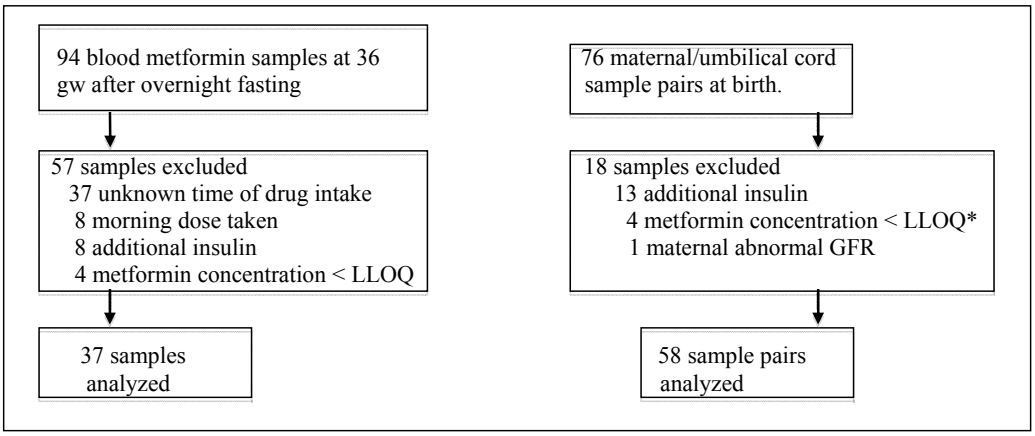
Before starting the medication in **Study III**, renal function was assessed by measuring the serum creatinine concentration; if outside the reference range, the patient was excluded. Metformin was stopped if significant side effects occurred. A product containing vitamin B complex (0.4 mg of folic acid) was initiated and continued throughout pregnancy along with metformin in **Study III**. In **Studies I** and **III** patients monitored their blood glucose by repeated finger-stick measurements: they measured their blood glucose at least four times daily, i.e. before and after breakfast and after the main meals at least 3 days a week. Additional insulin was initiated in both studies if target values were not met with metformin alone. In **Studies I** and **III** patients who were treated with metformin or insulin had several appointments with an obstetrician and a diabetologist, if necessary. All patients had a third-trimester ultrasound evaluation of fetal weight. At every visit to the outpatient maternity clinic an ultrasound examination was performed, and fetal growth and wellbeing were assessed.

To identify mothers needing additional insulin with metformin in **Study III**, the metformin-treated patients were, for purposes of statistical analysis, divided to

subgroups of metformin only (n=87) and metformin with additional insulin (n=23) (Fig. 5).

Blood samples were drawn from metformin-treated patients in the RCT to measure metformin concentrations (**Study IV**) (Figure 6). Maternal blood samples were drawn at gw 36 and immediately after delivery simultaneously from the maternal antecubital vein and the umbilical cord. All blood samples were centrifuged and the serum was stored at -70° C until metformin analysis. The glomerular filtration rate (GFR) of the patients who had submitted blood samples for metformin assessment was calculated by Cockroft-Gault's formula (Cockroft and Gault 1976).

To examine the association between metformin exposure and various outcomes, patients were divided into tertiles by metformin concentration at gw 36 and by the concentration ratio (umbilical cord / maternal serum mean metformin concentration) at birth.



*LLOQ = lower limit of reliable quantification

Figure 6. Allocation chart of **Study IV**.

4.1.2. Maternal data analyses

In **Studies I** and **III** maternal baseline characteristics of age, parity, smoking, BMI at first antenatal visit, values of OGTT, gw the test performed and HbA1c at time of OGTT (**Study I**) or at the time of randomization **(Study III**) were recorded. In **Study III** also fructosamine and c-peptide were measured at randomization and recorded.

In **Studies I** and **III** weight gain, hypertension and pre-eclampsia, gw at delivery, induction and the mode of delivery were recorded. In **Study III** HbA1c and fructosamine were measured at 36 gw and recorded. HbA1c was measured by high pressure liquid chromatography (HPLC) (Variant II, Bio-Rad Laboratories Ltd., Hemel Hempstead, UK). Fructosamine was determined with a colorimetric method based on the ability of

ketoamines to reduce nitrotetrazolium blue to formazan in alkaline solution. The levels of fructosamine were adjusted for the levels of total protein. Both assays were performed with the Modular P800 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Fasting plasma C-peptide was measured with an electrochemiluminescence immunoassay (ECLIA) on a Modular E170 automatic analyzer (Roche Diagnostics GmbH).

In **Study IV** the clinical efficacy of metformin was evaluated by assessment of maternal glycemic control (HbA1c and fructosamine values at 36 gw) and by the association between maternal weight gain and maternal serum metformin trough concentrations at 36 gw.

4.1.3. Fetal data analyses

Birth weight (in grams and SD-units), macrosomia (birth weight > 4500g and/or > 2 SD-units), LGA (birth weight >90th percentile), SGA (birth weight < 2SD-units), prematurity (delivery < 37 gw), shoulder dystocia, Apgar score at the age of 5 minutes and umbilical artery pH-value, neonatal hypoglycemia (P-gluc < 2.6 mmol/l and a need for intravenous-glucose), hyperbilirubinemia (need for phototherapy), respiratory distress syndrome (RDS) and need for intensive care treatment were recorded in **Studies I** and **III.**

In **Study IV** the ratio of the concentration of metformin in the umbilical cord serum and the maternal serum at birth was calculated to evaluate *in vivo* placental transfer of metformin. Association between maternal metformin concentration at 36 gw and birth weight and gw at birth was calculated. Additionally association between the concentration ratio at birth and same variables was calculated. Umbilical artery pH-value was studied in relation to the concentration ratio and cord serum metformin concentration separately. The incidence of neonatal hypoglycemia was studied in relation to the concentration of metformin in cord serum. The group of mothers treated with insulin in from Study III served as a reference group for these calculations. Metformin concentrations and the concentration ratio were analyzed by tertiles and the patients were divided in quartiles to evaluate the relation of the time from last drug intake to metformin concentration at birth.

4.1.4. Drug concentration analyses (Study IV)

Metformin concentrations were measured with a validated analytical method consisting of reversed phase liquid chromatography combined with mass spectrometric detection using an internal standard. The lower limit of quantification (LLOQ) was 100 ng/ml. The inter-assay accuracy bias% from quality control samples ranged from -4.2% to 7.5% and the inter-assay precision from 6.9% to 11.2%. The method is described in detail in publication IV.

4.1.5. Statistical methods

Table IX. Power calculations and statistical analyses.

Power calculations	
A two-sided test with 80% power and a significance level of 0.05 detected a 258 g mean difference in birth weights between the groups (45 patients on metformin and 45 on insulin). A two-sided test with 80% power and significance level of 0.05 identified a 25.9% difference in hypoglycemia incidence between the metformin and the insulin-treated group.	I
Non-inferiority was accepted if the 90% CI* for the birth weight was within −150 to 150 grams and within −0.45 to 0.45 SD-units. With a standard deviation of 1.5 SD and 80% power, 139 subjects were required in both groups.	III

Statistical analyses	**Study**
Linear mixed model	I
Conditional logistic regression analysis	I
Mann Whitney's U-test	III
Two-sample t-test	III
Poisson's regression analysis	III
Analysis of covariance	III, IV
Paired t-test	IV
Analysis of variance	IV
c^2 test	IV
Kruskall-Wallis's test	IV

*Confidence interval
**Standard deviation

In all studies *P< 0.05* was considered statistically significant. Statistical analyses were performed in **Studies I** and **III** by using the SAS System for Windows, version 9.1 and 9.2 (SAS Institute, Cary, NC, USA), and in **Study IV** by using GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA) and IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

From September 2009 onwards enrollment to **Study III** slowed down, and an interim analysis was performed. At that time the mean difference (90% CI) between the metformin (n=77) and insulin (n=84) groups was 0.11 SD-units (−0.16 to 0.38). CI was within the defined limits (−0.45 to 0.45), showing equivalence between the groups.

4.2. Perfusion of metformin in human term placenta *ex vivo* (Study II)

4.2.1. Placentas and perfusion system

An open (non-recirculating) human placental perfusion method (Schneider et al. 1972) was used (Figures 5 and 6). Thirty-four term placentas (at 38–42 weeks of pregnancy) were obtained after vaginal delivery (n=11) or caesarean section (n = 23). Pregnancies were uncomplicated and mothers were healthy

After delivery, the placenta was collected, and heparinized 0.9% NaCl solution was injected into the umbilical artery. An intact cotyledon was chosen and the corresponding distal branches of the chorionic artery and vein were cannulated. The cotyledon was excised from the placenta and transported to the perfusion laboratory, where it was placed on a metal plate with the maternal surface down and attached to the perfusion apparatus. All perfusions were initiated within 20 min of delivery of the placenta.

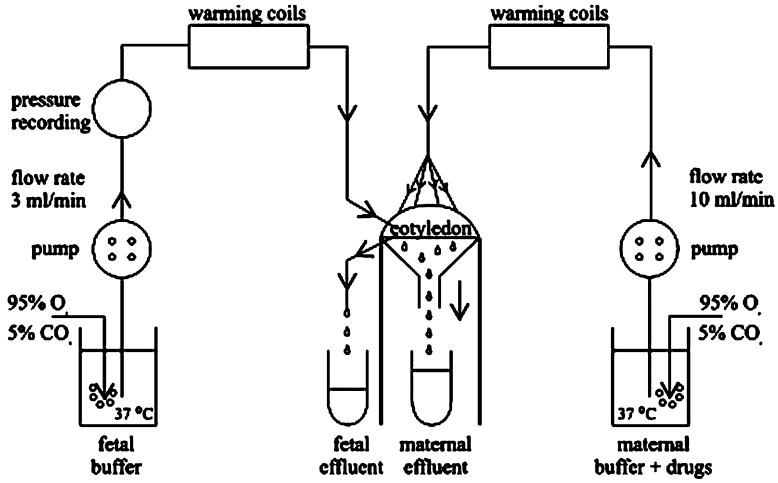


Figure 7. Schematic presentation of the perfusion method (Heikkinen et al. 2001), reproduced with permission.

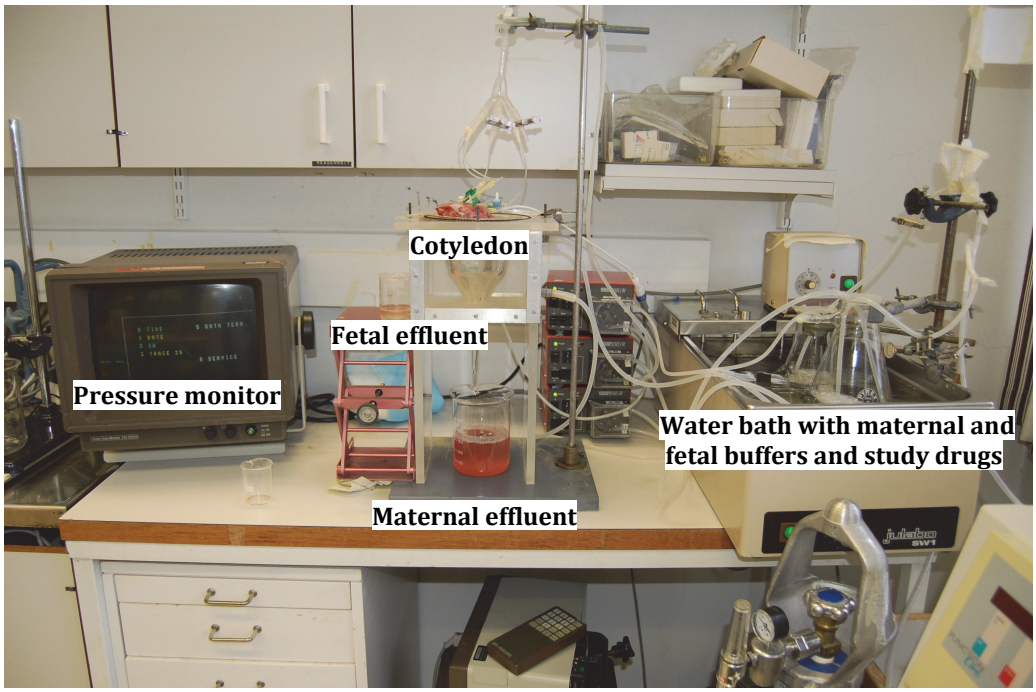


Figure 8. Picture of placental perfusion system.

4.2.1.1. Maternal-to-fetal perfusion

For the maternal perfusion, the infusion tube from the maternal perfusion solution was attached to the decidual surface of the placenta with butterfly needles (maternal arteries). For the fetal perfusion, the infusion tube from the fetal perfusion solution was attached to the cannulated fetal artery. Fetal effluent flowed through the cannulated fetal vein of the cotyledon. Krebs-Ringer bicarbonate buffer (Sigma–Aldrich, St Louis, MO, USA) containing albumin from bovine serum 8A-3912 (Sigma–Aldrich) 30 g/l was used as the perfusion solution on the maternal and fetal side. The perfusate was maintained at 37°C and equilibrated with 95% oxygen and 5% carbon dioxide mixture throughout the perfusion (Schneider and Proegler 1988). The perfusion was carried out at a flow rate of 10 ml/min on the maternal side and 3 ml/min on the fetal side.

There were two groups of perfused drugs. In addition to metformin (n = 13), metformin–cimetidine (n = 9) was used. Here, an OCT inhibitor, cimetidine (H_2-receptor antagonist) was used to study the role of OCT: s for the placental transfer of metformin. Cimetidine was used because it inhibits OCT1, OCT2 and OCT3 (Umehara et al. 2007, Kekuda et al. 1998). Cimetidine inhibits also the active renal tubular secretion of metformin by having a higher affinity for the OCT transporting system than metformin (Somogyi et al. 1987).

The drugs were infused into the maternal perfusion solution. To stabilize the perfusion system, all placentas (n = 22) were preperfused for 30 min with Krebs-Ringer bicarbonate solution containing albumin. The placentas in the metformin-cimetidine group were preperfused for an additional 30 min with cimetidine 100μg/ml (Hexal AG, Holzkirchen, Germany) to saturate the cotyledon with cimetidine. Then, all placentas were perfused for 2 h with metformin 2μg/ml (Sigma–Aldrich Chemie, Steinheim, Germany) and antipyrine 80μg/ml (Schneider et al.1985, Schneider and Proegler 1988) (Sigma–Aldrich). Antipyrine (phenazone, antipyretic analgetic drug) is commonly used as a reference drug for passive diffusion-dependent placental transfer because it diffuses rapidly, the rate of metabolism is negligible and it is minimally protein bound (Challier 1985). The concentration of metformin corresponded to the steady state serum levels of patients taking metformin. To ensure the function of cimetidine as an OCT inhibitor a concentration of cimetidine 100μg/mL was used, which exceeds the therapeutic concentration.

The total perfusion time was 2 h 30 min in the metformin group and 3 h in the metformin–cimetidine group, depending on the preperfusion time. Samples for the measurement of metformin and antipyrine concentrations were collected from the fetal venous effluent just before the perfusion, every 10 min for 60 min, and thereafter every 20 min for up to 120 min, and from the maternal venous effluent at 20, 60 and 120 min. Samples were obtained from the maternal arterial infusion line at 20, 60 and 120 min to control the initial concentrations of the perfused drugs. After the perfusion, the samples were centrifuged for 5 min at 2500 rpm to remove cellular debris and stored at −70°C until analysis.

4.2.1.2. Fetal-to-maternal perfusion (reversed perfusion)

Twelve placentas were obtained to study the transfer of metformin in the fetal-to-maternal direction. Six of these were also perfused with cimetidine. Fetal-to-maternal perfusions were performed as described above, but in a reverse manner, i.e. the drugs were infused into the fetal perfusion solution. Samples were collected from the maternal venous effluent at the same time points as described above. In addition, samples were collected from the fetal arterial infusion line at 20, 60 and 120 min after the start of perfusion. All placentas were perfused with metformin (2μg/ml) and antipyrine (80μg/ml) and six were also perfused with cimetidine (100μg/ml).

4.2.2. Viability of placentas

The viability of the placental tissue was tested by performing a blood gas analysis (pH, pO2, and pCO2) of maternal and fetal arterial and fetal venous perfusion solutions in the beginning and at the end of each perfusion. In reversed perfusions, a blood gas analysis of maternal and fetal arterial and maternal venous perfusion solutions was performed. The flow rates on the maternal and fetal side were measured to ensure stable flow, and the fetal inflow pressure was monitored throughout the perfusion.

4.2.3. Drug concentration analyses

Metformin and antipyrine concentrations were measured by a high performance liquid chromatography (HPLC) ultraviolet system. The method is described in detail in original publication II. The lower detection limit was 5.0 ng/ml for metformin and 100 ng/ml for antipyrine. The interassay variation (per cent coefficient of variation) was less than 10% at all metformin and antipyrine concentrations.

4.2.4. Data analysis

The time to reach the steady state concentration (t_{ss}) was estimated graphically for metformin and antipyrine for each perfusion. The concentration at steady state (C_{ss}) was calculated as the mean of the measured concentrations beyond t_{ss}. Transplacental transfer percentages (TPT%) in both directions were calculated using the equation published by Schneider et al. (1985) as described in original article (II).

The end-perfusion concentration of metformin at 120 min (TPT% at 120 min) was derived directly from the drug concentration data. The transplacental transfer index (TI), i.e. the ratio of transplacental transfer between metformin and antipyrine, was calculated by dividing the TPT% of metformin by the TPT% of antipyrine. All pharmacokinetic results are expressed as mean values and standard deviations (SD).

4.2.5. Statistical analysis

The paired *t*-test was used to examine the viability data (pH and perfusion pressure). An unpaired *t*-test was used for pharmacokinetic data, which were log-transformed because of skewness of the distribution before statistical analyses. The two-tailed level of statistical significance was set at $P < 0.05$. Statistical analyses were performed by using the GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

5. RESULTS

5.1. Clinical data (Studies I and III)

5.1.1. Study subjects

Table X. Maternal baseline characteristics in **Study I**.

Maternal baseline characteristics	Metformin (n=45)	Insulin (n=45)	p-value and OR (95% CI)
Age (years)	32.8 ± 5.0	32.7 ± 4.7	0.82
Primipara	10 (22.0)	19 (42.0)	0.05; 0.4 (0.2-1.0)
Smoking	7 (15.6)	7 (15.6)	1.0; 1.0 (0.3-3.0)
BMI (kg/m^2)	34.0 ± 6.4	33.2 ± 6.2	0.08
OGTT 0h (mmol/l)	5.9 ± 0.7	6.3 ± 0.8	0.005
OGTT 1h (mmol/l)	11.7 ± 1.7	12.7 ± 2.0	0.006
OGTT 2h (mmol/l)	8.3 ± 1.8	9.5 ± 2.2	0.003
Gw at OGTT	24.8 ± 5.5	24.3 ± 5.7	0.64
HbA1c%#	5.7 ± 0.4	5.7 ± 0.4	0.48
Dose of metformin (mg) and type of insulin	500-2000 (mean 1000)	short-acting only 29% (n=13), intermediate-acting only 20% (n=9), short- and intermediate-acting 51% (n=23)	

Data are expressed as mean ± SD or number and (%).
measured at the time of OGTT

Table XI. Maternal baseline characteristics in **Study III**.

Maternal baseline characteristics	Metformin (n=110)	Insulin (n=107)	p-value and RR (95% CI)
Age (years)	31.9 ± 5.0	32.1 ± 5.4	0.80
Primipara	42 (38.2)	48 (44.9)	0.45; 0.9(0.6-1.3)
Smoking	9 (8.5)	17 (16.0)	0.12; 0.5(0.2-1.2)
BMI (kg/m^2)	29.4 ± 5.9	28.9 ± 4.7	0.74
OGTT 0h (mmol/l)	5.5 ± 0.5	5.6 ± 0.4	0.41
OGTT 1h (mmol/l)	11.2 ± 1.5	11.2 ± 1.2	0.91
OGTT 2h (mmol/l)	8.3 ± 1.8	7.9 ± 1.7	0.11
Gw at OGTT	26.8 ± 2.5	26.9 ± 2.5	0.71
Gw at rand.	30.0 ± 2.0	30.4 ± 1.8	0.72
HbA1c%*	5.5 ± 0.3	5.5 ± 0.3	0.53
Fructosamine (µmol/l)*	209.5 ± 16.0	209.3 ± 15.4	0.92
C-peptide (nmol/l)*	1.07±0.33	1.04±0.27	0.98
Dose of metformin (mg) or/and insulin (iu)	500-2000 (mean 1500)	2-42 (mean 10)	
Type of insulin		short-acting only 60% (n=64), intermediate-acting only 14% (n=15), short- and intermediate-acting 26% (n=28)	

Data are expressed as mean ± SD or number and (%).
* measured at the time of randomization

In **Study III** one patient discontinued metformin after 5-weeks and continued tight diet treatment to maintain normoglycemia, which was successful. Two of the patients needing additional insulin discontinued metformin because of gastrointestinal side effects after two- and six-weeks of use and continued on insulin only. In the insulin group one of the 107 patients who completed the study discontinued insulin after one week and continued on diet only for rest of her pregnancy. For the analyses, all subjects were referred to their original treatment groups.

The baseline maternal characteristics are seen in Table X and in Table XI. In **Study I**, values in OGTT were significantly higher in the insulin group than in the metformin group. In **Study III** there were no differences in baseline maternal characteristics between the metformin and insulin groups.

5.1.1.1. Need for additional insulin among patients on metformin

In **Study I**, 18% of the patients in the metformin group (8/45) needed additional insulin to maintain adequate glucose control. In **Study III**, the figure was 21% (23/110).

Table XII. Maternal baseline characteristics in subgroups of **Study III** (patients on metformin).

Maternal baseline characteristics	Metformin only (n=87)	Metformin and insulin (n=23)	p-value and RR (95% CI)
Age (years)	31.4 ± 5.0	33.8 ± 4.9	0.04
Primipara	34 (39.1)	8 (34.8)	0.77; 1.1(0.5-2.4)
Smoking	5 (5.7)	2 (8.7)	0.64; 1.4(0.4-4.8)
BMI (kg/m^2)	29.8 ± 6.0	28.2 ± 5.8	0.37
OGTT 0h (mmol/l)	5.5 ± 0.5	5.7 ± 0.7	0.25
OGTT 1h (mmol/l)	11.2 ± 1.4	11.2 ±	0.51
OGTT 2h (mmol/l)	8.2 ± 1.8	8.5 ± 1.6	0.49
Gw at OGTT	27.1 ± 2.3	25.7 ± 3.2	0.01
Gw at rand.	30.6 ± 1.8	29.3 ± 2.2	0.004
HbA1c%	5.4 ± 0.4	5.6 ± 0.2	0.01
Fructosamine (µmol/l)	207.1 ± 15.8	218.4 ± 13.7	<0.001
C-peptide (nmol/l)	1.05±0.31	1.14±0.40	0.53
Dose of metformin (mg) or/and insulin (iu)	500-2000 (mean 1500)	1500-2000 (mean 2000) and 2-51 (mean 11)	
Type of insulin		short-acting insulin only 46% (n=10), intermediate-acting insulin only 22%(n=5), short- and intermediate-acting insulin 35% (n=8), initiation in gw 30-36	

Maternal baseline characteristics in the metformin subgroups (metformin only and metformin with additional insulin) are shown in Table XII. Compared to patients in the metformin only subgroup, patients in the combined treatment group were significantly older, OGTT and randomization were performed significantly earlier in pregnancy and

fructosamine and HbA1c values at randomization were significantly higher (Table XII). The differences in fructosamine and HbA1c values remained significant (p=0.03 and p=0.02, respectively) between the groups after adjustment for age, gw at OGTT and at randomization.

Among the patients with a fructosamine concentration above the median (207µmol/l), 34.6% needed additional insulin and among those with fructosamine concentrations below the median only 7.6% needed additional insulin (RR: 4.6, 95% CI: 1.6–13.6, p=0.006). Of the patients with a HbA1c%-value above the median (5.5), 30.2% needed additional insulin and of those with a HbA1c below the median the frequency was 14.9% (RR: 2.0, 95% CI: 0.9–4.6, p=0.09).

5.1.2. Maternal data

Table XIII. Maternal data and delivery.

Maternal data	Study I		p-value, OR (95%CI)	Study III		p-value, RR (95%CI)
	Metformin (n=45)	Insulin (n=45)		Metformin (n=110)	Insulin (n=107)	
Total weight gain (kg)	10.2 ± 6.7	9.7 ± 7.7	0.62	8.0 ± 5.3	7.9 ± 5.3	0.82
Weight gain (kg)	3.0 ± 3.6[1]	3.5 ± 5.2[1]	0.61	1.8 ± 2.6[2]	2.2 ± 3.0[2]	0.35
PIH #or pre-eclampsia	4 (8.9)	5 (11.1)	1.0; 0.78(0.2-3.1)	7 (6.4)	14 (13.1)	0.11; 0.6(0.3-1.8)
HbA1c % at 36 gw	-	-	-	5.68 ± 0.33	5.69 ± 0.36	0.84
Fructosamine at 36 gw (µmol/l)	-	-	-	204.6 ± 14.6	203.3 ± 14.6	0.55
Gw at labor	38.4 ± 1.4	38.1 ± 1.5	0.23	39.2 ± 1.4	39.3 ± 1.6	0.51
Labor induction	19 (42.2)	26 (57.8)	0.21; 0.5(0.2-1.2)	42 (38.2)	58 (54.2)	0.08; 0.7(0.5-1.0)
Vaginal delivery, non assisted	27 (60.0)	30 (66.7)	0.66; 0.8(0.3-1.8)	85 (77.3)	81 (75.7)	0.89; 1.0(0.8-1.4)
Vaginal delivery, assisted	5 (11.1)	5 (11.1)	1.0; 1.0(0.3-3.7)	9 (8.2)	8 (7.5)	0.85; 1.1(0.4-2.8)
Cesarean delivery	14 (31.1)	10 (22.2)	0.48; 1.6(0.3-1.8)	15 (13.6)	18 (16.8)	0.55; 0.8(0.4-1.6)

Data are expressed as mean ± SD or number and (%).
[1] Weight gain after diagnosis of GDM
[2] Weight gain after randomization
Pregnancy induced hypertension

As shown in Table XIII, there were no statistically significant differences in maternal data between metformin and insulin groups neither in **Study I** nor in **Study III**. And there were no differences between the metformin and diet-only groups in **Study I** (see original publication).

5.1.3. Neonatal data

Table XIV. Neonatal data.

Neonatal data	Study I		p-value, OR (95%CI)	Study III		p-value, RR (95% CI)
	Metformin (n=45)	Insulin (n=45)		Metformin (n=110)	Insulin (n=107)	
Birth weight (grams)	3761 ± 598	3759 ± 642	0.99	3604 ± 488	3589 ± 448	†
Birth weight (SD)	0.7 ± 1.3	0.9 ± 1.6	0.59	0.2 ± 1.0	0.2 ± 1.0	†
Macrosomia	7 (15.6)	10 (22.2)	0.59; 0.6(0.2-1.9)	5 (4.5)	1 (0.9)	0.15; 4.9(0.6-41.6)
LGA	14 (31)	14 (31)	1.0; 1.0(0.4-2.4)	16 (14.5)	17 (15.9)	0.80; 0.9(0.5-1.8)
Birth weight > 4000g	13 (29)	18 (40)	0.38; 0.6(0.3-1.5)	22 (20.0)	16 (15.0)	0.38; 1.3(0.7-2.5)
Apg at 5 min	8.6 ± 0.8	8.7 ± 1.2	1.0	8.7 ± 1.3[1]	8.9 ± 1.0[1]	0.71
Umbilical artery pH	7.3 ± 0.1	7.3 ± 0.1	0.80	7.3 ± 0.1[1]	7.3 ± 0.1[1]	0.47
Neonates to NICU	19 (42.2)	28 (62.2)	0.09; 0.4(0.2-1.0)	34 (31.2)[1]	39 (36.5)[1]	0.51; 0.9(0.5-1.4)
Hypoglycemia#	15 (34.1)	26 (57.8)	0.03; 0.4(0.2-0.9)	18 (16.5)[1]	18 (16.8)[1]	0.96; 1.0(0.5-1.9)
Hyperbilirubinemia	12(26.7)	11(24.4)	1.0; 1.1(0.4-2.9)	9 (8.3)[1]	10(9.4)[1]	0.79; 0.9(0.4-2.2)
RDS	0	1 (2.2)	1.0; 0.3(0.01-8.2)	1 (0.9)	0	1.0; 2.0(1.7-2.3)
Prematurity	2 (4.4)	5 (11.1)	0.43; 0.4(0.07-2.0)	6 (5.5)	4 (3.7)	0.75; 1.2(0.7-2.0)
SGA	1 (2.2)	1 (2.2)		1 (0.9)	0	1.0; 2.0(1.7-2.3)
Shoulder dystocia	0	0		1 (0.9)	2 (1.9)	0.62; 0.7(0.1-3.3)
Brachial nerve injury	0	1 (2.2)	1.0; 0.3(0.01-8.2)	0	2 (1.9)	0.24;0

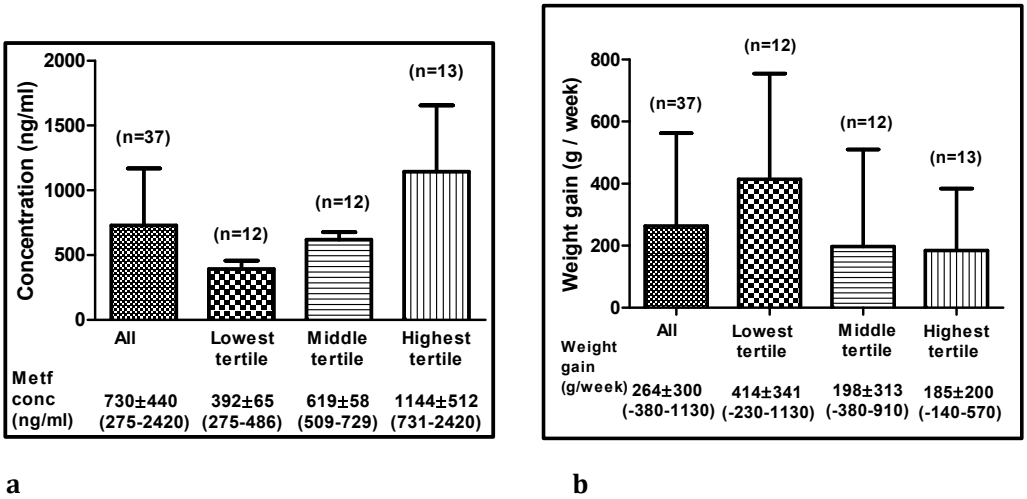
Data are expressed as mean ± SD or number and (%).
Macrosomia = birth weight > 2SD-units and/or >4500g
LGA = birth weight >90th percentile
Apg = Apgar scores 0-10 (0-2 point for heart rate, respiratory effort, color, muscle tone and reflex response)
# Neonatal P-gluc <2.6 mmol/l and a need for intravenous glucose
[1] n=109
† The mean difference (90% CI) in birth weight in grams was **+**15(**−**121 to 89) and in SD units **+**0.04 (**−**0.27 to 0.18). CI:s were within the non-inferiority limits, i.e. the birth weights were equivalent.

Neonatal data are presented in Table XIV. In **Studies I** and **III** there were no statistically significant differences in birth weight (grams or SD-units), macrosomia, the proportion of neonates with birth weight > 90th percentile or > 4000g between the metformin and insulin groups.

In **Study I** the incidence of neonatal hypoglycemia was significantly higher in the insulin treated group compared to the group treated with metformin. There were no other significant differences in terms of neonatal complications between the groups in either of the two studies. In **Study I** there were no significant differences between the metformin group and the diet only group (see original publication). In **Study I** no intrauterine deaths occurred but in **Study III**, there was one stillbirth at gw 39 in the metformin group, the likely cause of which was an umbilical cord complication. Data of this pregnancy were included in the results of maternal and neonatal data until birth.

5.2. *In vivo* placental transfer of metformin (Study IV)

5.2.1. Maternal and neonatal data by metformin concentration at 36 gestational weeks



a **b**

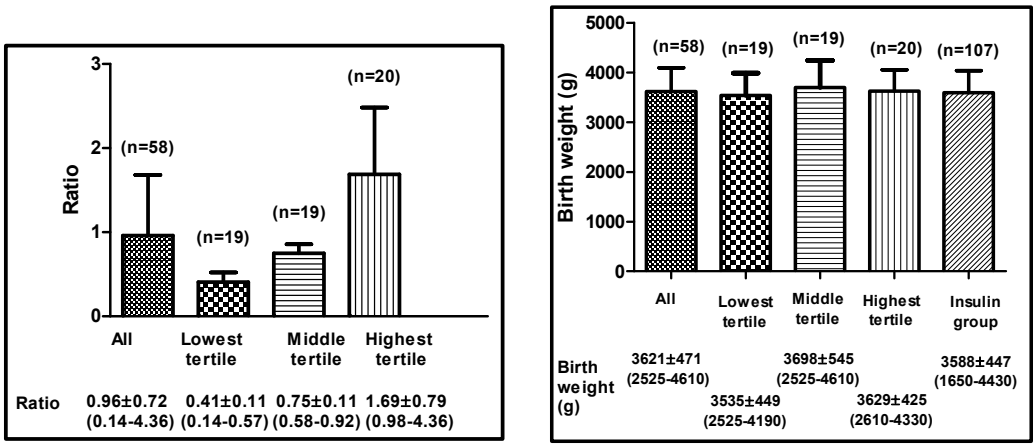
Figure 9. **9a:** Maternal mean trough serum metformin concentration at 36 gw by tertiles. **9b:** Maternal weight gain/week by tertiles and metformin concentration. Data are expressed as mean ±SD (range).

The mean trough serum metformin concentration at 36 gw was 730 ng/ml (range 275-2420 ng/ml) (Figure 9). The therapeutic concentration of metformin in the plasma is 1000-4000 ng/ml (Liu and Coleman 2009, Graham et al. 2011). Patients had normal eGFR-values, as assessed with the Cockroft-Gault formula (Cockroft and Gault 1976), indicating normal renal function.

There were no significant differences in HbA1c (p=0.25) or fructosamine (p=0.10) values between metformin concentration tertiles at 36 gw. Maternal weight gain (g/week) during medication was greater in the lowest metformin concentration tertile compared with that in other tertiles combined (p=0.04) (Figure 9). Analyses were adjusted for BMI at the first antenatal visit.

There were no significant differences in birth weight by grams (p=0.93) or SD-units (p=0.49), or gw at birth (p=0.71) between insulin treated patients and patients by metformin concentration tertiles at 36 gw. Nor were there any significant differences in birth weight (grams and SD-units) between the groups when adjusted for the fructosamine concentration at 36 gw (p=0.97 and p=0.74, respectively).

5.2.2. Neonatal data by metformin concentration at birth



a **b**

Figure 10. 10a: Metformin concentration ratio at birth by tertiles. **10b:** Birth weight by tertiles divided by the metformin concentration ratio. Insulin patients from Study III serve as a reference group. Data are expressed as mean ±SD (range).

At the time of labor the mean maternal serum metformin concentration was 553 ng/ml, the mean cord serum concentration was 457 ng/ml and the mean concentration ratio (umbilical cord / maternal serum mean metformin concentration) was 0.96 (Figure 10). There were no significant differences in birth weight measured in grams (p=0.74) (Figure 10) or SD-units (p=0.97), gw at birth (p=0.60) or arterial cord pH-value (p=0.39) between the patients in three different metformin concentration ratio tertiles and insulin group. There were no significant differences found in birth weight (grams and SD-units) in the corresponding comparisons when the data was adjusted for fructosamine (p=0.73, p=0.95, respectively).

The incidence of neonatal hypoglycemia and the arterial cord pH-value were studied. The patients were divided into tertiles by umbilical cord serum metformin concentration levels. Patients treated with insulin served as controls. There were no significant differences in neonatal hypoglycemia (p=0.92) nor arterial cord pH-value (p=0.78) between the groups.

5.2.3. Time of last drug intake and metformin concentration at birth

The values of the metformin concentration in umbilical cord serum, in maternal serum and their ratio at birth were divided into four groups (n=33) according to the time of known last drug intake [I: 6 to 10 hours (n=7), II: 11 to 15 hours (n=9), III: 16 to 20 hours (n=8=, and IV: over 20 hours (n=9)]. Umbilical cord and maternal serum concentrations were significantly higher (p=0.004, and p=0.022, respectively) in group I than in group IV, but there were no significant differences in the concentration ratios (p=0.62) between the four groups.

5.3. *Ex vivo* human term placental perfusion containing metformin (Study II)

5.3.1. Viability of placentas

Table XV. Viability of placentas in perfusions containing metformin.

	Maternal to fetal perfusions (n=17) *			**Fetal to maternal perfusions (n=12) ***		
	Perfusion pressure (mmHg)	pH FA	pH FV	Perfusion pressure (mmHg)	pH MA	pH MV
beginning	25.1(4.4)	7.31(0.04)	7.31(0.09)	25.9 (5.2)	7.36(0.04)	7.37(0.05)
end	23.1(5.0)	7.34(0.06)	7.31(0.08)	23.0 (9.9)	7.42(0.04)	7.36(0.07)

* Perfusions with metformin alone and metformin with cimetidine combined.
FA = fetal artery, FV = fetal vein, MA = maternal artery, MV = maternal vein
Data are expressed as mean and (SD).

Five of thirty-four perfused placentas were rejected because of abnormal antipyrine transfer. The results obtained with twenty-nine placentas were accepted for analyses.

In maternal-to-fetal perfusions, there were no physiologically or clinically significant changes in the pH values or perfusion pressure between the beginning and the end (Table XV). A small but statistically significant decrease in the fetal artery perfusion pressure (from 24.9 to 21.3 mmHg) in the metformin-only group ($p = 0.004$) was detected.

In fetal-to-maternal perfusions, there was a significant ($p = 0.03$) rise in pH (from 7.37 to 7.43) in the maternal artery, and a small but statistically significant decrease in the fetal artery pressure in the metformin-only group (from 25.0 to 18.3 mmHg) ($p = 0.03$).

All pH-values remained within the physiological range and the perfusion pressure was also within the accepted value of <60 mmHg (Bloxam and Bullen 1986). The flow rate was unchanged throughout the perfusions, and there was no mixing between the fetal and maternal circulation.

5.3.2. Placental transfer of antipyrine

Antipyrine is a good surrogate indicator of placental integrity and was transferred to a similar extent as in previously published studies (Schneider et al. 1972, Mölsä et al. 2005).

In maternal-to-fetal perfusions, the transfer of antipyrine was 10.0% and this value was not significantly affected by perfusion with cimetidine ($p = 0.69$). The steady state for antipyrine was reached on average after 25 minutes and was not significantly altered by cimetidine ($p = 0.38$).

In the fetal-to-maternal perfusions, the transfer of antipyrine was 42.3% and, again, not significantly affected by cimetidine ($p=0.28$). The steady state was reached after 32 min of perfusion and was not altered by cimetidine ($p = 0.87$).

5.3.3. Placental transfer of metformin

Metformin was transferred at a proportion of 3.7% from the maternal to the fetal side of the perfusion system, as measured by TPT% at steady state. It was increased insignificantly to 4.8% ($p=0.18$) when perfused with cimetidine. Steady state was reached on average after 29 minutes of perfusion, and was not affected by cimetidine ($p = 0.37$).

The proportion of metformin transferred in the fetal-to-maternal perfusion system was 15.5% as measured by TPT% at steady state. The transfer rose insignificantly to 18.6% ($p=0.44$) under the effect of cimetidine. The steady state was reached on average after 38 minutes of perfusion, and was not altered with cimetidine ($p = 0.26$).

The transfer of metformin from the fetus to the mother was significantly higher than the transfer in the opposite direction in the metformin ($p < 0.001$) and the metformin–cimetidine groups ($p < 0.001$). Similarly, the transfer of antipyrine from the fetus to the mother was also significantly higher both in the metformin ($p < 0.001$) and in the metformin–cimetidine groups ($p = 0.048$) than in the corresponding studies of transfer in the opposite direction. For metformin, the median time to reach the steady state was longer in the fetal-to-maternal than in the maternal-to-fetal perfusions in the metformin group ($p = 0.046$) and in the metformin–cimetidine group ($p = 0.004$).

6. DISCUSSION

Using medication during pregnancy requires consideration of the risks and benefits for the mother as well as for the unborn child. Possible long-term consequences that could affect the neonate and child must be evaluated. The medication must be essential, beneficial and cannot be harmful. If there is a well-documented pharmacological treatment for a given condition during pregnancy, an alternative drug must be documentedly sufficiently effective and safe before it can be considered for use during pregnancy. The use of metformin in gestational diabetes needs to fulfill these criteria. Thus, metformin must be confirmed to be safe for the mother and child and it must be effective in comparison to insulin.

6.1. Methodological and ethical considerations and study limitations

6.1.1. Retrospective, case-control study

The limitations of retrospective, case-control **Study I** lie in the selection and allocation of patients. The study was not randomized and it is presumed that insulin-treated patients were more hyperglycemic than metformin-treated patients, since all values in OGTT were significantly higher in the insulin group. There were no strict criteria for assigning patients to metformin or insulin unless the glucose values exceeded the limits for obligatory insulin treatment (fP-gluc >7.0mmol/l or pp-gluc >10.0 mmol/l). The study had limited power to detect differences between metformin and insulin treated groups in many of the outcome variables, especially of variables with a low incidence.

6.1.2. Randomized controlled trial

Study III was a RCT. It was open-label trial, which is appropriate for comparing the effectiveness of two similar treatments. Blinding was not achievable because of different routes of drug administration of metformin and insulin. A placebo-controlled group was not possible, since it would have been unethical not to treat GDM patients with medication at the prespecified level of hyperglycemia.

Instead of determining superiority of either of the two studied drugs in **Study III,** a noninferiority approach was taken, which aims to determine whether the new treatment (metformin) is less efficient compared with the reference treatment (insulin) or not. According to the noninferiority principle “the new treatment is recommended if it is similar to or better than an existing one” (Piaggio et al. 2006). Power calculations were made for analyzing the primary outcome, the birth weight, and calculations arrived at 139 subjects being required in both study groups. Because study enrollment slowed down, an interim analysis was performed in 2009 to assess non-inferiority of metformin with respect to birth weight. This interim analysis showed equivalence between the

groups when 77 metformin-treated and 84 insulin treated patients had undergone labor. To ensure the number of patients, recruitment still continued for 12 months, and the final numbers in the two groups were 110 and 107. **Study III** had limited power to detect maternal and neonatal complications, and it was not originally designed to characterize which metformin-treated patients needed additional insulin.

6.1.3. *In vivo* study

Collecting maternal and umbilical cord serum samples simultaneously after labor is fairly simple and ethically acceptable for all involved parties. This enables assessment of drug concentrations in the maternal and fetal circulation and determination of drug transfer (Bourget et al. 1995) at a certain time point, usually in late pregnancy. This circumstance is also a natural limitation of this method.

Umbilical arteries transfer blood from the fetus to the mother and the umbilical vein from the mother to the fetus. In **Study IV** blood samples were collected simultaneously from the vein of the parturient and from the umbilical cord, which contained mixed blood from the umbilical arteries and vein. To calculate drug concentrations in the fetal blood circulation, blood samples should - in theory - be collected from the umbilical artery. However, the results of **Study IV** are reliable for evaluation of the fetal blood circulation, since the literature contains reports of similar concentrations of metformin in samples from both umbilical artery and vein (Vanky et al. 2005, Eyal et al. 2010).

6.1.4. *Ex vivo* study

Ex vivo placental perfusion studies provide a non-invasive method to study human placental drug transfer. After delivery the placenta is not needed and it is normally discarded with other biological waste. To examine the placenta in this way causes, of course, no harm to the mother nor the fetus.

Ex vivo perfusion studies have limitations with respect to physiological and biochemical variables related to the mother, the placenta and the fetus. Interpretation of results from perfusion studies is complicated because perfusion *ex vivo* reflects conditions at only one time point and does not take into account short-term changes, e.g. in the hormonal and hemodynamic milieu and glycemia. Perfusion studies also reflect the situation in term pregnancy, and placental drug transfer in earlier phases of pregnancy may be different (Vähäkangas and Myllynen 2006).

Perfusion experiments can be performed by the closed (recirculating) or the open (noncirculating) method (Schneider et al. 1972, Miller et al. 1985); the latter was used in **Study II**. This setting of two methods complicates comparisons of results from different studies. The cells in the perfused placenta remain viable up to a certain point, and the longest reported approved perfusion time lasted 14 hours (Boal et al. 1997). The viability of placentas must be carefully controlled during the perfusion. In **Study**

II placental viability was tested by Astrup analysis of the pH, pO2 and pCO2 of the perfusion solutions in the beginning and at the end of each perfusion. Flow rates were set to mimic the situation in real life, and they were measured and followed to ensure stable flow. The fetal inflow pressure was monitored throughout the perfusion. The variables of viability in **Study II** showed that all cotyledons were perfused under viable physiological conditions.

The role of OCT-transporters in the metabolism of metformin has been confirmed in other human tissues (Shu et al. 2007, Graham et al. 2011), and it has been suggested - but not been verified - in the human placenta (Kovo et al. 2008b). Previous studies have shown that OCT3 is expressed abundantly, OCT1 to some extent and OCT2 at low levels in the human placenta (Sata et al. 2005, Staud et al. 2012). The density of OCT-transporters in the placentas was not quantified in **Study II**. OCT3 mediates metformin transfer across the rat placenta (Ahmadimoghaddam and Staud 2013). The role of transporters for drug transfer in tissues is often studied by inhibiting the function of the transporter by an appropriate competing substrate (Somogyi et al. 1987, Staud et al.2012). A lack of well-established inhibitors sets limitations to studies on the effect of transporters on drug transfer in the placenta. In **Study II**, cimetidine, an established substrate of OCT3, was used as an inhibitor of OCT-transporter to study the effects of OCTs on metformin transfer.

The possibility that a second, co-operative transporter could be operative in the human placenta, and that it could support metformin efflux from the trophobast cell to the recipient blood circulation was not studied. Based on recent results on studies with rat placentas (Ahmadimoghaddam and Staud 2013) OCT3 is considered only to take up the substrate to the trophoblast cell; a second transporter (e.g. MATE) is purportedly needed to transfer metformin from the cell to the circulation of a recipient, fetus or mother (Staud et al. 2012, Ahmadimoghaddam and Staud 2013). Instead of cimetidine a more potent inhibitor, (e.g. 1-methyl-4-phenylpyridinium iodide), would have been needed to study the existence and effect of MATE, if any, as a vectorial second transporter in the human placenta as was done in the study on rat placentas (Ahmadimoghaddam and Staud 2013).

6.2. Placental transfer of metformin - comparisons of *ex vivo* and *in vivo* studies

There is strong evidence that metformin crosses the placenta from mother to fetus. This has been demonstrated during recent years in *ex vivo* placental perfusion studies (Nanovskaya et al. 2006, Kovo et al. 2008a and 2008b), and in *in vivo* studies where the concentration of metformin has been measured in umbilical cord blood samples (Hague et al. 2003a, Vanky et al. 2005, Charles et al. 2006, Eyal et al. 2010). It has also been suggested, that the transfer of metformin through the placenta is mediated by a carrier (Kovo et al. 2008b, Ahmadimoghaddam and Staud 2013).

In the *ex vivo* **Study II**, the maternal-to-fetal transfer of metformin (4%) was lower than in previous studies (11–16%) (Nanovskaya et al. 2006, Kovo et al. 2008a). However, the transfer ratio between metformin and antipyrine (38%) was similar (34–50%) (Nanovskaya et al. 2006, Kovo et al. 2008a). Thus, the present results can be considered reliable. The fetal-to-maternal transfer of metformin and antipyrine was about 4-fold higher than the maternal-to-fetal transfer. Transfer of a substance across the placenta has been shown to depend on maternal and fetal flow rates: the higher the recipient flow, the higher the transfer of the substance (Schneider et al. 1985). In **Study II** a higher recipient flow in fetal-to-maternal direction compared to maternal-to-fetal direction (10 ml/min vs. 3ml/min) was used to mimic the physiological situation. The calculations of transfer kinetics were corrected for differences in flow rates, but this was not done in previous studies (Nanovskaya et al. 2006, Kovo et al. 2008a). This methodological difference in calculation may partly explain why the results in the present study differs from previous studies. The difference in transfer rates in opposite directions can partly be explained by the use of correction for differences in flow rates, but also by involvement of other active transporters of metformin than OCTs. Lower placental transfer of metformin compared to antipyrine can be also explained by slow passive diffusion of metformin, the evidence of which is shown in a recirculation perfusion study (Kovo et al. 2008a). Metformin may be transferred through the placenta dose dependently (Kovo et al. 2008b), but the metformin concentrations in **Study II** (2μg/ml) were comparable to previous studies (1μg/ml) (Nanovskaya et al. 2006, Kovo et al. 2008a).

In the *ex vivo* perfusion **Study II** the putative active mechanism of metformin placental transfer was studied further. The aim was to evaluate if there is a change in metformin placental transfer when the function of an assumed active OCT-transporter in the metformin carrier mechanism is inhibited. Cimetidine, an inhibitor of OCT3, did not cause any decrease in the transfer of metformin in the maternal-to-fetal nor in the fetal-to-maternal direction, in the transfer index nor in the clearance of metformin. The findings of present **Study II** suggest that OCT-transporters, and mainly OCT3 which is primarily expressed in placenta, are probably not mechanisms, which operate to transfer metformin in the placenta.

In the *in vivo* **Study IV** the results indicated that placental transfer of metformin from mother to fetus in late pregnancy is approximately 96%, and that the fractional transfer is independent of the degree of maternal metformin exposure from clinical doses. The time point of the last metformin intake did not influence the fractional transfer, but as presumed, maternal and umbilical metformin concentrations are the higher the sooner blood samples are taken after the last intake of metformin. Contrary to a small study (n=15) of Vanky et al. (2005) **Study IV** showed that the metformin concentration at birth is similar in maternal serum and in umbilical cord serum, which indicates that metformin does not seem to accumulate into the fetus. The results of previous studies are not comparable with those of the present study, because the measurements

in previous studies were not made from samples of taken simultaneously from the maternal veins and umbilical blood vessels (Hague et al. 2003a, Charles et al. 2006).

The clinical finding in **Study IV** indicates that the results from *ex vivo* placental perfusion studies strongly underestimate the *in vivo* fetal exposure to metformin.

6.3. Maternal outcome and effectiveness of metformin

In clinical **Studies I** and **III** the efficacy of metformin compared with insulin in GDM patients was evaluated. The target glucose values during medication in **Studies I** and **III** were the same (fP-gluc < 5.5 mmol/l, and pp-gluc < 7.8 mmol/l), and the proportion of patients who needed additional insulin was similar (18% and 21%). Still, the studies are not fully comparable. In addition to different study settings, maternal baseline data differed significantly between the studies. In the retrospective **Study I** patients were more obese, their HbA1c and fasting glucose values in OGTT were higher, and OGTT was performed earlier than for the patients in **Study III**.

Metformin does not increase the maternal risks of blood pressure problems, assisted vaginal delivery or cesarean sections according to **Studies I** and **III**, since there were no differences in maternal data between the metformin and insulin groups.

Comparison of the results of **Study III** with those from previous randomized trials sets limitations, because cut off values used for medical treatment, target glucose values during medication and population ethnicity between the studies vary. Ijäs et al. (2010) reported more assisted vaginal deliveries, and intralabor cesarean sections for prolonged labor in the metformin group than the insulin group. In other randomized trials, as also in present study, there were no differences in the mode of delivery (Moore et al. 2007, Rowan et al. 2008, Niromanesh et al. 2012, Spaulonci et al. 2013). Thus, metformin does not seem to increase delivery complications.

Metformin causes often gastrointestinal side effects. However, in **Study III** only 2 patients (1.8%) and in other randomized trials (Rowan et al. 2008, Moore et al. 2007, Ijäs et al. 2010, Niromanesh et al. 2012, Spaulonci et al. 2013) only 0-6% of patients have discontinued metformin because of gastrointestinal symptoms. In the study of Rowan et al. (2008) 77% of metformin-treated patients would choose metformin again.

Lower weight gain of patients treated with metformin compared with insulin has been reported in some studies (Rowan et al. 2008, Niromanesh et al. 2012). Also in **Study III** weight gain after starting medication was slightly, on average 0.5 kg, lower in the metformin group than in the insulin group, but this difference was not significant. There was a significant association between maternal trough metformin concentrations in late pregnancy and maternal weight gain in **Study IV**: the higher the metformin concentration in gw 36, the lower the maternal weight gain.

Comparing glycemic control between metformin and insulin groups is not fully relevant, because the metformin group included patients needing additional insulin. Still, in **Study III** maternal glycemic control was evaluated by HbA1c and by fructosamine values at 36 gw: there were no differences between the groups. Further on, when estimating the use of the metformin concentration as a predictor of maternal glycemic control (**Study IV**), it was seen that maternal late pregnancy metformin concentrations did not correlate with the HbA1c and fructosamine values. This is probably due to the practice that the metformin dose was individually titrated by the patients according to self-measured glucose values; mothers with a lower glucose concentration had a lower dose due to better glucose values. Two randomized trials have reported better glucose control among metformin users that than insulin users based on pp-glucose levels (Rowan et al. 2008, Spaulonci et al. 2013) and on both fasting and pp-values in an observational study of Rai et al. (2009).

6.3.1. Need for additional insulin

Some patients on metformin need additional insulin to meet glycemic targets. In **Study III** 21% of GDM patients in the metformin group needed additional insulin compared to 14-46% in previous randomized trials comparing metformin with insulin in GDM patients (Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Mesdaghinia et al. 2013, Spaulonci et al. 2013). In the study of Moore et al. (2005) none of the metformin-treated patients needed additional insulin. The varying results in different studies with respect to the need for additional insulin may be explained, first, by different glucose inclusion criteria in the OGTT and, second, by different fasting and postprandial glucose treatment targets. In **Study III** the patients needing additional insulin were significantly older, their OGTT had been performed earlier, they needed medication earlier in gestation, and HbA1c and fructosamine values at randomization were higher than among patients on metformin only. Based on our study, fructosamine seems to be more useful than HbA1c for predicting a need for additional insulin. Patients with a fructosamine concentration above the median (207 μmol/l) before starting medication had a 4.6-fold higher probability of requiring additional insulin than patients whose fructosamine was below the median. A useful cutoff point of the fructosamine concentration for clinical practice might be 217 μmol/l, as almost half (47%) of the patients with a fructosamine level above this needed additional insulin. **Study III** showed no association between BMI, glucose values in OGTT and a need for additional insulin, contrary to the findings of Ijäs et al. (2010). The reason for this different result may be chance, but the number of mothers in the study by Ijäs et al was smaller than that in the present study.

6.4. Pregnancy outcome and drug safety for the offspring

Since metformin does cross the placenta, it is essential to know whether this is neutral, beneficial or harmful to the fetus of mothers on metformin with GDM. The safety of

metformin was evaluated in **Studies I** and **III.** The present results indicate that there is no increased risk to the fetus nor the offspring when metformin is compared to insulin in GDM patients.

There were no significant differences in the data describing the condition of the newborns between the insulin and metformin groups in either study. According to **Study IV** the placental transfer or umbilical cord concentration of metformin do not influence fetal or neonatal wellbeing as judged by gestational age at birth, birth weight or umbilical artery pH. Additionally, the metformin concentration in cord serum did not affect the incidence of neonatal hypoglycemia. The mean birth weight (3604g), the incidence of LGA (14.5%) and the occurrence of neonatal hypoglycemia (16.5%) in the metformin group (**Study III**) were comparable with the results of Rowan et al. (2008) (3372g, 19.3%, and 15%, respectively). The level of metformin in the blood circulation of the mother had no influence on fetal growth (**Study IV**).

In randomized trials metformin has been compared to insulin in the treatment of GDM. Less neonatal hypoglycemia (Rowan et al. 2008, Spaulonci et al. 2013) and more preterm births (Rowan et al. 2008) were reported in the metformin group compared to the insulin group, but the difference in the rate of preterm births was not associated with other neonatal complications. In fact, there were more preterm births in the insulin group than in the metformin group in the study of Mesdaghinia et al. (2013). There was no difference in incidence of preterm births between the groups in other studies (Ijäs et al. 2010, Niromanesh et al. 2012, Spaulonci et al. 2013), nor in **Studies I** and **III.** The risk of RDS was low in **Studies I** and **III**, and was not increased by the use of metformin according to previous studies (Moore et al. 2007, Rowan et al. 2008, Niromanesh et al. 2012, Spaulonci et al. 2013). In the study of Mesdaghinia et al. (2013) RDS was reported even more often in the insulin group than in the metformin group.

There was one stillbirth in **Study III** apparently due to an umbilical cord complication in the metformin group. There was one stillbirth in the metformin group in the study of Moore et al. (2010) and one stillbirth in the insulin group in the study of Rowan et al. (2008). In other randomized trials there have been no stillbirths reported (Ijäs et al. 2010, Niromanesh et al. 2012, Mesdaghinia et al. 2013, Spaulonci et al. 2013).

The oldest children studied who have been exposed to metformin *in utero* are by now 7-9 years old; their mothers had been treated with metforming because of PCOS (Rø et al. 2012). The growth and body fat composition of these children was similar, but their fasting glucose level was slightly higher than of children not exposed to metformin *in utero* (Rø et al. 2012). Comparing children of PCOS-patients exposed to metformin *in utero* to those not exposed to metformin has shown normal growth, motor and social development up to 18 months (Glueck et al. 2004b), but increased weight at age 1 year (Carlsen et al. 2012). When children of GDM patients exposed to metformin *in utero* have been compared to children exposed to insulin, it has been found that their weight and total body fat was similar but metformin exposure was associated with more

subcutaneous fat in the arm at age of 2 years (Rowan et al. 2011). This may, in fact, suggest a favorable fat distribution relating to use of metformin.

6.5. Future considerations

There is relatively strong evidence that metformin therapy during pregnancy, starting from the first trimester of pregnancy (Gilbert et al. 2008), is safe for the mother, fetus and neonate (Moore et al. 2007, Rowan et al. 2008, Ijäs et al. 2010, Niromanesh et al. 2012, Mesdaghinia et al. 2013 Spaulonci et al. 2013). Metformin during lactation seems also to be safe for the neonate (Gardiner et al. 2003, Glueck et al. 2006). **Studies I, III** and **IV** confirm the safety of metformin for GDM patients and their offspring *in utero* and *post partum*.

There is a lack of long-term studies on children whose mothers have been exposed to metformin during pregnancy. Before metformin can be fully recommended as a first line antidiabetic treatment during pregnancy, the long-term effects on children and young people need to be scrutinized and the results achieved thus far need to be confirmed.

The proportion of metformin-treated GDM patients who need additional insulin varies from study to study from 14% to 46%. There is need for studies to establish better biological markers and other markers for prediction of which individuals will need additional insulin so that proper antihyperglycemic therapy can be instituted in a timely manner.

A follow-up study of the children born to mothers in **Study III** has been conducted. They have been tested with the psychomotoric Bayley 3 test and the Infant scale test at age of 2 years. The results will be available within a few months. Animal studies do not exclude the possibility that metformin may have harmful effects on the developing testes (Tartarin et al. 2012). A follow-up study examining the testes of the boys who were exposed to metformin *in utero* in **Study III** is being conducted.

Future human placental perfusion experiments should include studying the OCT3/MATE1 vectorial metformin transport. The perfusion technique is also suitable to confirm the role, if any, of Pgp and BCRP in transporting metformin through the placenta. Studies on the placental transfer of new antihyperglycemic agents are needed, as they may be potential drugs for GDM in the future.

If longterm results indicate no adverse effects of metformin to the offsprings exposed to metformin *in utero*, metformin could be considered to be the first line medication of GDM patients. However, in patients with a high risk of need of additional insulin to meet glycemic targets, starting metformin might be avoided not to delay instituting insulin therapy.

7. SUMMARY AND CONCLUSIONS

The mechanism of metformin placental transfer and the role of active organic cationic transporters (OCTs) were studied by *ex vivo* placental perfusion. Clinical use of metformin was compared retrospectively and prospectively with insulin to treat GDM patients by evaluating the influence of metformin on maternal and fetal outcome. The factors predicting the need of additional insulin with metformin were examined. Additionally, by measuring metformin concentrations from maternal and umbilical cord serum, the impact of different metformin exposure levels on maternal and fetal outcome was studied. The main findings are:

1. Placental transfer of metformin from mother to fetus was low, only 4% of the maternal metformin concentration (**Study II**). The *ex vivo* placental perfusion study indicated that OCTs do not seem to have any significant role in the placental transfer of metformin.

2. Contrary to the *ex vivo* results, *in vivo* maternal and umbilical cord serum metformin concentrations at labor indicate that the transfer of metformin from the mother to the fetus is high (96%) (**Study IV**). The level of maternal metformin exposure had no influence on the fractional transfer of metformin across the placenta from mother to fetus. The concentrations of metformin in the maternal serum at 36 gw and in umbilical cord serum at birth, and placental metformin fractional transfer at birth were not related to fetal outcome. Metformin does not accumulate in the fetus.

   There was an inverse association between maternal metformin concentration measured at 36 gw and maternal weight gain (**Study IV)**. Because of the favorable effect on maternal weight, a maximum clinical dose of metformin is to be recommended. Metformin concentration measurements in late pregnancy do not seem to have clinical value for predicting maternal glycemic control or for evaluating the safety of metformin therapy during pregnancy.

3. Metformin did not increase the maternal blood pressure problems during pregnancy, and did not influence delivery modes when compared with insulin (**Studies I** and **III).** Glycemic control measured by HbA1c and fructosamine was similar during metformin and insulin therapy (**Study III**).

   Every fifth of all metformin-treated patients needed additional insulin to achieve good glycemic control (**Study III**). These patients were older, their OGTT was performed and medication started earlier in pregnancy and their HbA1c and fructosamine values were higher than what was the case for patients on metformin only (**Study III**). Fructosamine seems to be more useful than HbA1c for predicting the need of additional insulin. When insulin is needed to meet

glycemic targets, it is worth continuing metformin because of its beneficial effects of maternal weight gain (**Studies III** and **IV**).

4. Metformin treatment in GDM patients was not associated with an increased occurrence of an adverse outcome for the fetus or neonate, which is an indication of the safety of metformin (**Studies I** and **III**).

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