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Elisa Paavilainen

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To my family

UNIVERSITY OF TURKU
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ABSTRACT

Gestational diabetes (GDM) is defined as a condition in which hyperglycaemia develops for the first time during pregnancy. Blood glucose-lowering pharmacological treatment is begun if, despite lifestyle changes, the blood glucose level remains above the target. Insulin, which has traditionally been the first-line option, is not transported to the foetus in commonly used doses. However, because insulin treatment is associated with practical inconveniences, tablet medications, particularly metformin, have been increasingly studied. In contrast to insulin, metformin is transported across the placenta into the foetal bloodstream. Although adverse effects on newborns from foetal metformin exposure have not been observed, data on the long-term outcomes of children are limited.

This study aims to investigate the safety of maternal metformin therapy in offspring at the age of 9 years. We studied 172 offspring of mothers who had been randomized to receive either metformin or insulin for GDM. The offspring were born between 2005 and 2010 in Turku or Oulu. We studied the anthropometry, blood pressure, glucose and lipid metabolism, low-grade inflammation, adiponectin, and leptin values of these offspring. To evaluate body composition, visceral adipose tissue volume, and liver fat, we used dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI). We studied the offspring's neuropsychological outcomes with the Wechsler Intelligence Scale for Children (WISC-IV); Developmental Neuropsychological Assessment (NEPSY II); Trail Making Test; Screening Test for Reading, Writing, and Calculus for First to Sixth Grades (Lukilasse 2); and Behavior Rating Inventory of Executive Functioning (BRIEF).

We found that 9-year-old offspring's anthropometry, body composition, metabolism, and neuropsychological outcomes were similar between the offspring of the mothers who had metformin and those who received insulin treatment for GDM. However, serum HDL cholesterol and adiponectin concentrations were slightly higher and the 2-hour serum glucose concentration in OGTT was slightly lower in boys whose mothers were treated with metformin.

Thus, metformin treatment for GDM seems to be safe for offspring in the long term.

KEYWORDS: gestational diabetes, metformin, long-term effect, anthropometry, metabolism, blood pressure, body composition, cognitive outcome, neuropsychological outcome, executive functions

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Lastentautioppi
ELISA PAAVILAINEN: Metformiini raskausdiabeteksen hoidossa - pitkäaikaisvaikutukset lapseen 9 vuoden iässä insuliiniin verrattuna
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TIIVISTELMÄ

Raskausdiabetes on sokeriaineenvaihdunnan häiriö, joka todetaan ensimmäisen kerran raskausaikana. Lääkitys aloitetaan, jos verensokeripitoisuus jää tavoitetta korkeammaksi elämäntapamuutoksista huolimatta. Insuliini ei kulkeudu käytetyillä hoitoannoksilla istukan kautta sikiöön ja on sen vuoksi ollut ensisijainen valinta raskausdiabeteksen hoidossa. Insuliinihoitoon liittyy käytännön haasteita, minkä vuoksi metformiinia on tutkittu vaihtoehtona. Metformiinin tiedetään siirtyvän istukan läpi sikiön verenkiertoon ja vaikka sikiöaikaisesta altistuksesta ei ole todettu aiheutuvan haittavaikutuksia vastasyntyneelle, on pitkäaikaista seurantatietoa vain niukasti.

Tämän tutkimuksen tarkoituksena oli selvittää metformiinin pitkäaikaisturvallisuutta syntyvän lapsen kannalta arvioimalla äidin raskausdiabeteksen hoidoksi käytetyn metformiinin mahdollisia vaikutuksia lapseen 9-vuoden iässä. Vuosina 2005–2010 Turussa tai Oulussa syntyneiden lasten äidit oli satunnaistettu saamaan raskausdiabeteksen hoidoksi joko metformiinia tai insuliinia. Lasten antropometriaa, verenpainetta, sokeri- ja rasva-aineenvaihduntaa, adiponektiini- ja leptiiniarvoja sekä matala-asteista tulehdusta selvitettiin. Lisäksi DXA ja MRI-kuvauksilla tutkittiin kehon koostumusta, vatsan alueen rasvakudoksen määrää ja maksan rasvoittumista. Neuropsykologista suoriutumista arvioitiin standardoiduilla testeillä (mm. WISC-IV, NEPSY II) ja toiminnanohjausta arvioivalla kyselyllä (BRIEF).

Tässä tutkimuksessa todettiin, että raskausdiabeteksen vuoksi metformiini- tai insuliinihoitoa saaneiden äitien 9-vuotiaiden lasten antropometria, kehon koostumus, aineenvaihdunta ja neuropsykologinen suoriutuminen eivät eronneet toisistaan. Lisäksi metformiinihoitoryhmän pojilla todettiin hieman korkeammat seerumin HDL-kolesteroli- ja adiponektiinipitoisuudet sekä hieman matalampi seerumin kahden tunnin glukoosipitoisuus sokerirasituskokeessa kuin insuliiniryhmän pojilla.

Näiden tulosten perusteella metformiini vaikuttaa turvalliselta syntyneelle lapselle myös pitkäaikaisseurannassa.

AVAINSANAT: raskausdiabetes, metformiini, pitkäaikaisvaikutus, antropometria, metabolia, verenpaine, kehon koostumus, neuropsykologinen suoriutuminen, toiminnanohjaus

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Abbreviations

ALT	alanine aminotransferase
ApoA1	apolipoprotein A1
ApoB	apolipoprotein B
BMI	body mass index
BP	blood pressure
BRI	Behavioral Regulation Index
BRIEF	Behavior Rating Inventory of Executive Functioning
CI	confidence interval
CVD	cardiovascular disease
DXA	dual-energy X-ray absorptiometry
FFMI	fat-free mass index
FMI	fat mass index
FSIQ	full-scale intelligence quotient
GDM	gestational diabetes mellitus
GlycA	glycoprotein acetyls
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
HbA1c	glycated haemoglobin
HDL	high-density lipoprotein
HOMA-IR	homeostatic model assessment of insulin-resistance
hsCRP	high sensitivity C-reactive protein
IL-6	interleukin-6
IQR	interquartile range
ISO-BMI	adult equivalent body mass index
LDL	low-density lipoprotein
LGA	large for gestational age
LUKILASSE	Screening Test for Reading, Writing and Calculus for 1st to 6th grades
MRI	magnetic resonance imaging
MRS	magnetic spectrometry
NEPSY II	Developmental Neuropsychological Assessment, 2nd edition
NICU	neonatal intensive care unit
OGTT	oral glucose tolerance test

PCOS	polycystic ovary syndrome
PI	ponderal index
RCT	randomized controlled trial
RDS	respiratory distress syndrome
ROI	regions of interest
SD	standard deviation
SGA	small for gestational age
T2D	type 2 diabetes
THL	Institute for Health and Welfare, Finland
TMT	Trail Making Test
VAT	visceral adipose tissue
WHtR	waist-to-height ratio
WISC-IV	Wechsler Intelligence Scale for Children, fourth edition

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Paavilainen E, Tertti K, Nikkinen H, Veijola R, Vääräsmäki M, Loo BM, Tossavainen P, Rönnemaa T, Niinikoski H. Metformin versus insulin therapy for gestational diabetes: Effects on offspring anthropometrics and metabolism at the age of 9 years: A follow-up study of two open-label, randomized controlled trials. *Diabetes, Obesity & Metabolism*, 2022; Mar;24(3):402–410. https://doi.org/10.1111/dom.14589

II Paavilainen E, Niinikoski H, Parkkola R, Koskensalo K, Nikkinen H, Veijola R, Vääräsmäki M, Loo BM, Tossavainen P, Rönnemaa T, Tertti K. Metformin versus insulin for gestational diabetes: Adiposity variables and adipocytokines in offspring at age of 9 years. *Diabetes Research and Clinical Practice*, 2023, Aug;202:110780. https://doi.org/10.1016/j.diabres.2023

III Paavilainen E, Nyman A, Niinikoski H, Nikkinen H, Veijola R, Vääräsmäki M, Tossavainen P, Rönnemaa T, Tertti K. Metformin versus insulin for gestational diabetes: Cognitive and neuropsychological outcomes of children at 9 years of age. *Journal of Developmental & Behavioral Pediatrics,* 2023 Dec 1;44(9):e642–e650. https://doi.org/10.1097/DBP.0000000000001233

1 Introduction

Gestational diabetes mellitus (GDM) is considered one of the most common metabolic disturbances during pregnancy. The prevalence of GDM is rising worldwide, along with maternal obesity, which has created concerns about a significant global health burden. Furthermore, research evidence has shown that untreated GDM causes significant short- and long-term complications for both the offspring and the mother. Short-term neonatal outcomes of GDM include an increased risk of macrosomia, birth trauma, and hypoglycaemia, while long-term offspring outcomes include an increased risk of obesity during childhood or adulthood and an increased risk of cardiometabolic disturbances.

Several international, national, and obstetric associations have defined clinical guidelines for screening, diagnosing, and managing GDM. There are variations among these guidelines, particularly related to medical treatment with glucose-lowering drugs such as metformin. According to the majority of these guidelines, insulin is still widely considered the first-line pharmacological choice for treating GDM. However, metformin has been increasingly studied as an optional treatment for GDM, and a few guidelines have already positioned metformin ahead of insulin. Moreover, metformin is an easy-to-use and more economically advantageous option.

Systematically collected long-term follow-up data that broadly assess children's health are needed to confirm the safety of metformin treatment for GDM during pregnancy regarding the health of developing offspring.

2 Review of the Literature

2.1 Gestational Diabetes Mellitus

2.1.1 Definition and prevalence

Gestational diabetes mellitus (GDM) is defined as a condition in which hyperglycaemia develops for the first time during pregnancy (ADA, 2020). It is currently one of the most common medical complications in pregnancy, and its prevalence has been increasing globally, along with obesity (ACOG, 2018). According to the latest estimates of the International Diabetes Federation (Wang et al., 2022), GDM affects 14% of pregnancies worldwide – a value that denotes approximately 20 million births annually (Wang et al., 2022). Occurrence rates of GDM can vary from 2% to 32% according to differences between countries in diagnostic criteria, screening practices, and population characteristics (Zhu et al., 2016). Wang et al. found that the highest standardised prevalence of GDM occurred in the Middle East and North Africa (27.6%), South-East Asia (20.8%), the Western Pacific (14.7%), and Africa (14.2%), while the prevalence in Europe was only 7.8% (Wang et al., 2022). In Finland, 66% of pregnant women participated in a screening program in 2019, and 20.6% were diagnosed with GDM (THL, 2019). Based upon this screening program, the estimated GDM prevalence is 13.6%. Indeed, the prevalence of GDM in Finland has doubled over the past decade, concurrent with an increase in the average age of first-time mothers and obesity among pregnant women (THL, 2019).

2.1.2 Screening and diagnosis

The criteria for screening and diagnosing GDM vary according to different international recommendations. In Finland, the current criteria have been used since 2008, when the screening standard changed from the previous risk factor–based model to screen all but low-risk pregnancies for GDM (Duodecim, Current Care Guidelines, 2022). Gestational diabetes is diagnosed with a 2-hour, 75-gram oral glucose tolerance test (OGTT) during pregnancy. The test is usually programmed in the second trimester (pregnancy weeks 24–28), although it can be conducted during

the first trimester if the mother has a particularly high risk for GDM or another type of diabetes (Duodecim, Current Care Guidelines, 2022).

The diagnostic criteria of GDM differ from the standard DM criteria; in GDM, a plasma glucose concentration ≥ 5.3 mmol/L (fasting), ≥ 10.0 mmol/L (1 hour), or ≥ 8.6 mmol/L (2 hours) is the diagnostic cut-off value at any of these measurement points (Duodecim, Current Care Guidelines, 2022). Fasting and 2-hour cut-off values in OGTT are slightly higher in Finland compared to those of the International Association of Diabetes and Pregnancy Study Group (IADPSG) or World Health Organization criteria, which use ≥ 5.1 mmol/L (fasting), ≥ 10.0 mmol/L (1 hour), and ≥ 8.5 mmol/L (2 hours).

International recommendations mainly include fasting glucose measurement as a screening procedure during the first trimester, aiming to find, for example, undiagnosed type 2 diabetes. However, optimal diagnostic OGTT values for GDM in early pregnancy are still unclear. Finnish guidelines recommend similar diagnostic OGTT values for early and late GDM because research evidence for optimal criteria for early GDM is lacking (Duodecim, Current Care Guidelines, 2022). A recent study by Simmons et al. (2023) found that immediate treatment of early GDM (diagnosed before 20 weeks' gestation) led to a modestly lower incidence of a composite of adverse neonatal outcomes than no immediate treatment.

2.1.3 Normal glucose metabolism in pregnancy

As pregnancy progresses, the mother's body undergoes several hormonal, physiological, and metabolic changes, the purpose of which is to ensure the appropriate development and growth of the foetus (Hadden et al., 2009). Furthermore, these changes provide the foetus with the energy stores needed after birth. The primary source of energy for the foetus is glucose in the mother's plasma (Baumann et al., 2002), which is regulated by maternal insulin production from her pancreatic beta cells and adequate insulin action of the maternal muscle, liver, and fat tissues (Hadden et al., 2009). During the second trimester of normal pregnancy, the maternal glucose metabolism is influenced by strongly increasing insulin resistance at the tissue level, which corresponds to an approximately 60% decrease in insulin sensitivity (Catalano et al., 1991).

2.1.4 Pathogenesis of GDM

GDM develops when maternal insulin production by beta cells fails to meet the increased insulin need induced by higher insulin resistance in tissues, resulting in elevated glucose concentration in maternal circulation (Levy et al., 1998). This process leads to increased placental transport of glucose among fatty acids and amino

acids, which stimulates the foetal endogenous production of insulin and insulin-like growth factor 1 (Plows et al., 2018). Further, these metabolic processes lead to foetal overgrowth and macrosomia at birth (Plows et al., 2018). Simultaneously, the placenta is exposed to other GDM-induced effects, such as increased inflammation, oxidative stress, altered hormone levels, and dyslipidaemia (Bedell et al., 2021). These changes affect placental function and may induce obstetric complications, including preeclampsia and stillbirth, as well as abnormal growth and development of the foetus (Bedell et al., 2021).

As mentioned above, both maternal beta cell dysfunction and tissue insulin resistance are critical components in the development of GDM. However, the group of mothers who develop GDM during pregnancy is heterogeneous, and different subtypes (pheno- or genotypes) can be found. GDM can be roughly divided into three subtypes: insulin-resistant, insulin-deficient, or mixed (Powe et al., 2020). Typically, obese mothers have an insulin-resistant subtype, while lean mothers have an insulin-deficient subtype of GDM (Powe et al., 2020). Women with obesity have a three- to four-fold higher risk of developing GDM during pregnancy compared to normal-weight women (Najafi et al., 2019).

2.1.5 The importance of intrauterine environment for offspring health

During the average of 40 weeks between conception and birth, tremendous development occurs as the offspring progress from a few cells to a newborn baby. Several factors can affect this process negatively, impact offspring development before delivery, or have adverse effects on offspring metabolism that may emerge in later life. One manifestation of the latter is described by the developmental origins of health and disease theory (DOHaD, aka the Barker hypothesis), which states that adverse intrauterine environments influence the risk of developing chronic disease in the offspring (Barker, 2007). Dr Barker's studies showed that impaired foetal growth due to insufficient maternal nutrition during pregnancy causes adaptation to limited nutrition. In other words, malnutrition permanently changes the physiology and metabolism of the foetus and predisposes the offspring to obesity, hypertension, diabetes, and cardiovascular diseases (Barker et al., 1993; Barker, 1997; Barker, 2007). Numerous subsequent studies, particularly the Dutch famine cohort of 1944–1945, have provided further strong evidence supporting the DOHaD theory (Roseboom et al., 2000, DeRooij et al., 2006).

The opposite situation, where the foetus is exposed to excess glucose and lipids due to the mother's high blood concentrations, is not in favour either. Pedersen (1952) first described the connection between maternal hyperglycaemia and foetal hyperinsulinemia leading to macrosomia. Freinkel (1980) later used the term "fuel-

mediated teratogenesis" to describe this phenomenon in which excess sugar and fat in the maternal diet alter offspring metabolism, glucose tolerance and insulin signalling and induce cardiometabolic diseases later in life. Further, the HAPO study group (2009) showed that neonatal adiposity was linked to increased levels of maternal glucose and cord serum C-peptide. Based on rodent studies, the mediators behind these changes are alterations in gene expression at the molecular level. These epigenetic modifications are hypothesized to link maternal nutrition to metabolic health in the offspring (Gluckman et al., 2008; Kereliuk et al., 2017).

2.1.6 Short-term maternal and neonatal complications

GDM is often mild and can be treated with lifestyle changes. The risk of pregnancy and offspring complications is lower in mild cases compared to GDM for which pharmacological treatment is needed. Approximately one third of women with GDM also require pharmacological treatment (Metzger et al., 2008). When GDM is combined with maternal obesity, the risk of adverse outcomes during pregnancy and delivery is increased (Metzger et al., 2008). Recent evidence has shown that both the subtype and severity of GDM affect the probability of adverse effects. Thus, the influence of these subtypes on perinatal complications can vary; for example, in the insulin-resistant subtype, in which postprandial glucose values are typically elevated, the risk for perinatal complications is higher than in the insulin-deficient subtype (Powe et al., 2020).

GDM is associated with complications throughout the course of pregnancy (pre-eclampsia, gestational hypertension), labour (risks of instrumental, traumatic, or caesarean labour), and breastfeeding (Sweeting et al., 2022).

The most common neonatal complications related to GDM are macrosomia, or being large for gestational age, and neonatal hypoglycaemia. Macrosomia develops when excess maternal glucose crosses the placenta, but maternal insulin does not (Freinkel, 1980). In this instance, the foetal pancreas responds to the increased glucose load by producing more insulin, which in turn promotes growth and excess adiposity. Other neonatal complications of GDM include preterm birth, birth trauma, perinatal asphyxia, hyperbilirubinemia, and respiratory distress syndrome (Boulet et al., 2003; Langer et al., 2005; Metzger et al., 2008; Esakoff et al., 2009; Billionnet et al., 2017; Sweeting et al., 2022). GDM-induced stillbirth is a rare complication of severe and untreated GDM. Additionally, obesity in the mother increases the risk of significant adverse foetal effects (Creanga et al., 2023).

Women who develop GDM early in pregnancy are likely to have chronic beta cell dysfunction and insulin resistance prior to pregnancy; therefore, the risk of congenital anomalies induced by hyperglycaemia and oxidative stress during early foetal development may also appear in GDM. Associations between GDM and

congenital anomalies were examined in a large population-based study (Wu et al., 2020). The study found that adjusted risk ratios of cyanotic congenital heart disease (1.50 [95% CI 1.43–1.58]) and hypospadias (1.29 [95% CI 1.21–1.36]) were elevated for GDM, although risk ratios were lower compared to offspring of mothers with pre-pregnancy diabetes (Wu et al., 2020).

2.1.7 Long-term offspring outcomes

While the uterus, together with placental circulation, typically provides an optimal environment for foetal development, several unfavourable factors can affect this intrauterine environment. One of these is maternal hyperglycaemia in GDM, which is shown to increase total and abdominal adiposity in 6-year-old offspring (Kearney et al., 2018) and induce longer-term metabolic effects in the offspring, such as impaired glucose tolerance and obesity at the age of 11 years (Lowe et al., 2019; Lowe et al., 2019).

The long-term outcomes of over 4,000 children in the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) cohort were studied at the age of 10–14 years (Table 1). The HAPO Study recruited a large, multinational, racially and ethnically diverse cohort of pregnant women who did not receive glucose-lowering medication. The HAPO Follow-up Study examined associations between maternal glucose balance during pregnancy and the long-term outcomes of mothers (n = 4697) and children (n = 4832) between 2013 and 2016 (Metzger et al., 2008). One of the follow-up studies of the HAPO group showed that exposure to higher levels of maternal hyperglycaemia in utero is associated with childhood glucose tolerance and insulin resistance, independent of child's or mother's body mass index (BMI), and family history of diabetes (Scholtens et al., 2019).

A large Danish population-based study evaluated the risk for early cardiovascular disease (CVD) in the offspring of mothers with diabetes (Yu et al., 2019). They studied the offspring's risk for CVD disease in different age groups from childhood until the age of 40 years and found that offspring of mothers with GDM had increased risk factor rates (1.19 [1.07 to 1.32]) of early onset of CVD in all studied age groups (Yu et al., 2019).

Intra-uterine foetal exposure to hyperglycaemia, hyperinsulinemia, and pro-inflammatory mediators in GDM can also affect the long-term neurodevelopment of children (Sousa et al., 2018). However, such findings have been contradictory (Ornoy et al., 1999; Ornoy et al., 2001; Fraser et al., 2012; Soepnel et al., 2022), and the study designs have been heterogeneous (Camprubi Robles et al., 2015; Adane et al., 2018). Two recent studies reported that GDM combined with maternal pre-pregnancy overweight or obesity may lead to transgenerational brain changes (Manuello et al., 2022) or weakened neurodevelopmental skills in offspring,

although these skills remain within the mean normative range in this population (Saros et al., 2023).

Table 1. Main results of the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Follow-up Study Cooperative Research Group.

AUTHORS	YEAR	MATERNAL MEASURES	AGE, YEARS	CHILDREN, n	CONCLUSION
Lowe et al.	2018	All measured glucose values*	10–14	4 832	Among children of mothers with GDM vs those without it, the difference in childhood overweight or obesity defined by BMI cut-offs was not statistically significant.
Lowe et al.	2019	GDM	10–14	4 160	GDM was significantly and independently associated with childhood IGT
Scholtens et al.	2019	All measured glucose values*	10–14	4 832	Foetal exposure to higher maternal glucose levels was significantly associated with childhood glucose and insulin resistance independent of maternal and childhood BMI and family history of diabetes.
Lowe et al.	2019	All measured glucose values*	10–14	4 832	Foetal exposure to higher maternal glucose levels was independently associated with childhood adiposity, including being overweight/obese, obesity, skinfold thickness, per cent body fat and waist circumference. Glucose levels less than those diagnostic of diabetes are associated with greater childhood adiposity.
Perak et al.	2021	Gestational CVH**	10–14	2 302	Better maternal CVH at 28 weeks' gestation was significantly associated with better offspring CVH at ages 10–14 years.
Josefson et al.	2021	All measured glucose values, BMI, + New-born adiposity***	10–14	4 832	Newborn adiposity was independently associated with childhood adiposity and, along with foetal hyperinsulinemia, mediates, in part, associations of maternal glucose and BMI with childhood adiposity.

* All measured glucose values during pregnancy are included in the study. **Gestational CVH was defined at week 28 of pregnancy based on five metrics: BMI, BP, total cholesterol level, glucose level, and smoking. Offspring CVH based on four metrics: BMI, BP, total cholesterol level and glucose level. *** Newborn adiposity was measured by weight, length, and flank, triceps, and subscapular skinfolds (age < 72 hours).
Abbreviations: BP, blood pressure; CVH, cardiovascular health; IGT, impaired glucose tolerance.

2.2 Treatment of GDM

After a GDM diagnosis, all mothers receive lifestyle guidance aimed at lowering glucose levels. This first-line treatment includes combined nutritional therapy and exercise. Pharmacological treatment is added if optimal glucose levels are not obtained through lifestyle interventions (ACOG, 2018; ADA, 2022). While the blood glucose balance of most mothers with GDM can be managed with counselling and diet modification, approximately 25–30% of these mothers fail to achieve sufficient glycaemic control without pharmacological therapy (ADA, 2022). Adequate treatment for GDM reduces the above-mentioned adverse effects (Guo et al., 2019; Tarry-Adkins et al., 2019).

In the US, three pharmacological therapies are used to treat GDM: insulin, metformin, and glyburide (ADA, 2022). Of these, glyburide is less used in other countries, and only insulin and metformin medications are used for GDM in Finland (Duodecim, Current care guidelines, 2022).

Insulin has been used to treat type 1 diabetes for 100 years, including during pregnancy. Traditionally, insulin has been the standard of pharmacological care for treating GDM—a practice that has been strongly supported by the knowledge that insulin does not cross the placenta. However, the use of insulin requires adequate storage possibilities, which can be challenging in resource-limited conditions, and multiple daily injections can reduce compliance. In addition, unlike metformin medication, insulin use is associated with an increased risk of hypoglycaemia, although hypoglycaemia in women with GDM is not common and typically not severe (Brown et al., 2017).

Of the national recommendations, only the National Institute for Health and Care Excellence (NICE) has elevated metformin to first-line treatment ahead of insulin (Table 2). A recent population-based cohort study from the UK showed a large increase in metformin prescriptions for GDM during the years 1998–2017, and metformin was the first-line treatment in over 85% pregnancies with pharmacotherapy in 2015 (Yu et al., 2023). In two studies on prescribing antidiabetic medicines for GDM in seven regions (between 2004 and 2010 or between 2006 and 2016), insulin was the most often dispensed, except in the US, where glibenclamide was most often used during both time periods (Cesta et al., 2019). Since 2008, the use of metformin instead of insulin has increased in Norway, Wales, and the UK (Charlton et al., 2016), as well as Australia, Norway, Sweden, Finland, and Iceland (Cesta et al., 2019).

Although metformin is increasingly used instead of insulin to treat GDM, insulin remains the first-line therapy recommended by the International Diabetes Federation, the American College of Obstetricians and Gynaecologists, and the American Diabetes Association (Table 2).

Table 2. The medical association recommendations regarding the pharmacological treatment of GDM.

ASSOCIATION	ABBREVIATION	INSULIN	METFORMIN
World Health Organisation	WHO	First-line	Second-line or first-line in resource limited areas
International Diabetes Federation	IDF	First-line	Second-line or first-line in resource limited areas
American College of Obstetricians and Gynaecologists	ACOG	First-line	Second-line, reserved for women unable or willing to use insulin
American Diabetes Association	ADA	First-line	Second-line
The British National Institute for Health and Care Excellence	NICE	Second-line when Metformin is contraindicated or unacceptable to patient	First-line
Society for Maternal-Fetal Medicine	SMFM	First-line	First-line
International Federation of Gynaecology and Obstetrics	FIGO	First-line	
Finnish Current care guidelines		First-line	First-line

2.3 Metformin

2.3.1 Mechanism of action

Metformin belongs to the biguanide group of drugs, and it has been used to treat type 2 diabetes for more than 60 years, since 1967 in Finland. This guanidine derivative was initially extracted from the plant *Galega officinalis* (French lilac). Metformin lowers blood glucose concentrations primarily by decreasing hepatic glucose production through inhibiting gluconeogenesis. Metformin also increases glucose uptake in skeletal muscle and adipocytes, decreases glucose absorption in the gastrointestinal tract (Romero et al., 2017), and affects the brain and appetite through enteroendocrine cells. As a combined effect of these functions, blood glucose and insulin concentrations diminish (Figure 1).

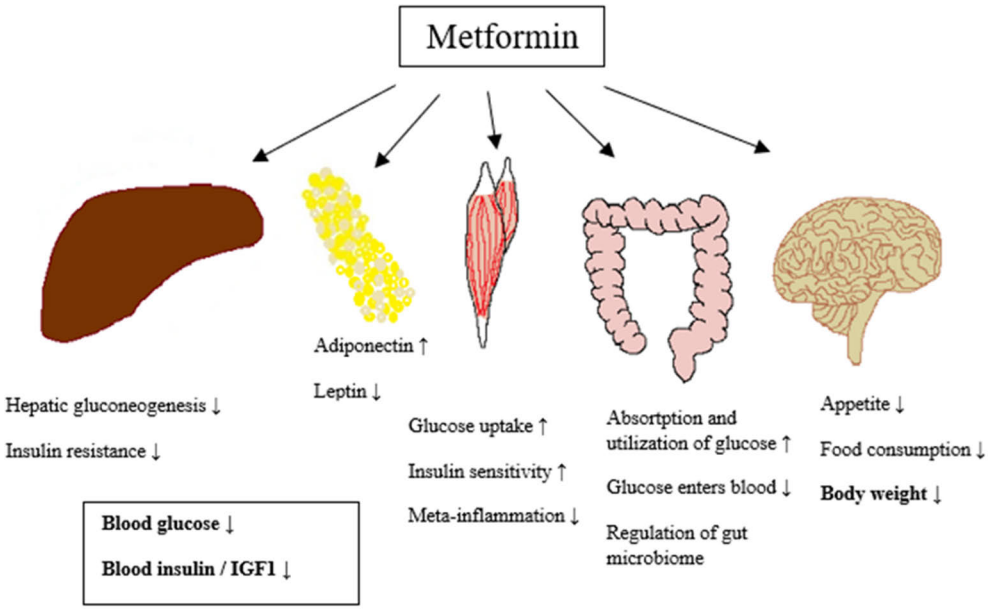


Figure 1. Systemic extra-pancreatic effects of metformin. Modified from Eibl et al., 2021 and Zhang et al., 2023.

The molecular mechanism via which metformin acts on mitochondrial function and regulates the gluconeogenic process has been studied for decades, but it still remains considerably controversial (Triggle et al., 2022). Mitochondria are intracellular organelles responsible for cell energy management. Basically, metformin inhibits the hepatic gluconeogenic process by regulating mitochondrial processes in liver cells. However, the action of metformin seems to be a complex, multidimensional process, and further studies are still needed to understand the complete mechanism. One of these processes is transmitted through a transient inhibition of mitochondrial complex 1, which decreases hepatic energy status. Thus, AMP-activated protein kinase acts to inhibit hepatic gluconeogenesis (Zhou et al., 2001). Another site of metformin action is in liver cell mitochondria through glycerol-3-phosphate dehydrogenase and liver kinase B1 (Madiraju et al., 2014).

In mothers with GDM, the use of metformin aims to improve an unfavourable metabolic state and thus secure as normal placental function and foetal growth as possible. Maternal obesity and GDM can both reduce nutrient and gas exchange in the placenta, causing chorionic villous immaturity (Bedell et al., 2021). However, metformin was found to reduce basal mitochondrial respiration and ATP synthesis in placental trophoblasts in a small (n = 10) study, findings that raised the possibility of its effects on foetal growth and metabolism (Tarry-Adkins et al., 2022). In a recent animal study (foetal sheep, foetal macaques, and juvenile macaques), Swenson et al.

(2023) suggested that metformin acts in foetal liver cells and is associated with disrupted signalling and metabolism in the foetal liver. They noted that metformin's action in foetal hepatocytes may cause decreased growth via reduced anabolic pathways and increased stress pathways (Swenson et al., 2023).

2.3.2 Metformin in Type 2 diabetes and related diseases

Metformin has been used to treat type 2 diabetes (T2D) in Finland for over 50 years, and it remains the first-line treatment for T2D (Duodecim, Current care guidelines, 2020), even though newer options have emerged (e.g., sodium-glucose co-transport inhibitor-2 [SGLT-2]). In contrast to the long history of metformin use in Europe, the drug was not adopted in treatment recommendations in the US until the 1990s (ADA, 2023).

Metformin has many positive effects when used to treat adults with T2D and obesity. Metformin does not increase insulin production, so hypoglycaemic side effects do not occur. It also has a favourable effect on body weight (Paneni et al., 2017). Studies have shown that metformin reduces visceral fat (Feng et al., 2018) and inhibits obesity-induced inflammation (Qi et al., 2017). Furthermore, metformin has been shown to significantly reduce macrovascular events in patients with T2D compared to other antidiabetic medications (Johnson et al., 2002; Paneni et al., 2017).

2.3.3 Metformin in Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a hormonal imbalance state that is highly correlated with obesity. PCOS causes reproductive difficulties due to ovarian dysfunction, excess secretion of male hormones (hyperandrogenism), insulin resistance, and neuroendocrine disruption (Hoeger et al., 2021). Metabolic disturbances in non-treated PCOS pregnancies cause placental inflammation and infarction (Koster et al., 2015), as well as an elevated incidence of miscarriage compared to metformin-treated pregnancies with PCOS (Nawaz et al, 2008). In a multicentre randomized controlled trial (RCT) comparing metformin and a placebo from the late first trimester for PCOS pregnancy (PregMet2; n = 487), Løvvik et al. found that metformin treatment until delivery did not prevent GDM but might reduce the risk of late miscarriage and preterm birth (Løvvik et al., 2019).

No risk for teratogenicity was found in follow-up studies of RCTs comparing metformin and placebo treatment in PCOS pregnancies in which metformin was used from the first trimester to delivery. In these follow-up studies (Løvvik et al., 2019 [n = 487], Vanky et al., 2010 [n = 257]; Vanky et al., 2004 [n = 38]), anthropometrics of neonates (Nilsen et al., 2023; n = 801); first-year weight gain (Carlsen et al., 2012;

n = 257); the risk of overweight at 4 years of age (Hanem et al., 2018; n = 182/295); steroid hormones at 5–10 (mean 7.4) years of age (Hanem et al., 2021; n = 117/255); growth, body composition, and metabolic profile at 7–9 years of age (Rø et al., 2012; n = 25/37); and cognitive function at 7 years of age (Greger et al., 2020; n = 93/292) were studied.

First, neonates in the metformin group had a larger head circumference than neonates in the insulin group, but other anthropometric measures (i.e., birth weight and length, BMI, ponderal index [PI]) were similar between the two groups (Nilsen et al., 2023). Second, 1-year-old children in the metformin group were heavier than those in the placebo group (10.2 ± 1.2 kg vs 9.7 ± 1.1 kg, $p = 0.003$; Carlsen et al., 2012). Third, 4-year-old children's weight and BMI z-scores were higher in the metformin group, and the children in that group were more often overweight or obese, but head circumference at the age of 1 year did not differ (Hanem et al., 2018). Fourth, metformin-exposed boys tended to have higher levels of 11-deoxycortisol compared to boys in the placebo group (Hanem et al., 2021). Fifth, a small follow-up study found no differences in weight, height, or body composition by dual-energy X-ray absorptiometry (DXA) between the groups, but did find higher fasting glucose concentration (4.93 mmol/L vs 4.60 mmol/L, $p = 0.04$), systolic blood pressure (106 mmHg vs 101 mmHg, $p = 0.05$), and a lower low-density lipoprotein (LDL) cholesterol level (2.42 mmol/L vs 2.99 mmol/L, $p = 0.07$) in the metformin group at 7–9 years of age. Sixth, no difference was observed in the mean full-scale intelligence quotient (FSIQ) in the children, but an association was found between metformin exposure in utero and the prevalence of borderline low FSIQ (between 70 and 85) in offspring (Greger et al., 2020).

2.3.4 Metformin in other potential conditions

Several studies described by Marshall et al. (2017) suggested that metformin has positive effects in treating obesity, non-alcoholic fatty liver disease, and metabolic syndrome. Metformin has also been studied in the prevention and treatment of several types of cancer (Evans et al., 2005; Eibl et al., 2021; Zhang et al., 2023). Because of its possible neuroprotective actions, metformin has recently been studied in several neurological incidences, such as traumatic brain injury, as well as diseases, including Parkinson's and Alzheimer's disease in adults (Cao et al., 2022). However, evidence of these recent findings in clinical use is still lacking.

2.3.5 Side effects and precautions

The gastrointestinal side-effects of metformin treatment are common but can be diminished by titrating the dose slowly and taking tablets with food. Long-term

metformin therapy may be associated with B12 vitamin deficiency in patients with type 2 diabetes (Kim et al., 2019). Furthermore, metformin is contraindicated in patients with severe renal impairment (DeFronzo et al., 2015). Metformin should also be avoided in any acute hypoxic state, such as cardiac, lung, kidney, or liver damage; febrile gastroenteritis; and other conditions with dehydration (Di Mauro et al., 2022). To date, as reviewed by Di Mauro et al. (2022), metformin alone is not directly associated with the risk of kidney injury and lactic acidosis. In connection with radiological imaging studies, iodine contrast agents are contraindicated within 48 hours of metformin administration in patients with even slightly impaired renal function.

2.4 Metformin treatment in GDM

Metformin is increasingly used to treat GDM. Practical differences between metformin and insulin treatment are listed in Table 2. Although current evidence suggests a beneficial effect of metformin in the treatment of GDM, the long-term data on the growth, anthropometrics, blood pressure, metabolism, body composition, and neurodevelopment of the offspring of metformin-treated patients with GDM are limited and controversial. Metformin has been documented to cross the placenta, with foetal levels similar to maternal concentrations (Vanky et al., 2005; Tertti et al., 2010). This has caused concerns about the possible influence of the foetal metabolic milieu and long-term metabolic effects in the offspring. However, no risk for teratogenicity has been observed in humans or in animal models when metformin is used from the first trimester of pregnancy onwards (Feig et al., 2007). Furthermore, metformin may be transported across the blood-brain barrier in the foetal brain, as in the adult brain (Cao et al., 2022). Thus, intrauterine metformin exposure might potentially influence the cognitive development of the offspring of mothers with GDM.

Metformin is easy to use and store. According to a study by Rowan et al. (2008), mothers with GDM preferred oral treatment over insulin, with patient satisfaction with metformin use being high (76.2%; Rowan et al., 2008). Metformin is generally effective for glycaemic control in patients with GDM, although some patients require supplemental insulin to achieve glycaemic control. In a recent meta-analysis of eight RCTs of metformin versus insulin for GDM, 3–46% of patients required supplemental insulin (Kattini et al., 2023). The highest count of participants needing combination therapy (46%) was found in a study by Rowan et al., in which this group of patients was found to have distinct baseline characteristics (i.e., higher BMI, glucose levels, and gestational age at diagnosis and a higher proportion of Maori or Pacific Islander Indigenous patients; Rowan et al., 2008).

The current scientific evidence on offspring health following maternal metformin treatment for GDM is presented in the following chapters.

Table 3. Comparison between insulin and metformin in the treatment of GDM.

	INSULIN	METFORMIN
Placental pass	No	Yes
Effectiveness	Effective	Effective, some patients need additional insulin
Risk of hypoglycemia	Yes (minor)	No
Administration	Subcutaneous injection	Oral tablet
Dosing	Several injections daily	2–3 times daily
Effect on maternal weight	Weight gain	No weight gain
Compliance	Poorer	Good
Storage	Cool temperature	No requirements
Cost	Costly	Low-cost

2.5 Studies of metformin versus insulin for GDM

At least seven RCTs on metformin versus insulin treatment for mothers with GDM have been published (Rowan et al., 2008; Ijäs et al., 2010; Niromanesh et al., 2012; Mesdaghinia et al., 2013; Tertti et al., 2013; Spaulonci et al., 2013). These studies aimed to investigate the possible adverse effects of prenatal exposure to metformin on pregnancy, labour, and the neonatal period.

To date, only three of these original randomized controlled trials comparing metformin and insulin treatment for GDM (Rowan et al., 2008; Ijäs et al., 2010; Tertti et al., 2013) have also reported longitudinal offspring outcomes (Table 4). The main findings of these three RCTs are briefly introduced in Table 4 and in the next chapter, concentrating on offspring health. Offspring outcomes after prenatal exposure to metformin have also been studied in two population-based cohort studies (Landi et al., 2019; Brand et al., 2022).

Several meta-analyses and systematic reviews of these RCTs have also been published (Table 5; Tarry-Adkins et al., 2019; Sheng et al., 2023; Kattini et al., 2023). The results of original RCT studies without offspring follow-up data are covered in the meta-analyses (Table 5).

In addition, a few meta-analyses and systematic reviews have concentrated on longer-term safety for the offspring of women who received metformin treatment for GDM (Table 5; Tarry-Adkins et al., 2019). Some of these meta-analyses have also included studies of metformin versus placebo for PCOS pregnancies and studies in which patients had continuous long-term metformin treatment for GDM or type 2 diabetes (Xu et al., 2019, He et al., 2019).

These studies (Tables 4 and 5) are summarized according to the offspring data in the following chapters.

Table 4. Original RCTs comparing metformin and insulin treatment for GDM and subsequent follow-up studies.

ORIGINAL RCTS		**FOLLOW-UP STUDIES**			
Authors, year	Study population, N M: metformin, N I: insulin, N	Authors, year	Study population, n (participation%*) M: metformin group n (%*) I: insulin group, n (%*)	Offspring age	Studied offspring outcome
Rowan et al., 2008	N = 733 women M:363 I:370	Rowan et al., 2011	N = 318 (43 %) Anthropometry: M:154 (42 %) I:164 (44 %) DXA: M: 57(16 %) I: 57 (15 %)	2 years	Body composition
		Battin et al., 2015	N = 170 (23 %) M:83 (23 %) I:87 (24 %)	2 years	Blood pressure
		Wouldes et al., 2016	N = 211 (29 %) M:108 (30 %) I:103 (28 %)	2 years	Neurodevelopmental outcome
		Rowan et al., 2017	N = 109 (15 %) M:58 (16 %) I:51(14 %) N = 99 (14 %) M:45 (12 %) I:54 (15 %)	7 years (Australia) 9 years (New Zealand)	Body composition and metabolic outcomes
Ijäs et al., 2011	N = 100 women M:50 I:50	Ijäs et al., 2015	N = 97 (97 %) M:47 (94 %) I:50 (100 %)	18 months	Growth and development
Tertti et al., 2013	N = 217 women M:110 I:107	Tertti et al., 2015	N = 146 (67 %) M:75 (68 %) I:71 (66 %)	2 years	Neurodevelopment
		Tertti et al., 2016	N = 52 (24 %) M:25 (23 %) I:27 (25 %)	Mean: 60 months (33–85 months)	Testicular size, Anthropometrics (BMI)

* Participation rate is calculated according to the number of participants in the original study.

Table 5. Meta-analyses of RCT and follow-up studies comparing offspring outcomes after metformin vs insulin treatment for GDM.

	NUMBER OF STUDIES (S), COUNTRIES (C), AND OFFSPRING (O)		**MAIN FINDINGS ACCORDING METFORMIN VS INSULIN TREATMENT**			
Authors, year	Neonatal outcomes	Child-hood	Maternal	Neonatal	Childhood	
Kattini et al., 2023	S = 14 C = 11 O = N/A		Maternal weight gain: lower (S = 7)	**Birth weight: lower** **Macrosomia: lower** Pre-term birth: higher (2 studies), lower (1study) **Neonatal hypoglycaemia: less** (4 studies) RDS: no increase NICU admission: no increase		
Sheng et al., 2023	S = 24 C = 10 O = 4355		N/A	**Birth weight: lower** (122.8 g) (S = 22) **Macrosomia: lower** (by 30%) (S = 20) LGA: no increase (S = 12) SGA: no increase (S = 12) **Neonatal hypoglycaemia: 45% lower** (S = 20) **NICU admission: lower** (S = 18) RDS: no increase (S = 14) Abnormal 5 min Apgar: no increase (S = 15) Hyperbilirubinemia: no increase (S = 9) Congenital anomalies: no increase (S = 9) Pre-term birth: no increase (S = 11) Abnormal pH of the umbilical cord: no increase (S=5) Neonatal sepsis: no increase (S = 4)		
Li et al., 2022	S = 50 C = 1 (China) O = 4663		Maternal glycaemic control: better	**Neonatal hypoglycaemia: lower** (S = 21, O = 1872) **RDS: lower** (S = 11, O = 976) **Pre-term birth: lower** (S = 6)		

	NUMBER OF STUDIES (S), COUNTRIES (C), AND OFFSPRING (O)		MAIN FINDINGS ACCORDING METFORMIN VS INSULIN TREATMENT			
Tarry-Adkins et al., 2019	S = 28 C = N/A O = 3976		N/A	**Birth weight: lower** (MD 108 g) (S = 19, O = 3723) **Ponderal index: lower** (S = 3) **Neonatal head circumference: lower** (S = 3) **Macrosomia: lower** (by 40%) (S = 19) **LGA: lower** SGA: no difference	Growth at 18–24 mths. **Weight: heavier** (MD 440g) S = 2, O = 411 Height: no difference	Growth at 5–9 yrs., S = 2, O = 301 **BMI: higher** (by 0.8 kg/m^2) Weight: no sign. difference Height: no difference

Abbreviations: BMI, body mass index; I, insulin; LGA, large for gestational age; M, metformin; NICU, neonatal intensive unit; RDS, respiratory distress syndrome; SGA, small for gestational age.

2.5.1 The short-term influence of prenatal metformin exposure on offspring

Several meta-analyses and systematic reviews of RCTs comparing metformin and insulin treatment for GDM have evaluated the possible effects on the foetus and during the first week after birth (Table 5). According to these meta-analyses, metformin seems to be an acceptable first-line medication when pharmacological treatment is needed.

2.5.1.1 Neonatal safety

Rowan et al. (2008) conducted a 10-center open-label prospective randomised controlled study comparing insulin (n = 370) and metformin (n = 363, of which 46.3% received supplemental insulin) in the treatment of GDM in New Zealand and Australian urban obstetric hospitals. The participants reported that they belonged to one of five different races or ethnic backgrounds. The median daily dose of metformin was 2500 mg (interquartile range, 1750–2500 mg). The baseline maternal and pregnancy characteristics did not differ between the metformin and insulin groups. The primary outcome was a composite of neonatal hypoglycaemia (glucose level < 1.6 mmol/L), respiratory distress syndrome, need for phototherapy, birth trauma, a 5-minute Apgar score less than 7, or prematurity (birth before 37 weeks of gestation). Secondary outcomes included neonatal anthropometric measurements, maternal glycaemic control, maternal hypertensive complications, postpartum glucose tolerance, and acceptability of treatment. No significant differences were found between the two medication groups in neonatal anthropometric measures. Severe hypoglycaemia was less common in the metformin group (p = 0.008), but preterm birth (< 37 weeks of gestation) was more common in the metformin group (p = 0.04). There was a clinically small difference in the mean gestational age at delivery between the metformin group (38.3 weeks) and the insulin group (38.5 weeks, p = 0.02).

Ijäs et al. (2010) conducted a two-centre open-label prospective randomised controlled study comparing insulin (n = 50) and metformin (n = 47, of which 31.9% received supplemental insulin) between June 2005 and June 2009 in the tertiary level Oulu University Hospital and secondary level Kainuu Central Hospital, Kajaani, Finland. The daily dose of metformin was 2250 mg. The baseline maternal and pregnancy characteristics did not differ significantly between the two groups, except that the incidences of both vacuum extraction deliveries and caesarean sections were higher in the metformin group compared with the insulin group (p = 0.041–0.047). The study aimed to examine the effectiveness of metformin in the prevention of foetal macrosomia and neonatal morbidity. They found no differences in the

incidence of large for gestational age, mean birthweight, mean cord artery pH, or neonatal morbidity between the insulin and metformin groups ($p = 0.145–0.976$).

Tertti et al. (2013) conducted a single-centre open-label prospective randomised controlled study comparing insulin (n = 107) and metformin (n = 110, of whom 20.9% received supplemental insulin) between June 2006 and December 2010 in Turku University Central Hospital, Turku, Finland. The median daily dose of metformin was 1500 mg (range 500–2000 mg). The baseline maternal and pregnancy characteristics did not differ between the metformin and insulin groups. The non-inferiority study design was applied in analysing birth weight as the primary outcome variable. No differences were found in mean birth weight expressed in grams (+15 [90% confidence interval (CI): −121 to +89]) or SD units (+0.04 [90% CI: −0.27 to 0.18]) between the metformin and insulin groups or in birth weights above the 90th percentile ($p = 0.80$). There were no differences in neonatal data (i.e., macrosomia, prematurity, Apgar score at 5 minutes, umbilical artery pH, need for neonatal intensive care unit [NICU] transfer, hyperbilirubinemia, or hypoglycaemia) between the groups.

In a recent systematic review of 14 RCTs (from 11 countries), Kattini et al. found equivalent or improved pregnancy outcomes in metformin-treated patients with GDM compared to insulin treatment (Kattini et al., 2023). Maternal weight gain was lower in the metformin group, and similarly, offspring birth weight and the risk of macrosomia, pre-term birth, and neonatal hypoglycaemia were lower compared to maternal insulin treatment. The risks of hyperbilirubinemia, respiratory distress syndrome (RDS), and NICU admission did not differ between the two medication groups (Kattini et al., 2023).

Another recently published systemic review of 24 RCTs (from 10 countries) involving 4,355 participants compared metformin and insulin treatment for GDM. In this review, offspring of the metformin group were found to have a lower birth weight and a lower risk of macrosomia, NICU admission, and neonatal hypoglycaemia (Sheng et al., 2023).

A Chinese meta-analysis comprising 50 Chinese RCTs (n = 4,663 patients) comparing metformin and insulin treatment was conducted to evaluate the efficacy and safety of metformin in the Chinese population with GDM (Li et al., 2022). Li et al. found that metformin was associated with better maternal glycaemic control in pregnancy and lower birth weight, lower risks of preterm birth, RDS, and neonatal hypoglycaemia compared to insulin. The risks of preterm birth, LGA, NICU admission, and neonatal death were not increased in the metformin group compared to insulin treatment (Li et al., 2022). However, the Chinese RCTs were mainly published in Chinese, which renders the evaluation of those studies difficult.

The above-mentioned meta-analyses and systemic reviews of metformin versus insulin treatment for GDM (Table 5) consisted partly of the same RCT studies, but

the combinations of the included studies differed between the analyses. In three of these analyses (Kattini et al., 2023; Sheng et al., 2023; Tarry-Adkins et al., 2019) including only GDM patients, the birth weight and risk of macrosomia were lower in the metformin group compared to the insulin group. One meta-analysis reported PI (from three studies) that was lower in the metformin group (Tarry-Adkins et al., 2019). In one meta-analysis (Li et al., 2022), risk of pre-term birth (reported in six studies) was lower in the metformin group. In the metformin group, the risk of neonatal hypoglycaemia was lower in all four analyses in which this risk was reported (Kattini et al., 2023; Sheng et al., 2023; Li et al., 2022). The risk of NICU admission was lower in one analysis (Sheng et al., 2023), and no increase was found in another analysis by Kattini et al. (2023).

2.5.1.2 Toddler and preschool age safety

Ijäs et al. reported no differences in body weight at 6 months of age (8.28 ± 0.99 kg vs 7.93 ± 0.99 kg; $p = 0.071$), but the children exposed to metformin prenatally were heavier at the age of 12 months (10.47 ± 1.49 kg vs 9.85 ± 1.26 kg; $p = 0.038$), as well as taller (83.9 ± 3.6 cm vs 82.2 ± 3.1 cm; $p = 0.023$) and heavier (12.05 ± 1.87 kg vs 11.32 ± 1.45 kg; $p = 0.04$) at 18 months than those whose mothers were treated with insulin. Results at the age of 18 months remained similar after adjusting for maternal pre-pregnancy BMI. However, the children's body composition, as defined by the PI, did not differ between the treatment groups. Furthermore, the mean weight for length and the proportion of overweight and obese children did not differ between the groups at the ages of 6, 12, and 18 months (Ijäs et al., 2015). Ijäs et al. also studied offspring's motor, social, and linguistic development at the age of 18 months. They utilized the Finnish maternal and child welfare clinics programme, in which the stage of a child's growth and development are evaluated by a general practitioner and/or a nurse who has been specially trained in child health care, achieving a high participation percentage (96%; 93 metformin and 97 insulin). They found no differences in 18-month-old children's development between the two groups (Ijäs et al., 2015).

Tertti et al. (2015) assessed the neurodevelopment of 2-year-old children whose mothers were treated with metformin (n = 75) or insulin (n = 71) for GDM. The development of these children was examined widely using the Bayley Scales of Infant and Toddler Development and the Hammersmith Infant Neurological Examination (Tertti et al. 2015). These standardised measures were used to evaluate neurological, cognitive, communicative, fine motor, and gross motor scores between the two groups. No differences in neurodevelopmental outcomes between the two medication groups were found (Tertti et al., 2015).

In another study by Tertti et al. (2016), the testicular size of 52 boys was assessed at the age of 5 years (33–85 months). The study was conducted after Tartarin et al.

(2012) reported that metformin administration to pregnant mice resulted in a decreased number of Sertoli cells and testicular size in the foetal and neonatal mice's testes. However, Tertti et al. (2016) found no differences between the study groups in terms of the offspring's testicle size or in height, weight, BMI, BMI z-score, or waist-to-hip ratio.

Follow-up studies of a large RCT of metformin versus insulin in the treatment of GDM from Australia and New Zealand were conducted at the age of 2 years (Rowan et al., 2008). Body composition (anthropometry [n =318], bioimpedance [n = 221], DXA [n = 114]), blood pressure, and neurodevelopmental outcomes were measured (Table 3; Rowan et al., 2011; Battin et al.; 2015; Wouldes et al., 2016). None of the measured growth parameters (weight, height, mid-upper-arm or waist circumference, and weight:height ratio) differed between the two treatment groups. However, in the metformin-exposed group, children had larger mid-upper arm circumferences (17.2 ± 1.5 cm vs 16.7 ± 1.5 cm; $p = 0.002$), as well as subscapular (6.3 ± 1.9 mm vs 6.0 ± 1.7 mm; $p = 0.02$) and biceps skinfolds (6.03 ± 1.9 mm vs 5.6 ± 1.7 mm; $p = 0.04$). However, no differences were found between the groups in arm fat by DXA or total fat by DXA or bioimpedance.

Battin et al. (2015) studied blood pressure values in 170 offspring and found no differences in BP between the offspring of the metformin and insulin groups at the age of 2 years.

Wouldes et al. (2016) studied the neurodevelopment of 211 2-year-old offspring using the Bayley Scales of Infant Development, which comprises a mental development index, a psychomotor development index, and a behaviour rating scale. However, only 37% of the original cohort were studied. They reported results separately for participants from New Zealand and Australia because the results on the Bayley Scales of Infant Development differed significantly between the offspring of these two countries. Nevertheless, neurodevelopmental outcomes were similar between the two treatment groups in both countries (Wouldes et al., 2016).

In a recent register-based cohort study from Finland, outcomes of 10,129 children (born 2004–2016) were studied. Their mothers received metformin or insulin treatment during pregnancy for GDM, PCOS, or type 2 diabetes. Of these children, 3,967 were exposed to metformin, 889 were exposed to combination treatment, and 5,273 were born to mothers who were treated with insulin. The median time of follow-up was relatively short in this cohort: 3.5 years (interquartile range [IQR] 1.6–6.4) for those exposed to metformin, 2.4 years (IQR 1.1–4.4) for those exposed to combination treatment, and 5.5 years (IQR 2.8–8.4) for those whose mothers received insulin treatment. Metformin treatment was not associated with increased risk of obesity, hypoglycaemia, hyperglycaemia, diabetes, or challenges in the motor–social development of the offspring at that age. The results remained

similar when the primary outcomes were investigated only among children with maternal gestational diabetes (Brand et al., 2022).

In a British birth cohort, offspring growth trajectories (i.e., weight, height, and BMI z-score) were studied until 60 months of age after maternal metformin (n = 76) or insulin (n = 420) treatment for GDM (Wright et al., 2013; Martine-Edith et al., 2023). Growth was studied at four age stages (0–60 months) and compared between the two medication groups. The study found no differences in growth trajectories during the first five years of life between the offspring of metformin- or insulin-treated mothers (Martine-Edith et al., 2023).

A retrospective population-based cohort study examined 3,928 New Zealand women treated with metformin (n = 1996) or insulin (n = 1932) for GDM (from 2005–2012) and their 4-year-old children to determine long-term outcomes (Landi et al., 2019). They used information gathered by a universal health and development screening programme, which is offered to all children in New Zealand at 4 years of age as routine well-child care prior to school entry at 5 years of age. The Strengths and Difficulties Questionnaire was used to assess a possible difference in behavioural development before entering school between the offspring of the two medication groups. In the study, the proportion of children with concerning scores in the Strengths and Difficulties Questionnaire and prosocial behaviour scores were similar in parent- and teacher ratings between the two groups. However, due to the wide confidence intervals, a potentially increased risk association with one medication could not be completely excluded. No differences between the children of the two medication groups were found in this study regarding growth (i.e., weight, height, BMI) or developmental assessments (Landi et al., 2019).

Neurocognitive, neurological, social, and behavioural development were studied in follow-up studies of RCTs at the age of 18 months (Ijäs et al., 2015) and in 2 years (Tertti et al., 2015; Wouldes et al., 2016). In addition, in population-based cohort studies at the ages of 3 and 5 years (Brand et al., 2022), as well as 4 years (Landi et al., 2019), no difference was found by different assessments between maternal metformin and insulin treatment regarding offspring development in mid-childhood.

2.5.2 The long-term influence of prenatal metformin exposure on offspring

Until now, the only published follow-up study of offspring health reaching school age was conducted on the MiG cohort (Rowan et al., 2018). The follow-up study participants in Australia (n = 109) were studied at the age of 7 years. Mothers of these children reported mostly (over 80%) European or Caucasian ethnicity, while in the original cohort, this ethnicity was represented only in 47% of the mothers. The maternal baseline BMI (measured before 20 weeks' of gestation) was similar in

metformin and insulin groups (31.3 ± 7.8 kg/m^2 vs 31.9 ± 8.3 kg/m^2, $p = 0.72$) in the 7-year subgroup. Rowan et al. found no differences in 7-year-old offspring's fasting plasma glucose concentration, anthropometric variables (i.e., weight, height, BMI, waist, and mid-upper-arm circumference, WHtR) or body composition by DXA or bioimpedance between the metformin and insulin treatment groups. Children of the New Zealand subgroup participated in the follow-up study at the age of 9 years (n = 99). The maternal baseline BMI of this cohort was at booking (before 20 weeks' of gestation) as 31.1 ± 8.8 kg/m^2 in metformin and 29.5 ± 6.4 kg/m^2 in the insulin group and did not differ significantly *(*$p = 0.32$) between the 9-year subgroup. Extensive measures in this subgroup included anthropometry, laboratory investigations (i.e., HbA1c, fasting plasma glucose, insulin, high-density lipoprotein [HDL], LDL, alanine aminotransferase [ALT], leptin, adiponectin, haemoglobin, and ferritin), body composition by DXA, and bioimpedance, together with assays of abdominal fat by magnetic resonance imaging (MRI) and liver fat by magnetic resonance spectroscopy (MRS).

The offspring (n = 45) of the metformin-treated mothers were found to have higher weight (37.0 ± 12.6 kg vs 32.7 ± 7.7 kg; $p = 0.049$), WHtR (69.1 ± 12.2 vs 64.2 ± 8.4; $p = 0.04$), waist (69.1 ± 12.2 cm vs 64.2 ± 8.4 cm; p = 0.04) and mid-upper arm (23.0 ± 4.3 mm vs 21.2 ± 2.9 mm; p = 0.02) circumference, and upper arm fat mass by DXA (1568 ± 801 g vs 1285 ± 534 g; $p = 0.047$).

Based on abdominal MRI, the children in the metformin group had a borderline difference in abdominal fat volume (4172 ± 2964 cm^3 vs 3120 ± 01898 cm^3; $p = 0.051$) and abdominal visceral fat volume (941 ± 629 cm^3 vs 722 ± 365 cm^3; $p = 0.051$). The proportion of abdominal fat volume to total abdominal volume (36.0 ± 14.4% vs 32.2 ± 10.9%; $p = 0.16$) and the percentage of abdominal subcutaneous fat (27.6 ± 12.3% vs 24.4 ± 9.7%; $p = 0.18$) and visceral fat (8.5 ± 3.1% vs 7.7 ± 1.9%; $p = 0.19$) of the total abdominal fat volume were similar between the two study groups.

The only difference in laboratory examinations was the higher ferritin concentration in the metformin group (n = 45) compared to the insulin group (n = 54) offspring (52 [40–70] µg/L vs. 40 [28–59] µg/L; $p = 0.009$). The authors reported that eight children had high ferritin concentrations (100–223.5 µg/L) for which infection could not be excluded.

Six children in this subgroup had signs of early puberty, and after excluding these children, the researchers conducted the analyses again. They found that the weight and arm fat mass measured by DXA were no longer different between the groups. The authors also reported that the 9-year-old study group was ethnically more heterogeneous than the total MiG cohort, but adjustment for ethnicity did not change the results.

In conclusion, the long-term safety of maternal metformin treatment for GDM from the perspective of offspring health has not been thoroughly studied, necessitating further large studies based on original randomized trials.

3 Aims

The main aim of the present study is to assess the possible anthropometric, metabolic, body composition-related, and neurocognitive long-term effects of prenatal metformin exposure on the offspring of women with GDM compared to insulin treatment, especially for factors known to be linked with obesity, type 2 diabetes, and CVD. The age of 9 years, just before the onset of puberty and after completing the second grade of primary school education, was considered the most appropriate age to compare these variables between the offspring of the two treatment groups.

To obtain evidence regarding the possible late effects of foetal exposure to metformin, the specific aims of the study were as follows:

1. To study possible late effects of prenatal metformin exposure on offspring growth and metabolism by comparing anthropometric variables, blood pressure, glucose and lipid metabolism (Study I)
2. To examine possible late effects of prenatal metformin exposure on offspring body composition and adiposity by investigating adipocytokines, markers of low-grade inflammation, volume of visceral adipose tissue, percentage of the liver fat, and regional fat distribution (Study II)
3. To assess the possible long-term effects of prenatal metformin exposure on cognitive and neuropsychological performance in offspring by assessing cognitive development as well as neuropsychological, executive, and academic functions (Study III)

4 Materials and Methods

This study was a longitudinal follow-up study of two previously published Finnish randomized controlled trials with similar study designs, comparing metformin and insulin treatment for GDM (Ijäs et al., 2011; Tertti et al., 2013). These two originally separate trials were combined in a follow-up setting to obtain a larger study population (Figure 1). In the two original trials, 321 women (221 women at Turku University Hospital and 100 women at Oulu University Hospital) with GDM were randomly assigned to receive either metformin (n = 161) or insulin (n = 160) between August 6, 2005 and October 14, 2010. Of these mothers, 314 completed the original trials (Ijäs et al., 2011; Tertti et al., 2013). From these participants, 311 offspring were eligible for this follow-up study.

4.1 Study subjects and design

All mothers of these 311 offspring who were eligible for follow-up studies were contacted and invited for a study visit with their 9-year-old children between 2015 and 2019. A total of 172 children participated in this follow-up study, representing 55% of all the eligible children (n = 311) from the two original RCTs (Figure 1). The participation rate was 59% (127/214) in Turku University Hospital and 46% (45/97) in Oulu University Hospital. Of the participating children, 82 (48%) were born to mothers who were randomly assigned at 17–34 weeks' gestation to treatment with metformin, and 90 (52%) were born to mothers assigned to insulin treatment. In the metformin group, 27% of the mothers (22/82) received additional insulin. The medication was continued until delivery. The population was almost entirely of white ethnicity (99%). In all analyses, children born to mothers originally randomized to receive metformin were handled as one group, including those whose mothers needed additional insulin.

The 9-year follow-up study was conducted at two sites, Turku University Hospital in Southwest Finland and Oulu University Hospital in Northern Finland, between August 2015 and November 2019. Examinations included measurements of growth, body composition, and blood tests for metabolism. Study examinations of the children were arranged during one day in the following order: in the morning, fasting blood samples, oral glucose tolerance tests, anthropometric measurements,

and blood pressure, and after lunch, neuropsychological assessments and radiological imaging studies assessing adiposity (Figure 1). Furthermore, anthropometric data (e.g., height and weight of the mothers) were gathered during the children's study visit. Parental demographic and lifestyle data, along with paternal anthropometric values and the children's lifestyle and school-related information, were collected from the parents before the study visits using questionnaires designed for the purposes of the present study. Additionally, teachers and parents filled out questionnaires about the children's executive functioning before the study visit. Children in this study were in the second or third grade in school. In Finland, basic education begins when a child turns 7 years old, and at the age of 9, they are completing their second or third grade at school.

One child was excluded from the laboratory analyses and body composition assessments because he had been diagnosed with type 1 diabetes. Four children refused blood tests. The OGTT was discontinued in three children because of difficulties drinking the total amount of glucose in two children and vomiting in one child. One child had serum concentration of the high-sensitivity C-reactive protein (hsCRP) above 10 mg/L, and two children had concentrations of interleukin-6 (IL-6) above 10 pg/ml. These concentrations were interpreted as being due to infection and were thus excluded from the respective analyses. DXA assessments were performed only in children followed up in Turku, although one child in Turku did not participate in the DXA study. MRS analysis was successful in 140/171 children; nine children refused to participate in MRI imaging, and for 21 children, MRS was unsuccessful due to technical reasons or the child's poor cooperation. Nine children, two from the metformin group and seven from the insulin group, were excluded from the neuropsychological assessments; one child had attended a similar psychological test less than a year ago, and eight children had Swedish as their school language. Furthermore, the neuropsychological assessment was not completed for three other children due to scheduling reasons, and one child was not able to attend the assessment because of nausea and feeling ill.

4.2 Ethical consideration

Written informed consent was obtained from each mother, child, and father. The assessors were blinded to the treatment allocation of the mothers. This 9-year follow-up study was registered in the Clinical Trials Registry (NCT02417090) and approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK 31/2015, 27 April 2015).

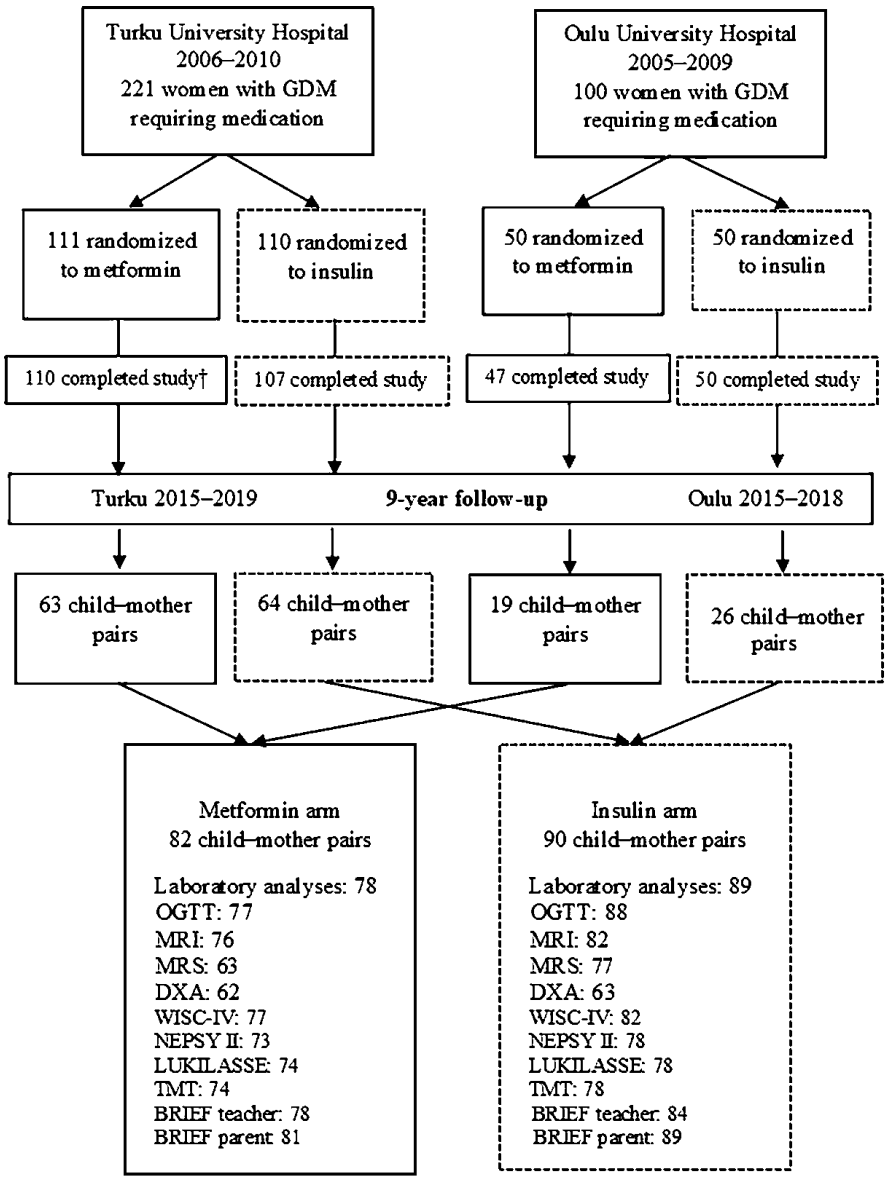


Figure 2. Participants of the two original randomized controlled trials and those of the 9-year follow-up study. † From the 110 participants who completed the original study in the metformin group in Turku, three offspring were excluded: one child had foetal valproate syndrome, one child had Down syndrome, and one child was stillborn. One child had type 1 diabetes and was excluded from laboratory analyses and body composition assessments. Abbreviations: BRIEF, Behavioural Rating Inventory of Executive Functioning; DXA, dual energy X-ray absorptiometry; GDM, gestational diabetes mellitus; Lukilasse 2, Screening Test for Reading, Writing, and Calculus for First to Sixth Grades; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NEPSY II, Developmental Neuropsychological Assessment; OGTT, oral glucose tolerance test; TMT, Trail Making Test; WISC, Wechsler Intelligence Scale for Children. Modified from Figure 1 in Study I, Figure 1 in Study II, and Figure 1 in Study III.

4.3 Clinical examinations

Clinical examinations at both sites were performed using well-established methods and included the measurement of the children's BP, height, weight, and waist circumference, as well as the height and weight of the mothers. BP was measured in a sitting position three times using a non-invasive oscillometric BP monitor (Critikon Dinamap, GE Medical Systems Ltd., London, UK) after a rest of 15 minutes and using the proper cuff size. Then, the mean values of the two final BP measurements were calculated. Height was measured using the Harpenden Stadiometer with a VR High Speed Counter (Holtain Ltd., Crosswell, UK) in a standing position three times, and the mean of the three measurements was calculated. Weight was measured with a Tanita WB-110MA (Tanita Corporation, Tokyo, Japan) digital scale. Waist circumference was measured three times in a standing position with a non-elastic tape (Seca 201, Seca Deutschland, Hamburg, Germany), and then the mean was calculated. Waist circumference was measured at the end of normal exhalation at the horizontal level of the midpoint between the lowest rib and iliac crest. The children wore only underpants during these measurements. The Tanner stage (G1-5, M1-5, P1-5) of pubertal development was assessed by a trained nurse or the study physician. Height was expressed as a standard deviation (SD) score indicating how many SD units a child's height was above or below the average height value according to age and sex (Sorva et al., 1984; Saari et al., 2011). BMI was calculated as weight (kg)/length (m) squared. To express the proportion of normal-weight, overweight, and obese children, we adopted the international cut-off points for BMI for overweight and obesity by sex and age used by Cole et al. (2000). At the age of 9 years, the BMI cut-off point for overweight is 19.10 kg/m^2 for boys and 19.07 kg/m^2 for girls, whereas the BMI cut-off point for obesity is 22.77 kg/m^2 for boys and 22.81 kg/m^2 for girls. In Finland, children's weight is evaluated using an adult equivalent BMI (ISO-BMI) that is calculated for each child with an ISO-BMI calculator (Duodecim, Terveyskirjasto, 2018).

WHtR was calculated, and a cut-off limit of 0.5 was used to describe the risk for mid-body obesity (Browning et al., 2010). The neonatal PI was calculated as birth weight (g) x 100/crown-heel length $(cm)^3$. All maternal and paternal baseline demographic and lifestyle data, as well as paternal height and weight values, were collected using questionnaires.

4.4 Laboratory analyses

Venous blood was collected after an overnight fast. A 2-hour OGTT with insulin and C-peptide determinations was performed on both the children and their mothers. The oral glucose load was 75 g, except for children weighing less than 43 kg, for whom the glucose load was 1.75 g/kg.

The blood samples used for the insulin assay were refrigerated at +4°C to +8°C immediately after sampling. Samples were centrifuged within 30–60 minutes after sampling. Whole blood (EDTA) was frozen as such for subsequent HbA1c analysis. EDTA plasma and serum samples were stored in aliquots at −70°C until further analysis. All blood samples were stored under similar conditions and analysed at the same time in one laboratory

Glucose, lipids, lipoproteins, ALT ferritin, and high sensitivity C-reactive protein levels were determined from the serum samples, while HbA1c was determined from haemolysed whole blood using the following system reagents: Glucose (HK), HDL-Cholesterol Plus, Cholesterol, LDL-Cholesterol, Triglycerides, Apolipoprotein A1, Apolipoprotein B, (ALT/GPT [IFCC]), Ferritin, hsCRP, and HbA1c (all from Thermo Fisher Scientific, Vantaa, Finland) on an Indiko Plus analyser (Thermo Fisher Scientific, Vantaa, Finland). Insulin and C-peptide levels were determined from plasma samples using the LIAISON® Insulin and C-peptide system reagent (DiaSorin S.p.A., Saluggia, Italy) on a LIAISON® immunoassay analyser (DiaSorin Deutschland GmbH, Dietzenbach, Germany). Adiponectin, leptin, and interleukin-6 (IL-6) were assayed from serum samples using Quantikine ELISA Human Total Adiponectin/Acrp30, Quantikine ELISA Human Leptin, and Quantikine HS Human IL-6 kits (all from R&D Systems, Minneapolis, USA). Glycoprotein acetyls (GlycA) were measured as part of a nuclear magnetic resonance metabolomics platform (Nightingale Health, Helsinki, Finland) as described by Soininen et al. (2009).

The calculated inter-assay coefficients of variation for these analyses were as follows: glucose 1.8%, HDL-C 2.7%, cholesterol 2.2%, LDL-C 1.2%, triglycerides 1.1%, ApoA1 1.2%, ApoB 0.5%, HbA1c 3.7%, insulin 3.3%, C-peptide 5.3%, adiponectin 6.0%, leptin 4.3%, ALT 3.7%, ferritin 3.4%, IL-6 3.8%, and hsCRP 5.5%.

Insulin resistance was calculated using a homeostatic model assessment based on fasting serum glucose and plasma insulin (HOMA-IR = glucose [mmol/L] x insulin [mU/L]/22.5; Wallace et al., 2004). The hsCRP concentrations were measured with a detection limit of 0.25 mg/L, and serum concentrations below this detection limit (n = 21) were set to 0.24 mg/L for the calculations. Serum concentrations above 10 mg/L for hsCRP (n = 1) and above 10 pg/ml for IL-6 were interpreted as being due to infection. For IL-6, we used values < 1.5 pg/mL as normal for healthy 9-year-old children (De Filippo et al., 2015; Tam et al., 2010).

4.5 Body composition

4.5.1 MRI and MRS acquisition

MRI and MRS scans were performed using a Siemens MAGNETOM Sola Fit 1.5 T MRI system (Siemens Healthcare, Erlangen, Germany) in Turku University Hospital and a Siemens MAGNETOM Aera 1.5 T MRI system (Siemens Healthcare, Erlangen, Germany) in Oulu University Hospital. A similar scan protocol was used at both sites. First, a two-point Dixon scan (see Study II online supplementary Table 1 for parameters) was performed on the thighs, and another one on the pelvic area. Then, the abdominal and thorax areas were scanned with respiration-compensated two-point Dixon sequences. Finally, single-voxel proton MRS was performed to determine the liver fat content. The parameters of the MRS sequence are listed in the Study II online Supplementary Table. To position the spectroscopic voxels in the liver tissue, three orthogonal views of the liver were produced with T2 HASTE sequences. In sum, the duration of the MRI and MRS scanning session was 30 minutes.

4.5.2 Visceral adipose tissue segmentation

For every child, the separate water and fat images produced by the Dixon sequences were combined into single water and fat images covering the entire abdominal cavity. This was achieved using Osirix (version 6.0.2, Pixmeo SARL, Bernex, Switzerland) software. Fat fraction maps (Bray et al., 2018) of the visceral area were constructed from the water and fat images by performing the following calculation for each voxel in Vinci (version 4.9) software (Vollmar et al., 2007): $F/(W + F)$, where F is the signal intensity of the fat image and W is the signal intensity of the water image. Thus, each voxel in the fat fraction map represents the fraction of fat signal intensity in relation to the signal intensity of both water and fat. Carimas (version 2.9, PET Centre, Turku University Hospital, Finland) software was used to define VAT volume from fat fraction maps. The VAT was segmented as follows: two-dimensional regions of interest (ROI) covering the VAT area were drawn on every six sagittal slices; typically, 22 ROIs were drawn for each subject. Then, a three-dimensional volume of interest covering the VAT region was constructed from the ROIs using the interpolation feature of the Carimas software. Artifacts or bright areas inside the gastrointestinal tract (stool and air) were segmented correspondingly and excluded from the volume of interest. Finally, the voxels with an intensity value above 0.6 (fat fraction over 60%) within the volume of interest were considered to represent VAT (Fig. 3). The segmentation of the VAT area was defined with anatomical landmarks for consistency: the most superior slice was chosen so that the

diaphragm formed a unified pattern and epicardial fat remained above the area. The most inferior slice was chosen on top of the S1 vertebra. The fat behind vertebral bodies was left outside of the ROI. Segmentations were generated by the author (EP), and 15% of the masks were validated by a senior radiologist (RP) with 32 years of experience with MRI. On average, constructing the segmentation for each patient took 1.5 hours. To further illustrate possible differences in the total VAT volumes between the two medication groups, the median VAT of all study offspring (250 cm^3) was used as a cut-off. To our knowledge, no reliable VAT reference values for 9-year-old children have been reported.

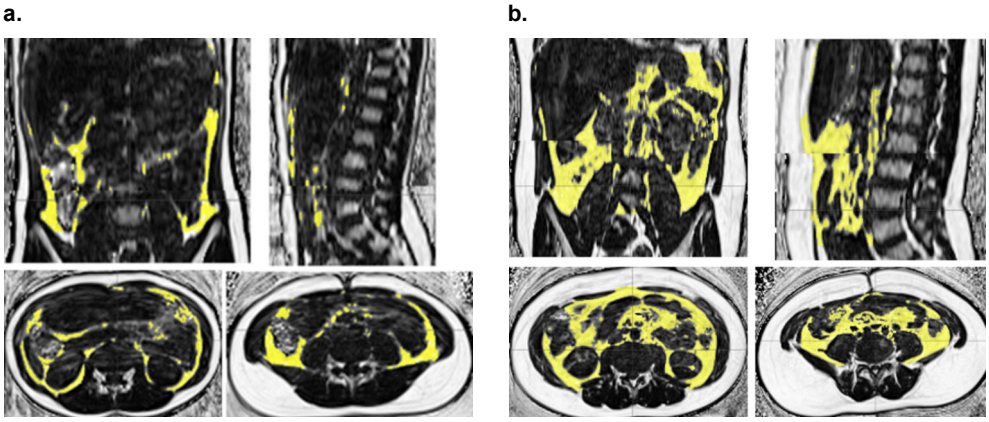


Figure 3. A 9-year-old child with low (**a.**) or high (**b.**) visceral adipose tissue (VAT). The yellow colour represents VAT (that is fat fraction over 60%) in the abdominal cavity. The axial slice on the left side is from the L2–L3 level, and the axial slice on the right side is from the L4–L5 level. Figure 3.a. The VAT volume is 195 cm^3 (BMI 17.6 kg/m^2, WHtR 0.43). Figure 3.b. The VAT volume is 1048 cm^3 (BMI 22.6 kg/m^2, WHtR 0.50). Abbreviations: BMI, body mass index; VAT, visceral adipose tissue; WHtR, waist-to-height ratio. According to Paavilainen et al., 2023. Reprinted from Diabetes Res Clin Pract. 2023 Aug;202:110780. Copyright 2023, with permission from Elsevier.

4.5.3 MRS data analysis

Liver MRS data were exported from the MR system in Siemens.rda-format and quantified using LCModel software (Version 6.3–1 N; Provencher, 1993). To determine a composite lipid signal amplitude, the lipid signal amplitudes from 0.9–2.8 ppm were summed, and the contribution of the lipid signals residing under the water peak was corrected by multiplying the sum by 1.086 (Hamilton et al., 2011). Water and the composite lipid signal amplitudes were corrected for T2 relaxation using the T2 times determined by Hamilton et al. (2009). The T1 correction was not applied since the TR time of 3000 ms was considered long enough to render the correction unnecessary (Qayyum et al., 2009; Leporq et al., 2013). The fat fraction

was calculated by dividing the corrected composite lipid signal amplitude by the sum of the corrected water and composite lipid signal amplitudes. We considered a liver fat level < 5% as normal in MRS since Di Martino et al. (2016) showed that 5% is a threshold value between healthy children and those with non-alcoholic fatty liver disease (Di Martino et al., 2016).

4.5.4 DXA

DXA assessments were performed only for the participants at the Turku University Hospital. Whole-body DXA for regional and total body fat, fat-free mass, and percent body fat was performed using the Discovery A System (Hologic, 123 Waltham, MA, USA) with standard imaging and positioning protocols. All metal items were removed before densitometry, and the children were examined wearing only underwear and a cloth gown. Android fat, gynoid fat, and the android/gynoid fat ratio as determined by DXA were reported because these values have been found to highly correlate to risk factors for both metabolic and cardiovascular diseases in normal-weight and overweight boys (Samsell et al., 2014; Lätt et al., 2018). To report more clinically relevant height-normalized indexes, fat mass index (FMI) and fat-free mass index (FFMI) were calculated as fat-free mass or fat mass divided by height squared, respectively (Shypailo et al., 2020).

4.6 Cognitive and neuropsychological assessments

For the purposes of this study, the neuropsychological test battery was designed to cover essential functions of development and school performance in 9-year-old children. The neuropsychological assessments were performed in Finnish. Under the guidance of an experienced psychologist, two psychologists and three final-stage psychology students conducted the assessments over a 4-year period.

4.6.1 Cognitive development

The children's cognitive development was assessed using the Finnish translation of the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler, 2011). FSIQ, which was used as a measure of general intelligence, comprises four indexes derived from 10 subtests. The Verbal Comprehension Index measures the verbal reasoning ability and acquired knowledge; the Perceptual Reasoning Index measures perceptual organization and logical reasoning; the Working Memory Index measures working memory and attention; and the Processing Speed Index measures the speed of mental and fine-motor processing. WISC-IV indexes were calculated

according to age-appropriate Finnish norms (mean 100, SD 15; Wechsler, 2011) and used as a continuous variable. Based on clinical significance, a cut-off was set to < 85 points (-1 SD) on the WISC-IV indexes to identify children whose results were at least slightly below normal (Wechsler, 2011). The FSIQ results were divided into three categories based on the cut-off levels of +1 and –1 SD (< 85, 85–115, > 115 std points) to increase understanding about the distribution of FSIQ results between the two groups. These three categories describe the proportion of children who have increased risk for difficulties (< –1 SD) in school performance or daily life and children who perform at least slightly above average (> +1 SD).

4.6.2 Neuropsychological performance

Language functions were assessed using the Developmental Neuropsychological Assessment (NEPSY II; Korkman et al., 2007, 2008) subtest called Comprehension of Instructions, which assessed the ability to receive and process oral instructions of increasing complexity.

Memory functions were assessed using the NEPSY II (Korkman et al., 2007, 2008) subtest called Narrative Memory, which evaluated memory for logical verbal stories under free and cued recall. NEPSY II scores were based on age-appropriate Finnish norms (mean 10, SD 3; Korkman et al., 2007, 2008) and used as a continuous variable. A cut-off was set to < 8 standard scores (–1 SD) to identify results at least slightly below normal (Korkman et al., 2007, 2008).

Attention regulation was assessed using the Trail Making Test (TMT) for children (Poutiainen et al., 2010), which consists of two parts: TMT A, in which the respondent is asked to connect randomly arranged circles containing numbers, requires visual tracking and simple set-sifting. TMT B, in which the respondent must alternate between numbers and letters, requires visual tracking and complex set-sifting. The time in minutes needed to complete each part as quickly as possible was used as a measure of performance and as a continuous variable.

Executive functions in daily life were assessed using both the teacher and parent forms of the Finnish translation of the Behaviour Rating Inventory of Executive Function (BRIEF; Gioia et al., 2000). BRIEF includes 86 items with a three-point Likert scale, and these items consist of eight subscales that form two indexes. The Behavioural Regulation Index is a composite score of inhibit (ability to resist impulse), shift (making transitions between tasks and mindsets), and emotional control (regulation of emotional responses). The Metacognition Index is a composite score of initiate (beginning an activity independently), working memory (holding information to complete a task), plan/organize (planning and organizing ahead for future events), organization of materials (sorting and organizing things), and monitor (assessing one's own performance for proper goal attainment). The Global Executive

Composite Score combines the Behavioural Regulation Index and Metacognition Index. The age- and gender-specific standardised T-scores on the subscales and index scores were used to measure outcomes (Gioia et al., 2000) and as a continuous variable. A pre-established cut-off T-score > 64 was used to indicate clinically significant symptoms (Gioia et al., 2000). Only consistently filled-in questionnaires were used in the analyses (Gioia et al., 2000).

4.6.3 Academic functioning

Reading fluency was assessed using a subtest of the Screening Test for Reading, Writing, and Calculus for First to Sixth Grades (Lukilasse 2; Häyrinen et al., 2013). For this test, the study participants read as many words as possible from the word list in 2 minutes, and correctly read words were counted (Häyrinen et al., 2013). Standard scores at or below –1.34 SD were considered to be below grade level (Häyrinen et al., 2013).

School-related information was collected from the parents, including the level of educational support received by each child. Educational support in Finland is divided into three levels: general, intensified, and special support. All students are covered by general support. Intensified support means part-time special education in a specific area, such as literacy or mathematics, while special support means full-time special education intended for children with a long-term need for support. Special support mainly includes individualized education plans in one or several subjects.

4.7 Statistical methods

Statistical power calculations were performed for two endpoints: offspring BMI and fasting serum glucose level at the age of 9 years. The mean (SD) BMI was assumed to be 18 (2.5) kg/m^2, and the mean (SD) fasting glucose level was assumed to be 4.8 (0.2) mmol/L, based on average childhood values. A two-sided test with a power of 80% and a significance level of 0.05 would, therefore, detect a 1.0 kg/m^2 mean difference in BMI between the 110 metformin and 110 insulin group subjects (71% of the combined cohort). Similarly, a two-sided test with a power of 80% and a significance level of 0.05 would detect a 0.12 mmol/L difference in the fasting serum glucose level.

Due to the relatively small number of participating children in both study groups, post-hoc power analysis was performed to evaluate the reliability of the WISC-IV results. Assuming at least non-inferiority between the study groups, the required sample sizes were calculated to attain 80% statistical power at a 95% significance level with 10 points as a non-inferiority margin using the observed group means and pooled standard deviation. We chose a 10-point difference because we were

interested in a clinically significant difference that might impact the children's performance. In this setup, we found that a sufficient total sample size to assess non-inferiority was 95 subjects for FSIQ and 216 subjects for the Perceptual Reasoning Index. This analysis was performed using R: A Language and Environment for Statistical Computing, version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria) and the epiR package, version 2.0.57 (EpiR).

The Kolmogorov-Smirnov test was used to analyse whether the variables were normally distributed, and the Shapiro-Wilk test was used to test the normality of the subgroups of boys and girls ($n < 50$). Between-group comparisons were performed using the student's t-test for normally distributed data and the Mann-Whitney U-test for skewed data, and the results were expressed as means ± SD or medians (interquartile range [IQR]). For categorical variables, the chi-square test was used for normally distributed data, and the Fisher's exact test was used for skewed data. Potential differences in boys and girls between the treatment groups were explored using subgroup analysis. Most variables were not normally distributed in the subgroups. Spearman's correlation coefficients were determined to estimate the correlation between HDL concentration and adiponectin concentration. The IBM SPSS Statistics version 25.0 (Study 1), 26.0 (Study 2), and 27.0 (Study 3) statistical software package (IBM Corp., Armonk, NY, USA) was used, and a p-value < 0.05 was chosen to indicate statistical significance.

5 Results

5.1 Baseline maternal and neonatal characteristics and parental characteristics at 9-year follow-up

Comparison of maternal and neonatal baseline characteristics between the participants and non-participants showed that the group of participants did not differ from the non-participants of the 9-year follow-up study (Table 6.a). Additionally, no differences in baseline data were found between the metformin and insulin groups in participants of the 9-year follow-up study (Table 6.b).

Table 6.a. Baseline maternal and neonatal characteristics of the participants of the 9-year follow-up study and the group of non-participants in the study of GDM treatment (n = 311).

	PARTICIPANTS N = 172	NON-PARTICIPANTS N = 139	*P*-VALUE
Insulin / Metformin	90(52.3) / 82(47.7)	67(48.2) / 72(51.8)	0.47
Maternal			
Age at randomization (years)	33.0 (29.0–36.0)	32.0 (27.0–35.0)	0.06
Smoking in pregnancy	21 (12.4)	24 (17.4)	0.21
BMI (kg/m²), 1st antenatal visit	28.8 (25.0–33.0)	29.0 (26.0–33.0)	0.13
Normal weight (BMI < 25.0 kg/m²)	31 (18.0)	20 (14.4)	0.53
Overweight (BMI 25.0–29.9 kg/m²)	68 (39.5)	52 (37.4)	
Obese (BMI ≥ 30.0 kg/m²)	73 (42.4)	67 (48.2)	
Total weight gain during pregnancy (kg)	8.51 ± 4.94	7.98 ± 5.24	0.37‡
Gestational weeks at randomization	30.6 (29.3–31.9) [16.7, 34.3]	30.4 (28.9–32.1) [19.0, 34.0]	0.41
75g OGTT results at enrollment			
fasting plasma glucose (mmol/L)	5.4 (5.1–5.7)	5.5 (5.2–5.9)	0.12
2-h plasma glucose (mmol/L)	8.1 (7.0–9.4)	7.8 (6.5–9.2)	0.28
HbA1c at randomization (mmol/mol)	38 (36–40)	38 (36–41)	0.13
HbA1c at 36 gestational weeks (mmol/mol)	39 (36–41)	39 (37–42)	0.11
Duration of medication (weeks)	8.5 (7.0–10.6)	8.7 (7.0–10.9)	0.41
Gestational weeks at birth	39.1 (38.4–40.1)	39.1 (38.1–40.1)	0.56
Prematurity (delivery < 37 gestational weeks)	10 (5.8)	5 (3.6)	0.36
Caesarean delivery	37 (21.5)	25 (18.0)	0.44
Neonatal			
Boy/Girl (n)	85/87	68/71	0.93
Birth weight (g)	3608 (3305–3930)	3670 (3390–3930)	0.51
Birth weight (SD)	0.12 ± 1.12	0.25 ± 1.18	0.32‡
Birth weight < -2 SD	4 (2.3)	4 (2.9)	0.98
Birth weight > 2 SD	9 (5.2)	7 (5.0)	
Crown-heel length (cm)	50.8 ± 2.20	51.0 ± 1.98	0.45‡
Crown-heel length (SD)	0.43 ± 1.12	0.57 ± 1.03	0.26‡
Ponderal index (g/cm³)	2.73 ± 0.27	2.73 ± 0.26	1.00‡
Apgar score at 1 minutes	9.0 (9.0–9.0)	9.0 (8.0–9.0)	0.89
Apgar score at 5 minutes	9.0 (9.0–9.0)	9.0 (9.0–9.0)	0.83
Apgar score at 15 minutes	9.0 (9.0–10.0)	9.0 (9.0–9.0)	0.41
Umbilical artery pH	7.28 (7.22–7.33)	7.27 (7.22–7.33)	0.69
Hypoglycaemia*	35 (20.3)	29 (20.9)	0.91

Data are expressed as n (%), [min, max], mean ± SD or median (IQR). T-test (‡) or Mann-Whitney U-test was used for continuous and Chi-square or Fisher's exact test for categorical variables. Abbreviations: BMI, body mass index, OGTT, oral glucose tolerance test. Ponderal index = birth weight (g) x 100/crown-heel length (cm)³. *Need for intravenous glucose. Modified from Supplemental Table S2 in Study I and Supplemental Table S1 in Study III.

Table 6.b. Baseline maternal and neonatal characteristics of the participants of the 9-year follow-up study (n = 172). Comparison between groups treated with metformin or insulin for GDM.

	METFORMIN N = 82	INSULIN N = 90	*P*-VALUE
Maternal			
Age at randomization (years)	33.5 (29.0–36.0)	33.0 (29.0–36.0)	0.80
Smoking in pregnancy	6 (7.4)	15 (16.9)	0.06
BMI (kg/m²), 1st antenatal visit	29.0 (25.0–33.0)	28.0 (25.5–33.0)	0.68
Normal weight (BMI < 25.0 kg/m²)	18 (22.0)	13 (14.4)	0.12
Overweight (BMI 25.0–29.9 kg/m²)	26 (31.7)	42 (46.7)	
Obese (BMI ≥ 30.0 kg/m²)	38 (46.3)	35 (38.9)	
Total weight gain (kg) during pregnancy	8.4 ± 4.62	8.6 ± 5.24	0.85‡
Gestational weeks at randomization	30.4 (29.4–31.9) [16.7, 34.0]	30.6 (29.3–32.0) [20.3, 34.0]	0.66
75g OGTT results at enrollment			
fasting plasma glucose (mmol/L)	5.4 (5.0–5.7)	5.4 (5.2–5.8)	0.28
2-h plasma glucose (mmol/L)	8.4 ± 1.93	8.1 ± 1.79	0.36‡
HbA1c at randomization (mmol/mol)	38 ± 4	38 ± 4	0.59‡
HbA1c at 36 gestational weeks (mmol/mol)	38 ± 4	39 ± 4	0.56‡
Duration of medication (weeks)	8.6 (7.0–10.6) [4.3, 22.2]	8.4 (6.8–10.6) [2.0, 18.7]	0.69
Gestational weeks at birth (weeks)	39.0 (38.4–40.1)	39.1 (38.4–40.3)	0.70
Prematurity (delivery < 37 gestational wks)	6 (7.3)	4 (4.4)	0.42
Cesarean delivery	18 (22.0)	19 (21.1)	0.89
Neonatal			
Boy/Girl (n)	42/40	43/47	0.65
Birth weight (g)	3611 ± 489	3576 ± 519	0.65‡
Birth weight (SD)	0.15 ± 1.08	0.09 ± 1.16	0.71‡
Birth weight < -2 SD	3 (3.7)	1 (1.1)	0.41
Birth weight > 2 SD	5 (6.1)	4 (4.4)	
Crown-heel length (cm)	51.0 (50.0–52.0)	51.0 (49.5–52.0)	0.54
Crown-heel length (SD)	0.45 (-0.2–1.2)	0.40 (-0.2–1.2)	0.77
Ponderal index (g/cm³)	2.73 ± 0.26	2.73 ± 0.28	0.94‡
Apgar score at 1minutes	9.0 (9.0–9.0)	9.0 (8.0–9.0)	0.22
Apgar score at 5minutes	9.0 (9.0–9.0)	9.0 (9.0–9.0)	0.87
Apgar score at 15 minutes	9.0 (9.0–10.0)	9.0 (9.0–10.0)	0.56
Umbilical artery pH	7.27 ± 0.09	7.27 ± 0.08	0.92‡
Hypoglycemia*	18 (22.0)	17 (18.9)	0.62

Data are expressed as n (%), [min, max], mean ± SD or median (IQR). T-test (‡) or Mann-Whitney U-test was used for continuous and Chi-square or Fisher's exact test for categorical variables. Abbreviations: BMI, body mass index; OGTT, oral glucose tolerance test. Ponderal index = birth weight (g) x 100/crown-heel length (cm)³. *Need for intravenous glucose. Modified from Supplemental Table S3 in Study I and Supplemental Table S2 in Study III.

Furthermore, parental characteristics at the 9-year time point were similar between the two treatment groups (see Study I for details). Most of the mothers of these 9-year-old children were overweight or obese, and only 15% of the mothers and 25% of the fathers had a normal BMI (Table 7). In the Finnish population, 41% of women and 27% of men in the same age range (40–49 years) have a normal weight (Lundqvist et al., 2018). The 9-year overall prevalence of type 2 diabetes was 14% (24/172) in the mothers, 13% (11/82) in the metformin group, and 14% (13/90) in the insulin group.

Parents reported that five children (3.1%) had a diagnosis that could potentially affect their learning at school; two children in both medication groups were diagnosed with attention and hyperactivity disorder (ADHD), and one child in the metformin group was diagnosed with a developmental language disorder.

Table 7. Proportion of normal weight and overweight or obese mothers and fathers in the 9-year follow-up study and in the Finnish population at the same age group.

	MOTHERS PARTICIPATING THE STUDY	FINNISH WOMEN (40–49 YEARS)	FATHERS PARTICIPATING THE STUDY	FINNISH MEN (40–49 YEARS)
Normal weight (BMI < 25 kg/m^2)	15%	41%	25%	27%
Overweight or obese (BMI ≥ 25 kg/m^2)	85%	59%	75%	73%

5.2 Anthropometry, growth, and blood pressure (Study I)

At the 9-year assessment, no significant differences were found between the metformin and insulin groups in terms of the offspring's age, weight, height, BMI, proportions of overweight or obese children, waist circumference, WHtR, distribution of WHtR over 0.5, or systolic or diastolic BP (Table 8). In the total cohort of children (n = 179), a tendency towards lower WHtR values (0.43 [0.4–0.5] vs 0.45 [0.4–0.50]; $p = 0.06$) was found in the boys of the metformin group compared to those of the insulin group (see Study I for details). When we studied only those children who had laboratory analyses (n = 167) available, the difference in WHtR between the metformin and insulin group boys was more apparent ($p = 0.032$, Table 8). No differences were found between the medication groups in the proportion of overweight or obese children when overweight/obesity was defined using international age- and sex-specific adjusted BMI cut-off points, according to Cole et al. (2000).

Table 8. Offspring age, height, weight, BMI, proportions of overweight or obese children, waist circumference, WHtR, distribution of WHtR over 0.5, and systolic and diastolic BP. Children whose laboratory results have been obtained are included (n = 167).

	ALL CHILDREN			**BOYS**			**GIRLS**		
	Metformin n = 78	Insulin n = 89	*p*-value	Metformin n = 39	Insulin n = 43	*p*-value	Metformin n = 39	Insulin n = 46	*p*-value
Age (years)	9.0 (9.0–9.1)	9.0 (9.0–9.1)	0.45†	9.0 (9.0–9.1)	9.0 (9.0–9.1)	0.31†	9.1 (9.0–9.1)	9.1 (9.0–9.1)	0.98†
Height (cm)	136.6 ± 6.0	136.3 ± 6.7	0.77	137.4 ± 6.1	137.7 ± 7.5	0.80	135.9 ± 5.9	135.0 ± 5.6	0.49
Relative height (SD)	0.10 ± 1.1	0.08 ± 1.1	0.93	0.12±1.1	0.24±1.3	0.66	0.07 ± 1.0	-0.06 ± 0.9	0.51
Weight (kg)	32.6 (29.7–37.7)	32.6 (28.5–40.3)	0.87†	32.0 (29.3–38.2)	33.5 (28.4–40.4)	0.56†	33.5 (30.0–37.3)	32.0 (28.6–39.5)	0.40†
BMI (kg/m²)	17.5 (16.3–19.3)	17.6 (16.1–20.1)	0.83†	17.3 (16.2–18.6)	17.8 (16.2–20.8)	0.39†	18.0 (17.0–19.6)	17.6 (16.0–20.0)	0.65†
Normal weight (adjusted BMI < 25.0)§	58 (74.4)	60 (67.4)	0.33	31 (79.5)	31 (72.1)	0.44	27 (69.2)	29 (63.0)	0.55
Overweight or obese (adjusted BMI ≥ 25.0)§	20 (25.6)	29 (32.6)		8 (20.5)	12 (27.9)		12 (30.8)	17 (37.0)	
Waist circumference (cm)	59.3 (56.3–64.2)	59.6 (55.8–66.7)	0.57†	59.2 (56.1–63.6)	61.5 (58.2–69.1)	0.12†	59.8 (56.3–65.0)	58.2 (54.8–65.5)	0.56†
Waist:height ratio	0.43 (0.41–0.47)	0.44 (0.43–0.49)	0.19	**0.43 (0.41–0.47)**	**0.44 (0.43–0.49)**	**0.032†**	0.44 (0.41–0.47)	0.44 (0.42–0.47)	0.77
Waist:height ratio < 0.5	67 (85.9)	76 (85.4)	0.93	35 (89.7)	36 (83.7)	0.42	32 (82.1)	40 (87.0)	0.53
Waist:height ratio > 0.5	11 (14.1)	13 (14.6)		4 (10.3)	7 (16.3)		7 (17.1)	6 (13.0)	
BP systolic (mmHg)	105.4 ± 9.2	104.6 ± 9.7	0.58	105.7 ± 8.7	106.9 ± 9.9	0.57	105.1 ± 9.7	102.5 ± 9.2	0.20
diastolic BP(mmHg)	59.2 ± 7.3	60.2 ± 7.6	0.39	59.8 ± 7.4	61.8 ± 7.6	0.22†	58.6 ± 7.2	58.7 ± 7.4	0.97

Data are expressed as mean ± SD, median (IQR) or n (%). T-test or Mann-Whitney U-test (†) was used for continuous variables and Chi-square test for categorial variables. § According Cole et al. (2000). Abbreviations: OGTT, oral glucose tolerance test. Modified from Table 1 in Study I.

Some, although statistically insignificant, differences were seen in the proportion of normal weight, overweight, and obese children between the two medication groups in terms of boys and girls when obesity/overweight was defined by ISO-BMI. These ISO-BMI percentages are presented in Figure 4 along with Finnish reference values (age 7–12 years; Lundqvist et al., 2018).

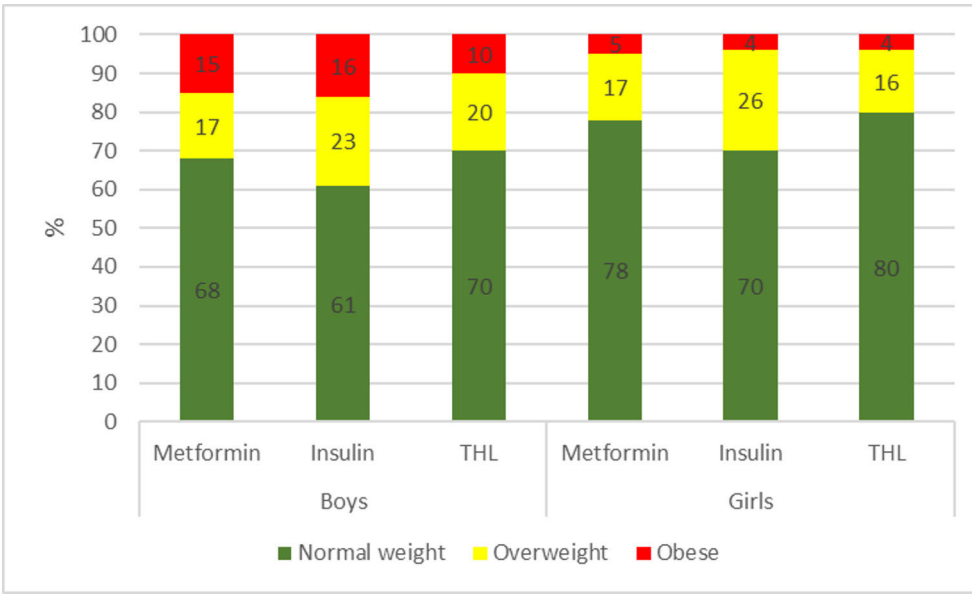


Figure 4. Proportion of normal weight, overweight, and obese boys and girls in the study and of Finnish reference values (Lundqvist et al., 2018) defined by adult-equivalent body mass index (ISO-BMI). Abbreviations: THL, Institute for Health and Welfare, Finland.

5.3 Glucose metabolism (Study I)

Fasting serum glucose concentrations were similar in the two treatment groups (Study I). The glucose values collected from the OGTT were within the normal reference range in both sexes, but the two-hour glucose value was 0.6 mmol/L lower ($p = 0.015$) in the boys of the metformin group (5.3 ± 1.02 mmol/L) than in the boys of the insulin group (5.9 ± 1.12 mmol/L; Table 9). Serum insulin and c-peptide concentrations in OGTT, HbA1c, and insulin resistance assessed by HOMA-IR were similar in the two treatment groups, as well as the subgroups of boys and girls (Table 9).

Table 9. Serum glucose, plasma insulin, and plasma c-peptide concentrations in OGTT, HbA1C values, and insulin resistance measured by homeostatic model assessment (HOMA-IR) in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			BOYS			GIRLS		
OGTT:	Metformin n = 78	Insulin n = 89	*p*-value	Metformin n = 39	Insulin n = 43	*p*-value	Metformin n = 39	Insulin n = 46	*p*-value
Fasting Glucose (mmol/L)	5.0 (4.8–5.0)	5.1 (4.8–5.3)	0.32	5.0 (4.8–5.3)	5.2 (4.9–5.4)	0.13	5.0 (4.8–5.2)	4.9 (4.8–5.3)	0.96‡
0.5h-Glucose (mmol/L)	8.3 ± 1.44	8.5 ± 1.67	0.54‡	8.4 ± 1.78	8.4 ± 1.57‡	0.92	8.2 ± 1.00	8.5 ± 1.78	0.40‡
2-h Glucose (mmol/L)	5.5 ± 1.08	5.7 ± 0.98	0.23‡	**5.3 ± 1.02**	**5.9 ± 1.12**	**0.015**	5.8 ± 1.09	5.6 ± 0.83	0.32‡
Fasting Insulin (mU/L)	7.5 (4.8–12.1)	8.5 (5.3–13.6)	0.52	6.6 (4.5–10.6)	7.2 (4.4–13.0)	0.57	9.0 (5.6–13.0)	9.5 (5.7–14.9)	0.76
0.5-h Insulin (mU/L)	57.4 (43.0–84.3)	61.7 (38.0–88.4)	0.93	54.2 (43.9–75.8)	61.6 (36.6–79.2)	0.86	57.9 (42.9–90.5)	63.2 (47.5–101.6)	0.79
2-h Insulin (mU/L)	29.8 (20.2–44.3)	31.8 (20.8–47.4)	0.62	25.9 (14.4–36.6)	29.1 (20.0–38.3)	0.16	37.9 (28.4–61.4)	33.4 (25.9–52.6)	0.47
Fasting C-peptide (ng/mL)	1.17 (1.0–1.6)	1.23 (0.9–1.7)	0.69	1.16 (0.9–1.4)	1.23 (0.9–1.7)	0.41	1.17 (1.0–1.7)	1.22 (0.9–1.7)	0.85
0.5-h C-peptide (ng/mL)	5.03 (3.9–7.1)	5.51 (4.0–6.7)	0.93	4.95 (3.9–6.8)	5.27 (3.7–6.7)	0.90‡	5.08 (4.3–7.3)	5.58 (4.5–7.2)	0.88
2-h C-peptide (ng/mL)	4.44 (3.3–6.0)	4.55 (3.3–6.0)	0.91	4.12 (3.0–5.1)	4.49 (3.5–5.7)	0.13	5.55 (4.0–6.9)	4.55 (3.3–6.3)	0.20
HbA1c (mmol/mol)	36.2 (34.5–37.6)	35.7 (34.7–37.6)	0.40	36.7 (34.7–37.6)	36.7 (34.5–37.6)	0.84	35.7 (33.8–37.1)	35.7 (35.7–37.6)	0.32
HOMA-IR	1.71 (1.0–2.7)	1.86 (1.1–3.1)	0.45	1.55 (0.9–2.5)	1.73 (1.0–3.0)	0.51	1.92 (1.2–2.8)	2.06 (1.2–3.2)	0.70

Data are expressed as mean ± SD, median (IQR), or n (%). T-test (‡) and Mann-Whitney U-test were used. Abbreviations: HbA1C, glycated haemoglobin; HOMA-IR, homeostatic model assessment of β-cell function and insulin resistance; OGTT, oral glucose tolerance test. Modified from Table 2 in Study I.

5.4 Lipid metabolism (Study I) and adipocytokines (Study II)

The offspring of the metformin group had more favourable lipid and adipocytokine profiles than the offspring of the insulin group (Table 10). That is, their median HDL cholesterol concentration (1.72 [1.5–1.9] mmol/L vs 1.54 [1.4–1.8] mmol/L; $p = 0.039$) and median adiponectin concentration (10.37 [8.7–14.5] µg/ml vs 9.50 [6.8–12.0] µg/ml, $p = 0.016$) were higher, whereas their median LDL cholesterol (2.39 [2.1–2.8] mmol/L vs 2.58 [2.2–2.9] mmol/L; $p = 0.046$) and apolipoprotein B (0.63 [0.6–0.7] g/L vs 0.67 [0.6–0.8] g/L; $p = 0.043$) concentrations were lower than those of the children in the insulin group. In a detailed analysis, differences in the mean serum HDL cholesterol and mean serum adiponectin concentrations were evident only in the boys (1.85 [1.6–2.0] mmol/L vs 1.54 [1.5–1.8] mmol/L; $p = 0.003$ and 12.13 [9.7–14.7] µg/ml vs 7.50 [5.8–11.3] µg/ml, $p < 0.001$), but not in the girls (Table 10).

The mean serum concentrations of total cholesterol and apolipoprotein A1 did not differ between the two treatment groups. Neither did the median serum concentrations of triglycerides and leptin (Table 10). However, a tendency towards lower median serum triglyceride concentration was seen in boys in the metformin group compared to those of the insulin group (0.52 [0.4–0.7] mmol/L vs 0.63 [0.5–0.8] mmol/L; $p = 0.059$).

Table 10. Serum lipid values and adipocytokine concentrations in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

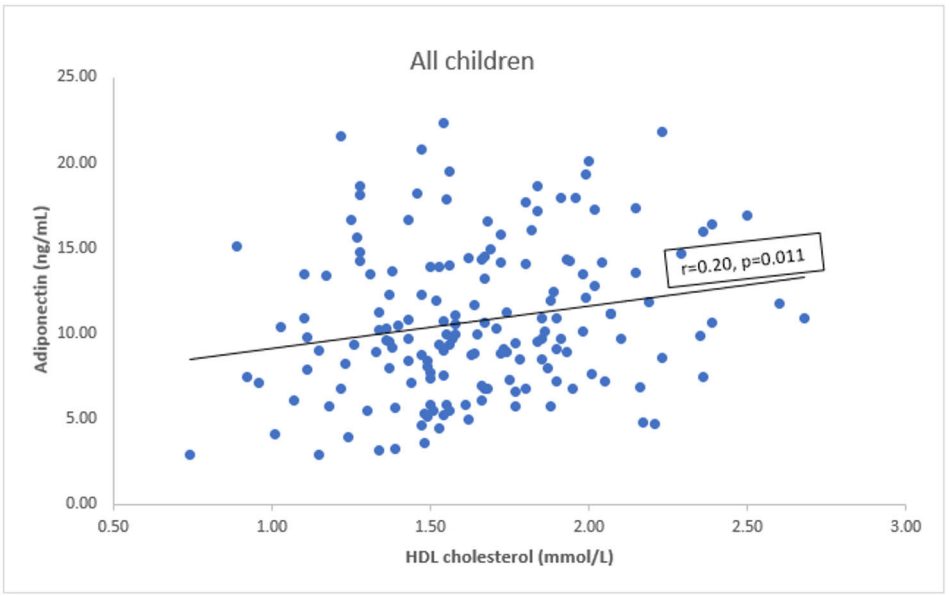
	ALL CHILDREN			**BOYS**			**GIRLS**		
	Metformin n = 78	Insulin n = 89	*p*-value	Metformin n = 39	Insulin n = 43	*p*-value	Metformin n = 39	Insulin n = 46	*p*-value
Total cholesterol (mmol/L)	4.45 ± 0.71	4.56 ± 0.69	0.35	4.48 ± 0.67	4.55 ± 0.65	0.62	4.43 ± 0.76	4.56 ± 0.72	0.34†
LDL cholesterol (mmol/L)	**2.39 (2.1–2.8)**	**2.58 (2.2–2.9)**	**0.046†**	2.41 (2.1–2.8)	2.57 (2.2–2.9)	0.21	2.38 (2.1–2.7)	2.64 (2.3–2.9)	0.12†
ApoB (g/L)	**0.63 (0.6–0.7)**	**0.67 (0.6–0.8)**	**0.043†**	0.62 (0.5–0.7)	0.66 (0.6–0.8)	0.19	0.64 (0.6–0.7)	0.68 (0.6–0.8)	0.15†
HDL cholesterol (mmol/L)	**1.72 (1.5–1.9)**	**1.54 (1.4–1.8)**	**0.039†**	**1.85 (1.6–2.0)**	**1.54 (1.5–1.8)**	**0.003†**	1.55 (1.3–1.8)	1.55 (1.4–1.8)	0.89
ApoA1 (g/L)	1.46 ± 0.17	1.47 ± 0.18	0.69	1.50 ± 0.15	1.50 ± 0.17	0.95	1.42 ± 0.17	1.44 ± 0.18	0.59
HDL/Total cholesterol ratio	**0.39 ± 0.09**	**0.36 ± 0.07**	**0.015**	**0.41 ± 0.09**	**0.36 ± 0.07**	**0.002†**	0.36 ± 0.09	0.35 ± 0.07	0.59
HDL/LDL ratio	**0.73 (0.6–0.9)**	**0.61 (0.5–0.7)**	**0.006†**	**0.76 (0.6–0.9)**	**0.59 (0.5–0.7)**	**0.003†**	0.66 (0.5–0.8)	0.63 (0.5–0.7)	0.32
Non-HDL-cholesterol (mmol/L)	**2.66 (2.4–3.1)**	**2.97 (2.5–3.4)**	**0.028†**	2.57 (2.3–3.1)	2.96 (2.5–3.4)	0.07	2.75 (2.5–3.0)	3.00 (2.4–3.3)	0.21†
Triglycerides (mmol/L)	0.58 (0.4–0.8)	0.61 (0.5–0.7)	0.24†	0.52 (0.4–0.7)	0.63 (0.5–0.8)	0.059†	0.63 (0.5–0.8)	0.61 (0.5–0.7)	0.73†
Adiponectin (µg/mL)	**10.37 (8.7–14.5)**	**9.50 (6.8–12.0)**	**0.016**	**12.13 (9.7–14.7)**	**7.50 (5.8–11.3)**	**< 0.001**	9.37 (7.7–14.3)	9.98 (7.8–12.5)	0.85
Leptin (ng/mL)	6.13 (2.7–11.7)	6.69 (3.1–11.8)	0.63	4.01 (2.1–6.8)	6.66 (2.3–10.8)	0.21	8.71 (4.7–14.5)	6.69 (3.7–13.8)	0.47
Leptin/ adiponectin ratio	0.48 (0.2–1.1)	0.70 (0.3–1.4)	0.18	**0.30 (0.2–0.7)**	**0.75 (0.3–1.3)**	**0.016**	0.75 (0.4–1.7)	0.70 (0.3–1.6)	0.44

Data are expressed as mean ± SD, median (IQR), or n (%). T-test was used unless stated otherwise (Mann-Whitney U-test [†]). Abbreviations: ApoB, apolipoprotein B; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Modified from Table 2 in Study I and Table 1 in Study II.

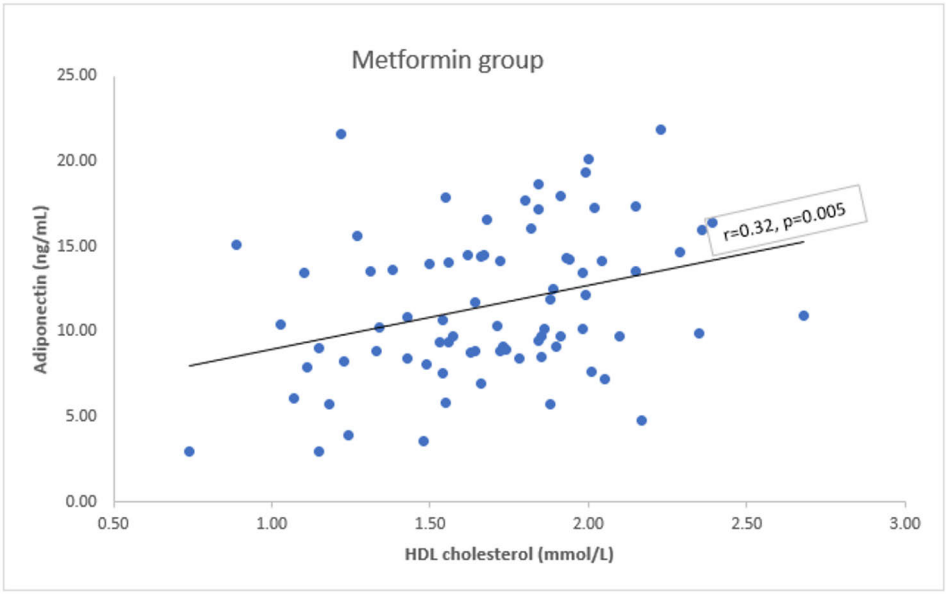
Since both HDL cholesterol concentrations and adiponectin concentrations were higher in metformin-exposed children compared to those in the insulin group, exploratory analysis was used to study the correlation between these two assays. The correlation coefficient was 0.20 ($p = 0.011$) in all children, 0.32 in the metformin

group (p = 0.005), and 0.02 (p = 0.87) in the insulin group. Thus, the correlation between HDL and adiponectin concentrations was significant only in the metformin group.

a.



b.



c.

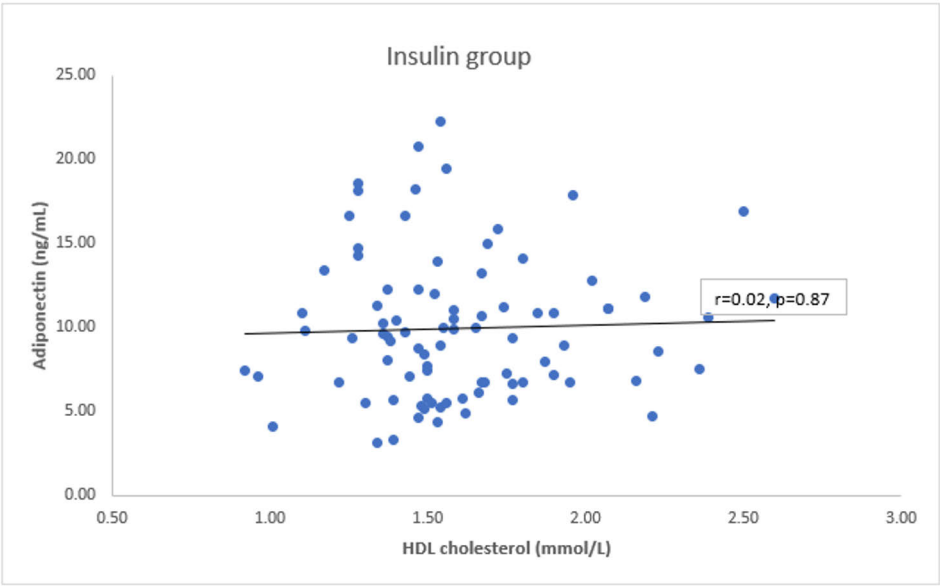


Figure 5. Correlation between serum HDL cholesterol and adiponectin concentration in all children (**a.**), in the metformin group (**b.**), and in the insulin group (**c.**).

5.5 Low-grade inflammation (Study II)

We used hsCRP, IL-6, ferritin, and GlycA as biomarkers to assess low-grade inflammation. Concentrations of these inflammation markers were similar in the two treatment groups, and they were also within normal, age-appropriate limits (Schlenz et al., 2014; Tam et al., 2010; Christiaki et al., 2022; Haapala et al., 2023). Furthermore, no sex-related differences were found in the inflammation markers.

Table 11. Markers of low-grade inflammation in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			BOYS			GIRLS		
	Metformin n = 78	Insulin n = 89	*p*-value	Metformin n = 39	Insulin n = 43	*p*-value	Metformin n = 39	Insulin n = 46	*p*-value
HsCRP (mg/L)	0.24 (0.2–0.7)	0.24 (0.2–1.1)	0.34	0.24 (0.2–0.4)	0.24 (0.2–0.8)	0.83	0.24 (0.2–0.8)	0.30 (0.2–1.3)	0.31
IL-6 (pg/mL)	1.18 (0.7–1.8)	1.31 (0.8–1.9)	0.23	0.87 (0.6–1.5)	1.30 (0.8–1.6)	0.23	1.44 (0.7–2.0)	1.31 (1.0–2.2)	0.63
Ferritin (ug/L)	27.0 (20.9–40.1)	31.0 (24.0–41.4)	0.11	26.5 (21.3–36.3)	28.4 (23.5–40.5)	0.29	27.2 (20.8–43.9)	32.1 (24.5–44.9)	0.27
GlycA (mmol/L)	0.80 (0.8–0.9)	0.81 (0.7–0.9)	0.81	0.78 (0.7–0.9)	0.80 (0.7–0.8)	0.94	0.84 (0.8–0.9)	0.83 (0.7–0.9)	0.61

Data are expressed as medians (IQR), and Mann-Whitney U-test was used. Abbreviations: HsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; GlycA, glycoprotein acetyls. Modified from Table 1 in Study II.

5.6 Body composition, liver fat, and ALT (Study II)

The children's body composition was compared between the two study groups by investigating VAT volume measured by MRI, liver fat percentage measured by MRS, and total and regional body fat percentages measured by DXA. All studied variables were similar between the offspring of the mothers treated with metformin and those treated with insulin.

5.6.1 Visceral adipose tissue

In Study II, VAT volume was also presented as a categorical variable by dividing the values into two groups based on the median VAT of all studied offspring (250 cm^3). However, because of the wide range of variation in VAT volumes (from 44.17 cm^3 to 1160.98 cm^3), these values were also divided into two groups based on the value of 500 cm^3 to assess possible differences between the two groups in larger VAT volumes. A VAT volume < 500 cm^3 was found in 87% of the metformin group boys compared to 69% of the insulin group boys ($p = 0.057$). This tendency was not seen in girls (Table 12).

Table 12. Median (IQR) and proportions of VAT volumes by MRI in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			BOYS			GIRLS		
	Metformin n = 76	Insulin n = 82	*p*-value	Metformin n = 38	Insulin n = 42	*p*-value	Metformin n = 38	Insulin n = 40	*p*-value
VAT volume (cm^3)	246.3 (178.9–423.3)	254.5 (153.3–516.4)	0.82	204.7 (135.7–336.3)	286.0 (132.4–530.1)	0.46	284.3 (213.4–501.4)	216.5 (167.4–483.9)	0.16
VAT volume < 500.0 cm^3	62 (82)	60 (73)	0.21	33 (87)	29 (69)	0.057	29 (76)	31 (78)	0.90
VAT volume ≥ 500.0 cm^3	14 (18)	22 (27)		5 (13)	13 (31)		9 (24)	9 (23)	

Data are expressed as median (IQR) or n (%). Mann-Whitney U-test or Fisher's exact test was used. Abbreviations: GDM, gestational diabetes mellitus; MRI, magnetic resonance imaging; VAT, visceral adipose tissue. Modified from Table 1 in Study II.

5.6.2 Liver fat and ALT

Five (7.9%) children in the metformin group and 11 (14.3%) children in the insulin group (Table 13) had a liver fat percentage higher than 5%. The proportion of boys with high liver fat content was numerically lower in the metformin group versus the insulin group (1 [3.3%] vs 6 [15.4%], $p = 0.10$).

Serum ALT concentrations, which are considered a simple and widely available marker of fatty liver, did not differ between the groups or sexes. Exploratory analysis

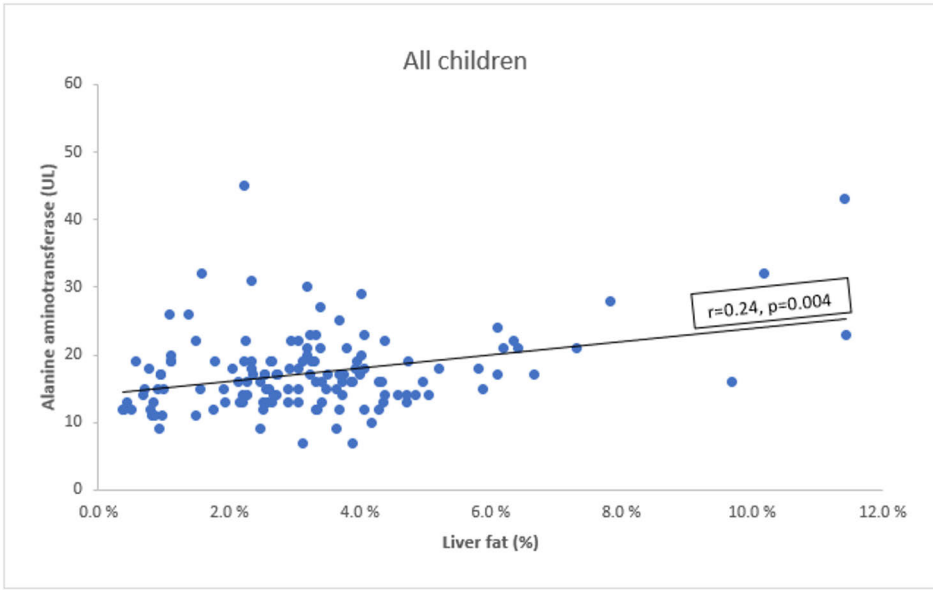
was used to study the correlation between serum ALT concentrations and liver fat percentage in this cohort. The correlation coefficient between ALT and liver fat percentage was 0.24 ($p = 0.004$) in all children. The correlation coefficient between ALT concentration and VAT volume was of the same magnitude, 0.25 ($p = 0.002$). Thus, the correlations between ALT concentration and liver fat percentage and between ALT concentration and VAT volume were moderately low. However, the correlation between VAT volume and liver fat percentage was slightly higher (correlation coefficient 0.29, $p = 0.001$).

Table 13. Median (IQR) for liver fat percentages by MRS and ALT in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

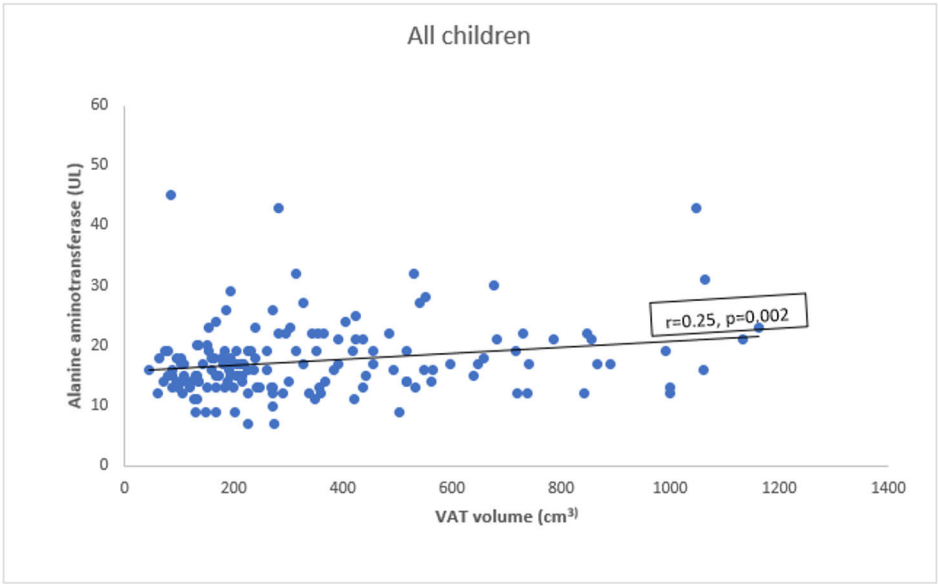
	ALL CHILDREN			BOYS			GIRLS		
	Metformin n = 63	Insulin n = 77	*p*-value	Metformin n = 30	Insulin n = 39	*p*-value	Metformin n = 33	Insulin n = 38	*p*-value
Liver fat (%)	3.1 (2.5–4.1)	3.1 (1.7–4.0)	0.39	2.9 (2.5–4.1)	3.1 (1.5–3.9)	0.73	3.3 (2.5–4.1)	3.2 (1.9–4.0)	0.43
Liver fat < 5.0%	58 (92)	66 (86)	0.20‡	29 (97)	33 (85)	0.10‡	29 (88)	33 (87)	0.90‡
Liver fat ≥ 5.0%	5 (8)	11 (14)		1 (3)	6 (15)		4 (12)	5 (13)	
Alanine amino-transferase (U/L)	16.0 (13.0–19.0)	17.0 (14.0–21.0)	0.22	16.0 (14.0–19.0)	17.0 (15.0–22.0)	0.07	16.0 (13.0–19.0)	16.0 (13.0–20.3)	0.99

Data are expressed as median (IQR) or n (%). Mann-Whitney U-test or Fisher's exact test (‡) was used. Abbreviations: ALT, alanine aminotransferase; GDM, gestational diabetes mellitus; MRS, magnetic resonance spectroscopy. Modified from Table 1 in Study II.

a.



b.



c.

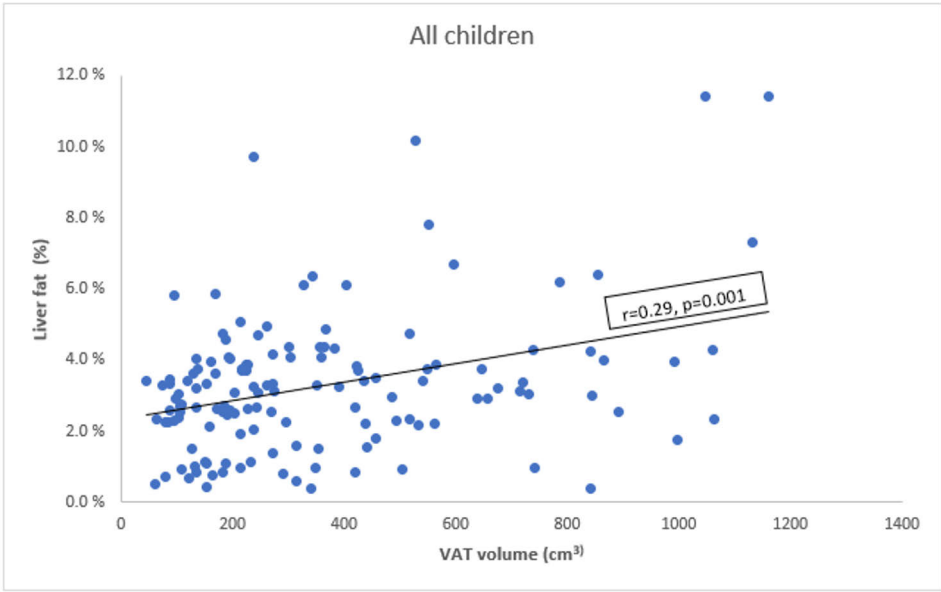


Figure 6. Correlation between serum ALT concentration and liver fat percentage (**a.**), between serum ALT concentration and VAT volume (**b.**), and between VAT volume and liver fat percentage (**c.**) in all children.

5.6.3 DXA – total fat and fat distribution

The fat distribution measured by DXA and the total fat-free mass of the offspring were similar between the groups. Similarly, clinically more representative variables — body size-adjusted fat mass and fat-free mass indexes (FMI and FFMI) — did not

differ between the two treatment groups (Table 14). No significant differences were found between sexes in the MRI, MRS, or DXA studies, but a statistically borderline difference was found in android/gynoid fat ratio by DXA between the metformin and insulin group boys (0.293 [0.26–0.35] vs 0.342 [0.29–0.40], $p = 0.063$).

Table 14. Body composition variables measured by DXA in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			BOYS			GIRLS		
	Metformin n = 62	Insulin n = 63	*p*-value	Metformin n = 32	Insulin n = 30	*p*-value	Metformin n = 30	Insulin n = 33	*p*-value
FMI (kg/m²)	5.41 (4.4–6.4)	5.88 (4.6–7.7)	0.15†	4.63 (4.1–5.9)	5.71 (4.2–6.8)	0.23†	5.78 (5.0–6.88)	5.88 (4.8–7.9)	0.55†
FFMI (kg/m²)	12.71 ± 1.47	12.66 ± 1.20	0.84	13.12 ± 1.37	13.10 ± 0.19	0.96	12.28 ± 1.46	12.27 ± 1.21	0.34
Total fat percentage (%)	30.25 (25.6–34.7)	32.1 (28.3–36.7)	0.14‡	27.0 (24.6–31.2)	31.2 (24.1–34.7)	0.17	32.4 (29.3–35.5)	33.1 (29.4–38.0)	0.27‡
Android/ gynoid fat ratio	0.293 (0.27–0.35)	0.340 (0.28–0.40)	0.073†	0.293 (0.26–0.35)	0.342 (0.29–0.40)	0.063†	0.308 (0.27–0.37)	0.332 (0.28–0.40)	0.55†

Data are expressed as median (IQR), mean ± SD, or n (%). T-test was used unless stated otherwise [Mann-Whitney U-test (†), Chi-squared, or Fisher's exact test (‡)]. DXA values are from Turku. Abbreviations: DXA, dual energy X-ray absorptiometry; FMI, fat mass index; FFMI, fat-free mass index; GDM, gestational diabetes mellitus. Modified from Table 2 in Study II.

5.7 Neuropsychological performance (Study III)

The parents reported that five children (3.1%) had a diagnosis that might potentially affect their learning at school. Moreover, two children in both medication groups were diagnosed with attention and hyperactivity disorder (ADHD), and one child in the metformin group was diagnosed with developmental language disorder. The results of these children were included in the analyses.

5.7.1 Cognitive development

The cognitive development (Table 15, Figure 7 and in detail study III, Table 2) results between the metformin and insulin groups were similar, and adjustments for maternal and paternal education did not change the results. When comparing the proportion of children who performed below the average level in FSIQ (< 85 standard points; < -1 SD) in FSIQ, 28.6% of the children belonged to the metformin group, while 16.5% belonged to the insulin group ($p = 0.070$; Study III). When the FSIQ in three cognitive development categories were compared (Table 15), offspring of the metformin group were more strongly represented in both the lower and higher FSIQ groups than offspring of the insulin group. Three children had FISQ < 70 corresponding to severe cognitive impairment (standard points between 50 and 69).

Table 15. Full-scale IQ results (WISC-IV) in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			BOYS			GIRLS		
	Metformin n = 77	Insulin n = 82	*p*-value	Metformin n = 39	Insulin n = 38	*p*-value	Metformin n = 38	Insulin n = 44	*p*-value
FSIQ	96.0 ± 15.0	97.8 ± 13.4	0.43	92.3 ± 12.8	93.4 ± 11.0	0.68	99.9 ± 16.2	101.7 ± 14.3	0.60
FSIQ > 1 SD[A]	**10 (13)**	**5 (6)**		1 (3)	0 (0)		9 (24)	5 (12)	
FSIQ -1 SD – +1 SD	**45 (58)**	**61 (77)**	**0.041**	25 (64)	29 (78)	0.40‡	20 (53)	32 (76)	0.088
FSIQ < -1 SD[B]	**22 (29)**	**13 (17)**		13 (33)	8 (22)		9 (24)	5 (12)	

Data are expressed as mean ± SD or n (%). T-test, Chi-squared, or Fisher's exact test (‡) was used. [A] FSIQ > 115 standard points. [B] FSIQ < 85 standard points. Abbreviations: FSIQ, full-scale intelligence quotient; GDM, gestational diabetes mellitus; WISC-IV, Wechsler Intelligence scale for Children, Fourth Edition. Modified from Table 2 and Supplemental Table S3 in Study III.

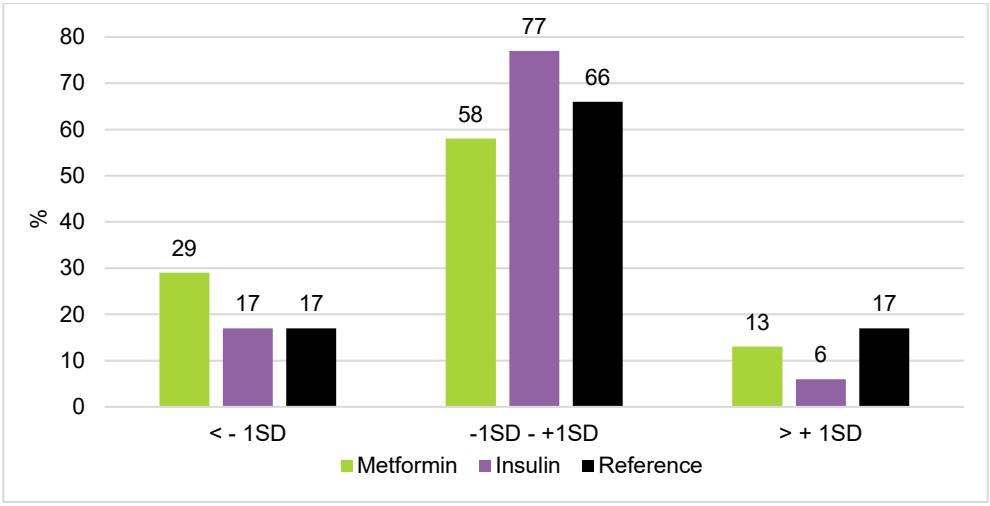


Figure 7. Full-scale IQ (WISC-IV) distribution of offspring of the metformin and insulin groups and standard reference.

5.7.2 Neuropsychological functions

The results of the two subtests of NEPSY II (comprehension of instructions and narrative memory) and TMT A and B were similar in the children of the metformin and insulin groups (Table 16). Additionally, executive function profiles at school and at home, as well as the proportion of children who were reported to have clinically significant symptoms, were similar in the two study groups (Table 17).

Table 16. Neuropsychological performance in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			BOYS			GIRLS		
	Metformin n = 77	Insulin n = 82	*p*-value	Metformin n = 39	Insulin n = 38	*p*-value	Metformin n = 38	Insulin n = 44	*p*-value
Comprehension of instructions[A]	10.0 (8.0–12.0)	10.5 (8.8–12.0)	0.26‡	8.9 (7.0–11.0)	10.0 (8.0–11.0)	0.34‡	11.0 (8.3–12.0)	11.0 (9.0–12.0)	0.61‡
Narrative[A] memory	7.1 ± 3.4	7.6 ± 3.9	0.48	6.8 ± 3.3	6.6 ± 3.7	0.77	7.4 ± 3.4	8.3 ± 4.0	0.29
Trail making test A (s)	23.0 (19.0–30.0)	22.5 (18.0–29.3)	0.55‡	23.0 (18.0–28.0)	23.0 (17.0–32.0)	0.99‡	23.0 (19.5–30.0)	21.0 (19.0–28.0)	0.41‡
Trail making test B (s)	47.0 (40.0–74.0)	50.0 (38.0–67.3)	0.81‡	45.5 (40.0–74.0)	55.0 (39.0–70.0)	0.47‡	48.0 (39.5–71.5)	48.0 (38.0–63.0)	0.77‡

Data are expressed as median (IQR) or mean ± SD, and T-test or Mann-Whitney U-test (‡) was used. [A]Two subtests of the Developmental Neuropsychological Assessment (NEPSY-II) test battery. Abbreviations: GDM, gestational diabetes mellitus. Modified from Table 2 in Study III.

Table 17. Executive functioning in daily life at 9 years of age, as rated by the teacher and parent. Medians of BRIEF indexes and proportion of children who had clinically significant problems (T-scores above 64) at school or at home. Comparison between the offspring of the mothers treated with metformin or insulin for GDM.

	ALL CHILDREN		
	Metformin	**Insulin**	***p*-value**
Executive functioning at School (n = 162)	N = 78	N = 84	
Behavioral Regulation Index	48.5 (45–60)	48.0 (45–57)	0.55
Clinically significant problems at school, n (%)	11 (14)	7 (8)	0.24
Metacognition Index	51.0 (44–60)	50.0 (45–60)	0.68
Clinically significant problems at school, n (%)	13 (17)	14 (17)	0.97
Global Executive Composite Scores	50.0 (45–61)	49.5 (45–60)	0.93
Clinically significant problems at school, n (%)	13 (17)	12 (14)	0.65
Executive functioning at Home (n = 170)	N = 81	N = 89	
Behavioral Regulation Index	43.0 (39–48)	42.0 (38–50)	0.54
Clinically significant problems at home, n (%)	4 (5)	2 (2)	0.34†
Metacognition Index	44.0 (39–52)	44.0 (38–48)	0.37
Clinically significant problems at home, n (%)	2 (3)	2 (2)	0.93†
Global Executive Composite Scores	45.0 (38–50)	43.0 (38–48)	0.39
Clinically significant problems at home, n (%)	2 (3)	3 (3)	0.72†

Data are expressed as the median (IQR) or n (%). Scores above 64 are used to indicate clinically significant problems. The Mann-Whitney U-test was used for continuous variables, and the Chi-squared or Fisher's exact test (†) for categorical variables. Abbreviations: BRIEF, The Behaviour Rating Inventory of Executive Function; GDM, gestational diabetes mellitus. Modified from Table 3 in Study III.

5.7.3 Academic functions

Reading skills assessed using Lukilasse 2 were similar in the metformin and insulin groups. Eleven (14.3%) children in the metformin group and seven (8.5%) in the insulin group received intensified or special support at school (p = 0.063 between groups, Table 18). For reference, 15.3% of pupils in second grade and 27.4% in third grade received intensified or special support at school in 2017 in Finland (Statistics Finland). At the time of the neuropsychological assessment, half of the children were completing second grade, and half of the children had started third grade at school.

Table 18. Academic functions in the 9-year-old offspring of metformin- or insulin-treated mothers with GDM.

	ALL CHILDREN			**BOYS**			**GIRLS**		
	Metformin n = 77	Insulin n = 82	*p*-value	Metformin n = 39	Insulin n = 38	*p*-value	Metformin n = 38	Insulin n = 44	*p*-value
Reading fluency[A]	71.8 ± 19.9	68.4 ± 18.0	0.28	67.4 ± 20.1	66.8 ± 16.1	0.90	76.2 ± 18.9	69.9 ± 19.7	0.16
Educational support[B]:									
Intensified, n (%)	3 (3.9)	6 (7.3)		3 (7.7)	4 (10.5)		0 (0)	2 (4.5)	
Special, n (%)	8 (10.4)	1 (1.2)		4 (10.3)	0 (0)		4 (10.5)	1 (2.3)	
Intensified or special, n(%)	11 (14.3)	7 (8.5)	0.063	7 (18.0)	4 (10.5)	0.13	4 (10.5)	3 (6.8)	0.28

Data are expressed as mean ± SD or n (%). A T-test or Fisher's exact test (for categorical variables) was used. [A] Subtest of the Screening Test for Reading, Writing, and Calculus for First to Sixth Grades (Lukilasse 2) and [B] information about received support at school. Abbreviations: GDM, gestational diabetes mellitus. Modified from Table 2 in Study III.

6 Discussion

The results of several short-term follow-up studies on metformin treatment for GDM support the use of metformin during the second and third trimesters of pregnancy, but the safety of metformin treatment needs to be confirmed considering possible negative effects on offspring throughout the lifespan.

This thesis discovered that metformin, compared to insulin treatment for GDM, did not cause negative effects on prepubertal 9-year-old offspring's well-being as evaluated with measurements of anthropometrics, body composition, lipid and glucose metabolism, and cognitive and neuropsychological development.

6.1 Metformin exposure and offspring growth, body composition and development before school-age

To date, researchers have often suggested that metformin treatment for GDM may have unfavourable effects on offspring growth, referring to meta-analyses with findings of children being smaller at birth but larger during childhood in the metformin group compared to the insulin group (e.g., Jorquera et al., 2020; Sciacca et al., 2023; Martine-Edith et al., 2023). These findings have found support from the DOHaD theory (Barker et al., 1993; Barker, 1997; Barker, 2007) and studies of the Dutch famine cohort (Roseboom et al., 2000, DeRooij et al., 2006).

According to several meta-analyses on metformin or insulin treatment for GDM, metformin-exposed neonates weigh approximately 100 g (103–123 g) less at birth than neonates of insulin-treated mothers (Tarry-Adkins et al., 2019; Wang et al., 2022; Sheng et al., 2023) without an increase in the risk of being born with SGA. In the RCTs, birth weight was reported as a number in grams without combining information with pregnancy weeks, the standard deviation of birth weight, or the length of the neonate (PI).

That some studies found a lower birth weight in neonates in the metformin group than in the insulin group does not confirm the significance of this finding because the above-mentioned context has not been considered. In contrast, the risk of macrosomia has been found to be 30–40% lower when a mother's GDM is treated with metformin compared to insulin (Tarry-Adkins et al., 2019; Sheng et al., 2023),

which can be interpreted as a positive finding. However, the interpretation of these meta-analyses (Tarry-Adkins et al., 2019; Sheng et al., 2023) should consider the study limitations related to heterogeneity in diagnostic criteria for GDM and metformin dosing. Additionally, the proportion of maternal obesity was not reported in most of the RCT studies. Therefore, its possible effect on neonatal and childhood measures is unknown. Moreover, in a meta-analysis by Sheng et al. (2023), two studies of the same cohort were included (Tertti et al., 2013; Huhtala et al., 2020). Conversely, a recent study by Swensson et al. (2023) suggested that metformin may have a direct reductive effect on foetal growth via foetal liver cells. The clinical importance of this finding must be confirmed by further research, but this might be one mechanism by which metformin could affect perinatal growth.

Two Finnish RCTs (Ijäs et al., 2010; Tertti et al.; 2013) comprising data on 314 pregnancies found no difference in the birth weight of neonates between metformin- or insulin-treated mothers. Study I of this thesis, based on the two RCTs mentioned above, found that birth weight, the proportion of children with birth weight < -2 SD, PI, Apgar scores, umbilical artery pH, and rates of hypoglycaemia did not differ between the two medication groups.

Only a few meta-analyses (Tarry-Adkins et al., 2019) of follow-up studies (Rowan et al., 2011; Ijäs et al., 2015; Tertti et al., 2016) or population-based cohort studies have been conducted with this research setup and have included school-age children. To conclude the findings of these studies, although the weight of metformin-exposed children and the children of insulin-treated mothers was similar at 6 months, the metformin-exposed children were heavier at the ages of 12 and 18 months. However, PI relating the children's weight to their height at 6, 12, or 18 months of age did not differ between the two groups (Ijäs et al., 2015). At the age of 2 years, weight, height, mid-upper arm or waist circumference, and WtHR were similar between the two groups, but offspring in the metformin-exposed group had larger mid-upper arm circumferences and subscapular and biceps skinfolds (Rowan et al., 2011). However, no differences were found between the groups in arm fat by DXA or total fat (Rowan et al., 2011). At the age of 5 years (33–85 months), the BMI, BMI z-score, and waist-to-hip ratio of the boy offspring (n = 56) were similar between the two medication groups (Tertti et al., 2016). In population-based cohort studies, metformin treatment was not associated with an increased risk of obesity, either at the age of 3.5 years (Brand et al., 2022) or 4 years (Landi et al., 2019). Furthermore, growth trajectories did not differ among 0–60-month-old children from these two medication groups (Martine-Edith et al., 2023).

In a meta-analysis by Tarry-Adkins et al. (2019), infant growth data (aged 18–24 months) from two studies (Ijäs et al., 2015; Rowan et al., 2011) were combined, and they found that metformin-exposed children were heavier (mean difference 440 g, 95% CI 50–830 g, $p = 0.03$) than children of insulin-treated mothers. They

also reported weight and BMI in mid-childhood (5–9 years). No significant difference was found in weight or height, but the mean BMI was higher (mean difference 0.78 kg/m^2, 95% CI 0.23–1.33, p = 0.005) in the metformin group. Another meta-analysis by Xu et al. (2019) combined data on offspring weight in mid-childhood from GDM and PCOS studies, and they found that children in the metformin group were heavier (mean difference 480 g, 95% CI 240–730 g; p = 0.0001) than children in the insulin group, but no difference was found in the children's BMI.

To conclude the findings of these RCTs and meta-analyses on the growth of the offspring before school age, in some RCT studies, children in the metformin group weighed more than children in the insulin group, but the clinical significance of this finding might not be remarkable, especially when considering that no systematic differences in PI or BMI were observed between these two medication groups.

Cognitive, motor, social- and behavioural development of these children has been studied by different assessments in follow-up studies of RCTs at the age of 18 months (Ijäs et al., 2015) and 2 years (Tertti et al. 2015; Wouldes et al., 2016), as well as in population-based cohort studies at the age of 3.5 years (Brand et al., 2022) and 4 years (Landi et al., 2019). These studies are described in the literature review section. No differences were found between maternal metformin and insulin treatment in the offspring's development before school age.

6.2 Metformin exposure and offspring growth, body composition and development at school age

To date, only one RCT on maternal metformin or insulin treatment for GDM (Rowan et al., 2008) has reported offspring data at school age (i.e., anthropometry, metabolism, adipocytokines, markers of low-grade inflammation, adiposity measures, regional fat distribution, and liver fat percentage; Rowan et al., 2018).

Furthermore, only two follow-up studies on metformin treatment for PCOS during pregnancy have reached school age. Rø et al. (2012) published a follow-up study of a small RCT pilot (Vanky et al., 2004), and Greger et al. (2020) conducted a follow-up study of the cognitive function of offspring at 7 years of age. There are several differences between the metformin studies examining PCOS patients and GDM patients. First, in PCOS studies, metformin treatment was begun during the first trimester, and in GDM studies, it was begun, on average, during the second trimester. Second, in PCOS studies, comparisons were performed between the metformin and placebo groups. Third, some mothers in the PCOS studies also had GDM (Vanky et al., 2010). These issues mean that the intrauterine environments of mothers in GDM studies and PCOS studies, despite metformin exposure in both, are

only partially comparable. For these reasons, the PCOS studies are not discussed further.

6.2.1 Anthropometry, body composition, and blood pressure

Obesity, particularly abdominal obesity, causes several health disadvantages for children when it develops, especially at a young age. Because previous findings indicate that foetal exposure to metformin treatment may increase excess offspring weight before school age, longer-term follow-up data on anthropometry and body composition are important. At the age of 9 years (n = 99), the offspring of the metformin-treated mothers were found to be heavier and had a greater waist circumference, WHtR, and mid-upper arm circumference than the offspring of the insulin-treated mothers (Rowan et al., 2018). Six children in this follow-up subgroup had signs of early puberty, and after excluding these children, the researchers redid the analyses when the weight was no longer different between the groups. Additionally, offspring in the metformin group had a higher BMI ($p = 0.051$) and triceps skinfolds ($p = 0.05$) with a slight difference. In DXA measures, offspring of the metformin group had a trend towards higher fat-free mass ($p = 0.07$) and fat mass ($p = 0.07$; Rowan et al., 2018). In addition to a larger mid-upper arm circumference and biceps skinfolds in the offspring in the metformin group, Rowan et al. (2018) found higher arm fat by DXA in the metformin group. However, at the age of 7, Rowan et al. (2018) found no differences between the metformin and insulin groups in corresponding variables.

In the present study, we observed no significant differences between the two homogenous treatment groups (n = 172) at 9 years of age in the anthropometric variables (i.e., weight, height, BMI, proportions of overweight or obese children, waist circumference, WHtR, or prevalence of WHtR over 0.5). Skinfold measurements were not examined in present study.

The percentage of parental overweight and obesity as well as the BMI of mothers and fathers were similar in our two study groups, which may indicate a similar lifestyle environment and genetic background between the study groups and, therefore, similar future growth and development of overweight or obesity in the offspring. Only 15% of the mothers and 25% of the fathers had a normal weight. Despite this, 70% (120/172) of the offspring had a normal weight at the age of 9 years.

Neither we nor Rowan et al. (2018) found differences between treatment groups in total body, leg, or abdominal (or android) fat or total fat-free mass as measured by DXA. In the present study, FFMI and FMI, which are related to a child's height, were similar between the two groups.

In the study by Rowan et al. (2018), the blood pressure was not measured at the age of 7 or 9 years. In that cohort, blood pressure values were published only at the age of 2 years, and no difference between the medication groups was found (Battin et al., 2015). We measured blood pressure in all 9-year-old children, and we found no difference between the medication groups.

In sum, in present study at the age of 9 years and in the study by Rowan et al. (2018) at the age of 7 years, no difference between the two treatment groups was found in anthropometry or body composition variables. However, Rowan et al. (2018) found some differences in anthropometry and body composition between the groups which were unfavourable to the metformin group at the age of 9 years. After excluding children with signs of early puberty, Rowan et al. (2018) found no difference in arm-fat mass by DXA between the two groups. The authors also reported that the 9-year-old study group was ethnically more heterogeneous than the original cohort, but adjustment for ethnicity, offspring sex, maternal pregnancy BMI, or maternal weight gain during pregnancy did not change the overall significance of the results. However, they noted that the numbers in each subgroup were small for reliable adjustments (Rowan et al., 2018).

6.2.2 Abdominal fat, liver fat, and ALT

Abdominal fat refers to both the subcutaneous fat and the intra-abdominal fat around the internal organs in the abdominal cavity. The fat in the abdominal cavity has a stronger association with CVD morbidity than subcutaneous fat. Waist circumference has been suggested as a good predictor of VAT (Brambillia et al., 2006), and BMI is associated with subcutaneous abdominal fat in children and adolescents (Brambilla et al., 2006). Rowan et al. (2018) found slightly higher abdominal fat volume and visceral fat volume in the metformin group offspring than in the insulin group offspring at the age of 9 years, the difference being slightly significant. When the authors analysed the data according to the child's size using percentage values (i.e., abdominal fat volume of the total abdominal volume, percentage of abdominal subcutaneous fat, and visceral fat of the total abdominal fat volume), the results did not differ between the two study groups.

In the present study, we found no differences in median VAT volumes between the two treatment groups. When using the median VAT volume (250 cm^3) as a cut-off value, the proportion of smaller and higher VAT volumes was similar between the two groups. Although half of the children had low VAT volumes ($\leq$ 250 cm^3), several children had very high VAT volumes. When assessing the difference between the two groups using a higher cut-off (500 cm^3), a slight difference towards fewer boys in the high-fat group was seen in the metformin group versus the insulin

group, but a similar difference was not seen in the girls. The details of the differences between the sexes are presented in Section 6.2.5.

Liver fat percentages higher than 5% are considered to indicate high liver fat content in MRS (Lätt et al., 2018). In the present study, only a few offspring—five (7.9%) in the metformin group and 11 (14.3%) in the insulin group (Table 13)—had a liver fat percentage higher than 5%. Neither we nor Rowan et al. (2018) found differences between treatment groups in liver fat percentage at the age of 9 years. In the present study, the median liver fat percentage was slightly higher (3.1% both in the metformin and insulin groups) compared to that in the study by Rowan et al. (2018; 2.5% and 1.8% respectively). Methodological reasons can explain the higher numbers in the present study than in the study by Rowan et al. (2018). The estimation of liver fat percentage might be dependent on the selected region for analysis in MRS; thus, the percentage values between these two studies are not necessarily comparable. Serum ALT concentration has been used as a simple, cheap, and widely available indicator for fatty liver (Schwimmer et al., 2009). Serum concentrations of ALT in present study were low, similar to the study by Rowan et al. (2018), and they did not differ between the treatment groups in either of the studies.

In summary, in both 9-year follow-up studies, the metformin and insulin groups were similar in abdominal or visceral fat, liver fat, and ALT measures when the body size of the offspring was noted in the analyses of the abdominal fat.

6.2.3 Glucose and lipid metabolism and adipocytokines

Rowan et al. (2018) found no differences either in the fasting plasma glucose levels between the metformin and insulin groups in 7-year-old offspring or in fasting glucose, fasting insulin values, HbA1c, and HOMA-IR in 9-year-old offspring. In the present study, insulin ja C-peptide concentrations were assessed along with glucose values by OGTT to form an accurate and comprehensive estimate of the glucose metabolism of the offspring. We found that glucose metabolism values in OGTT, HbA1c, and HOMA-IR were similar between the groups, even though some of the above-mentioned values tended to be slightly lower in the metformin group boys than in the insulin group boys ($p = 0.015–0.13$).

Although they reported the children in the metformin group to be heavier than those in the insulin group, Rowan et al. (2018) found that the concentrations of LDL cholesterol, HDL cholesterol, and triglycerides were similar between the groups, and the sex of the offspring did not impact the results. In the present study, a detailed lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B) was determined to describe the state of lipid metabolism. We found that the HDL cholesterol concentration was higher and the LDL cholesterol and apolipoprotein B concentrations were lower in

the metformin group than in the insulin group, suggesting a more advantageous lipid profile in the offspring of the metformin group.

Adipose tissue is a highly active endocrine organ, and the characteristics of adipose tissue have been shown to determine adiponectin secretion more than the amount of it (Zhao et al., 2021). Optimal adiponectin concentration enhances insulin sensitivity, and a low adiponectin/leptin ratio is a reliable, predictive biomarker for several metabolic disorders, such as type 2 diabetes and cardiovascular disorders (Zhao et al., 2021). Indeed, Frithioff-Bøjsøe et al. (2020) suggested that the ratio of adiponectin and leptin is a more important risk marker for cardiometabolic comorbidities in children than adiponectin or leptin alone. Contrary to Rowan et al. (2018), we found higher serum adiponectin values in the offspring of the metformin group.

In contrast to the study of Rowan et al. (2018), we found differences in lipid metabolism and adipokine results that were favourable for the metformin group. In the present study, the higher serum HDL cholesterol and adiponectin concentrations in the metformin group was observable only in boys. Differences between sexes are discussed in more detail in chapter 6.2.6.

6.2.4 Low-grade inflammation

Low-grade inflammation is considered a risk factor for CVD development. Obesity has been connected with low-grade inflammation, and obese children and adults have higher hsCRP than lean individuals (Christaki et al., 2022). For an exact assessment of low-grade inflammation, we used hsCRP, IL-6, ferritin, and GlycA as biomarkers and found no differences in inflammation markers between the two treatment groups. Of these low-grade inflammation markers, Rowan et al. (2018) reported only ferritin values. The differences between our results and those of Rowan et al. (2018), who found higher ferritin in the metformin group, might be partly due to differences in ethnicity (Tahmasebi et al., 2020). Rowan et al. (2018) found a difference of 162.8 pmol/L (72.45 µg/L) versus 97.37 pmol/L (43.33 µg/L, $p = < 0.001$) in mean ferritin concentration in males of East Asians and Caucasian ethnicity, respectively. In the present study, 99% of the mothers were Caucasian, whereas more than 50% of the mothers in the study by Rowan et al. were of East Asian descent.

6.2.5 Cognitive and neuropsychological performance

Metformin has been shown to cross the placenta (Tertti et al., 2010) and enter foetal circulation in a similar concentration as in the mother's blood. Furthermore, metformin may also be transported across the blood-brain barrier in the foetal brain,

as in the adult brain (Cao et al., 2022). Thus, metformin might potentially influence the cognitive development of the offspring of mothers with GDM. However, metformin has been increasingly studied in adults because of its possible neuroprotective actions in several neurological incidences, such as traumatic brain injury or diseases, such as Alzheimer's and Parkinson's disease (Cao et al., 2022).

To our knowledge, the present study is the first to report the long-term cognitive and neuropsychological outcomes of children born to mothers randomized to either metformin or insulin treatment for GDM.

Based on previous results, foetal exposure to metformin does not seem to affect children's motor, social, behavioural, linguistic, or cognitive development compared to insulin treatment when examining children before school age (Ijäs et al., 2015; Tertti et al., 2015; Wouldes et al., 2016; Brand et al., 2022; Landi et al., 2019). However, mild cognitive and neuropsychological difficulties might become evident with age and increasing demands during the school years. In the present study, however, neurocognitive outcomes were similar in 9-year-old children between the metformin and insulin groups. The results of the present study align with previous studies at younger age groups (Ijäs et al., 2015; Tertti et al., 2015; Wouldes et al., 2016; Landi et al., 2019; Brand et al., 2022).

In the present study, the FSIQ results were divided into three categories based on cut-off levels of -1 SD and +1 SD (< 85, 85–115, > 115 std points) to increase understanding about the distribution of FSIQ results between the two groups. These cut-offs describe at least slightly below average (< -1SD) and at least slightly above average (> +1SD). In the metformin group, a slightly larger proportion of children were categorized into FSIQ < -1.0 SD and FSIQ > +1.0 SD when compared to the insulin group. Furthermore, a tendency was found for a larger proportion of children in the metformin group that received intensified or special support at school compared to insulin group children ($p = 0.063$). However, at the time the study was conducted, the children were completing either second or third grade, which may have affected these results. According to data from Finland's national statistical institute, 15.3% of pupils in second grade and 27.4% in third grade received intensified or special support at school during the year 2017. In present study, the percentages in both groups were lower than the average in Finland at that time. These stages of support for learning were first adopted in 2012, and implementation was varied in different regions at first.

When metformin is used to treat PCOS, treatment is begun during the first trimester and may be continued throughout the pregnancy. In contrast to PCOS pregnancies, medication in GDM is typically started during the second or third trimester. However, in a Norwegian follow-up study of mothers with PCOS randomized to metformin or placebo treatment from the first trimester, the mean FSIQ in the metformin and placebo group in 5–14 (mean 7)-year-old offspring

(n = 93) was similar, but an association between the offspring's metformin exposure in utero and borderline FSIQ (between 70 and 85) was found (Greger et al., 2020). Nevertheless, the participation rate in that study was only 32%.

6.2.6 Differences between Boys and Girls

Some metabolic and adiposity-related variables (higher serum HDL cholesterol and adiponectin concentrations, and lower leptin/adiponectin ratio and 2-hour serum glucose concentration) were more favourable in the boys of the metformin group compared to the boys of the insulin group, but such differences were not seen in girls.

In addition, we found few tendencies towards more favourable anthropometric, metabolic and body composition measures in the metformin group boys compared to the insulin group boys. These were lower values in triglyceride concentration ($p = 0.059$), lower android/gynoid fat ratio ($p = 0.063$), lower VAT volume (< 500 cm^3; $p = 0.057$) and lower WHtR ($p = 0.06$). If the difference in WHtR between the two groups was calculated only in boys whose laboratory assessments were analysed, the p-value was 0.032. All the above-mentioned differences are regarded as beneficial. Among the girls, there were no such tendencies. However, without statistical significance, these tendencies might be connected with the higher adiponectin and HDL concentrations in the metformin group boys, although the correlation between the serum HDL cholesterol concentrations and the serum adiponectin concentrations was not high.

Whether metformin exposure during pregnancy could have a different long-term influence on lipid and glucose metabolism among boys than girls, either independently or mediated by the anthropometric variables, is unknown. Several mechanisms might exist by which metformin treatment for GDM during pregnancy could affect prepubertal boys differently than girls. In addition to adiposity variables, inherited genetic factors, other postnatal environmental factors, and epigenetics might also have been involved in these sex-associated differences in the serum lipids of the offspring of metformin- and insulin-treated mothers with GDM. Lu et al. (2021) showed sex-discordant differences in adult mice offspring after exposure to maternal GDM. They found that the sexes can develop different metabolic phenotypes after similar exposure during pregnancy. Regarding epigenetics, the non-coding RNA 866 (nc866) epiallele methylation status might be one possible explanation for our findings regarding the HDL cholesterol concentration differences between sexes. In general, the methylation status of a nc866 epiallele in offspring is dependent on the maternal nc866 epiallele methylation and the ambient conditions during gestation. Indeed, Marttila et al. (2021) found that boys, but not girls, with a nonmethylated nc866 epiallele had higher ($p < 0.05$) estimated HDL cholesterol levels during childhood (ages 6–12 years) than boys with a hemi-

methylated nc866 epiallele. Furthermore, in studies of very-preterm-born children, boys are more vulnerable than girls to early risk factors that might affect cognitive and neuropsychological development (Kuban et al., 2016).

6.3 Methodological strengths and limitations

The major strength of the present follow-up study is that the 9-year-old offspring represent the original cohort well, enabling valid comparisons between the treatment groups. Moreover, the baseline data were similar between the 9-year study participants and the group of non-participants. Among the participating children, both sexes and medication groups were evenly distributed, and all the children were prepubertal. All measurements were performed using strict procedures, and the neuropsychological assessment covered clinically essential functions of 9-year-old children, reflecting the overall picture of their neurocognitive outcomes. All blood samples were stored under similar conditions and analysed simultaneously in one laboratory. In addition, the study protocol was similar at the two study sites, as well as at baseline and follow-up. Currently, this follow-up cohort of 172 nine-year-old offspring whose mothers received either metformin or insulin treatment for GDM is the largest published cohort to compare the long-term effects of prenatal metformin exposure and maternal insulin treatment. Power calculations for fasting glucose concentration and BMI were performed before the study was performed. The follow-up rate of 55% obtained for the total cohort was satisfactory considering the long period of 9 years between birth and follow-up, although it was slightly lower than expected in the power calculation. The follow-up rate of the RCT by Rowan et al. was 43–23% at the age of 2 years and 14–15% at the age of 7–9 years (Table 4). However, the results of present study were reassuring, as no detrimental influence of metformin treatment for GDM was observed on the health of the 9-year-old offspring.

The study also had some limitations. First, at the time the original RCT studies were initiated, no longer-term follow-up studies were planned, which, together with the number of participants in the original studies and the suboptimal follow-up rate, may have led to some potential and milder differences not being detected between the treatment groups. Second, DXA assessments were performed only in children followed-up in Turku. These children, however, represented 74% of all participants. Third, the neuropsychological assessments were performed by five psychologists or final-stage psychology students, although no differences in test score medians between the psychologists were found. The narrative memory test (NEPSY II) results were lower than average in both medication groups. This may have been related to the ceiling effect (Uttl, 2005; Wang et al., 2009) further compounded by the exhaustion from prolonged examinations. Fourth, the children were examined at

the age of nine years, which led to a situation in which the studied children were in either the second or third grade at primary school. This may have affected the level of educational support received. However, the distribution of children in the second and third grades was similar in both medication groups. Last, the participants were almost entirely of white Caucasian ethnicity, which may have affected the applicability of the results to other ethnic groups. Although neither the present study nor the study by Rowan et al. were able to find small differences in 9-year-old offspring outcomes, these studies add knowledge to this topic, which has only been studied a little.

6.4 Clinical implications and future perspectives

GDM is currently one of the most common medical complications in pregnancy, and its prevalence has been increasing globally along with that of obesity (ACOG, 2018). Annually, GDM affects approximately 14% of pregnancies, or approximately 20 million births worldwide (Wang et al., 2022). Many of these women have limited access to health care services. Traditionally, insulin has been the standard of pharmacological care for the treatment of GDM. However, compared to insulin treatment, use of metformin to treat GDM reduces health care costs (Xu et al., 2017; Weile et al., 2015), facilitates treatment in many ways, and is, as an oral drug, better adopted and more accessible to everyone. Metformin is safe for the mother, and it does not cause hypoglycaemia.

The results of this thesis will likely reassure the use of metformin for treating GDM. The study has been noted in the recent Finnish national guidelines for GDM treatment (Duodecim, Current Care Guidelines, 2022).

Of mothers with GDM, 3–46% (Kattini et al., 2023) fail to achieve optimal glucose balance with metformin and require supplemental insulin; therefore, insulin still has an important role in the treatment of GDM. However, especially in obese mothers, treatment of GDM with metformin seems to have a positive effect on lipid metabolism, and it also reduces excess weight gain (Feig et al., 2020).

Maternal BMI and glycaemic profile during pregnancy may be relevant factors affecting which group of mothers and offspring benefit most from the metformin treatment. In present study, the number of study subjects was relatively small, and therefore, a reliable comparison between the offspring variables considering maternal pregnancy BMI or glycemic profile categories in the two medication groups was not possible. Nevertheless, further studies are needed to explore whether any specific group of mothers with GDM, such as obese mothers with insulin resistance, benefits the most from metformin treatment.

Previous studies found that maternal metformin use for GDM does not affect the foetus or newborn adversely (Feig et al., 2007; Kattini et al., 2023). In present study,

we found no untoward effects in long-term follow-up regarding anthropometry, lipid and glucose metabolism, body composition, or cognitive development in 9-year-old children exposed antenatally to metformin due to maternal GDM.

Future research should investigate more detailed metabolic profiles (i.e., metabolomics) of these children and mothers. Although the growth of offspring of metformin- and insulin-treated women was similar during the first 9 years of life, possible later differences, such as those in pubertal development or reproductive health, cannot be ruled out. The encouraging results of neuropsychological outcomes in this study would be confirmed if a registry-based study of this cohort was performed, for example, at the end of basic education at the age of 16 years.

We found some, albeit fairly subtle, differences in the effects of metformin between the sexes. Indeed, boys exposed to metformin seemed to have a healthier lipid and adipokine profile than insulin-exposed boys. There was no such finding in girls. However, although not reaching statistical significance, metformin group boys seemed to perform slightly less well in some cognitive variables than insulin group boys. Again, there was no such tendency in girls. Based on our results, the possibility that exposure to metformin during pregnancy may influence boys and girls differently requires further study.

7 Summary/Conclusions

Two Finnish randomized studies have shown the safety and efficacy of metformin treatment in GDM mothers, but the long-term safety of offspring has been scarcely studied. This follow-up of those two randomized studies consisted of 172 prepubertal 9-year-old children whose mothers were treated with metformin or insulin for GDM.

The main findings of this thesis can be summarized as follows:

1. Anthropometric measurements and body composition were similar between the groups, and metformin-exposed offspring were not more often overweight or obese than offspring of insulin-treated mothers.
2. Metformin treatment did not influence the children's glucose metabolism differently than insulin treatment for GDM.
3. Children in the metformin group had higher HDL cholesterol and adiponectin concentrations, whereas their LDL cholesterol and apolipoprotein B concentrations were lower than those of the children in the insulin group.
4. In detailed analysis, adiponectin and HDL cholesterol concentrations were similar in the two treatment groups in girls but significantly higher in boys in the metformin group than in the insulin group. Similarly, boys in the metformin group had lower 2-hour glucose values and WHtR than boys in the insulin group.
5. Cognitive and neuropsychological outcomes were similar based on standardised tests or executive function ratings by teachers and parents. In this study, clinically significant differences that could affect children's academic or daily lives were not found.

In conclusion, metformin treatment for GDM does not appear to have negative effects on prepubertal 9-year-old offspring's anthropometry, metabolism, body composition, or cognitive and neuropsychological outcomes compared to insulin

treatment. Furthermore, no differences were found in known markers of CVD risk in metformin-exposed children compared to children of insulin-treated mothers at the age of 9 years. In sum, maternal metformin treatment for GDM seems safe for offspring in the long term.

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