Gestational Diabetes Mellitus- Future risk for mother and child

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Gestational Diabetes Mellitus

Future risk for mother and child

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Abstract
Gestational diabetes mellitus (GDM) occurs as a complication in 2% of all pregnancies in Sweden. Women with GDM have a substantial risk of developing type 2 diabetes later in life, but the risk of developing type 1 diabetes is also increased. GDM increases the risk for macrosomia and caesarean delivery. However, long term prognosis and eventual future risks for children born to mothers with a previous GDM are less well studied. In this thesis women who had GDM during 1995-2010 and their children were investigated.

Aims Paper I-III: Determine how many women with GDM that have beta-cell specific autoantibodies such as glutamic acid decarboxylase antibodies (GADA), tyrosine phosphatase antibodies (IA-2A) and zink transporter 8 antibodies (ZnT8A) during pregnancy, and follow these women after delivery to estimate the risk for later development of type 1 diabetes. Evaluate C-peptide levels in women with GDM as a predictor for future development of diabetes.

Aims Paper IV: Investigate the effects of maternal GDM on childhood body mass index (BMI) compared to the age-specific reference values in Sweden and to their siblings born after a non-GDM pregnancy.

Results Paper I-III: Up to 8% of women with GDM had GADA or IA-2A during pregnancy, and 50% of these women developed type 1 diabetes later in life. GADA was the most frequent autoantibody. When adding ZnT8A as an autoimmune marker in GDM, the number of autoantibody positive women increased by 2%. C-peptide analyses did not add any valuable information for development of either type 1 or type 2 diabetes.

Results Paper IV: BMI for boys was higher at ages 7-10 and for girls at birth and ages 4-12 compared to Swedish reference values. The same BMI pattern was found in siblings born after a non-GDM pregnancy.

Conclusions Paper I-III: Since 50% of women with autoantibodies during GDM develop type 1 diabetes later in life, at least GADA analyses should be performed in all women with GDM by routine.

Conclusions Paper IV: Children to women with a prior GDM have a high risk for overweight and obesity. This is thought to be due to life style habits in the family rather than prenatal factors, even if genetic factors could not be tested in this study, since similar BMI pattern was found in siblings. Early life style intervention is therefore very important in these families.

Key words
Gestational Diabetes Mellitus, autoantibody, GADA, ZnT8A, C-peptide, overweight, offspring

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Cover picture from Wikipedia, created by Isaac Yonemoto, showing six insulin molecules assembled in a hexamer.

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To my wonderful parents

Table of contents

Original papers

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text. The papers are appended at the end of the thesis.

I. **Nilsson C**, Ursing D, Törn C, Åberg A, Landin-Olsson M.
Presence of GAD antibodies during gestational diabetes predicts type 1 diabetes. Diabetes Care 2007;30:1968-1971

II. Dereke J, **Nilsson C**, Landin-Olsson M, Hillman M.
Prevalence of Zinc transporter 8 antibodies (ZnT8A) in gestational diabetes mellitus. Diabetic Medicine 2012;29:436-439

III. **Nilsson C**, Hillman M, Ursing D, Strevens H, Landin-Olsson M.
Clinical use of C-peptide and beta-cell specific autoantibodies during gestational diabetes mellitus. Practical Diabetes 2012;29:105-108

IV. **Nilsson C**, Carlsson A, Landin-Olsson M.
Increased risk for overweight among children born to mothers with gestational diabetes mellitus. Submitted.

Abbreviations

ACHOIS	Australian Carbohydrate Intolerance Study
ADA	American Diabetes Association
BMI	Body mass index
EASD	European Association for the Study of Diabetes
ELISA	Enzyme linked immunosorbent assay
GDM	Gestational diabetes mellitus
GADA	Glutamic acid decarboxylase antibodies
GLUT	Glucose transporter
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes study
HbA1c	Haemoglobin A1c
HLA	Human leukocyte antigen
HOMA	Homeostasis model assessment
IAA	Insulin autoantibodies
IA-2A	Tyrosine phosphatase antibodies
IADPSG	International Association of Diabetes in Pregnancy Study Groups
ICA	Islet cell antibodies
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
JDF-U	Juvenile Diabetes Foundation units
LADA	Latent autoimmune diabetes in adults
NS	Not significant
NT	Not tested
OGGT	Oral glucose tolerance test
SD	Standard deviation
WHO	World Health Organization
ZnT8A	Zink transporter 8 antibodies

Background

Diabetes is defined as a group of metabolic disorders characterized by defects of insulin secretion and/or insulin action which leads to hyperglycaemia. There are different forms of diabetes, but the long term negative side effects of chronic hyperglycaemia on different organs such as kidneys (nephropathy), eyes (retinopathy), blood vessels (angiopathy), nerves (neuropathy) and heart remain the same (1).

History of diabetes mellitus

Clinical features of diabetes were first described by the ancient Egyptians about 1550 BC. In the Tomb of Thebes a papyrus was discovered where polyuria was mentioned. It was sold to the German Egyptologist Georg Ebers in 1872 and named after him as the Ebers Papyrus. Even though the Ebers papyrus was written about 1550 BC, evidence suggests that it was copied from a series of books from 3400 BC (2, 3).

Aretus of Cappodocia from ancient Greece (81-133 AD) was first to use the term "diabetes", which came from the Greek word for siphon (4). The clinical diagnosis of diabetes with polyuria and glycosuria was described by the Hindu physicians Charaka, Susruta and Vaghbata. They found that the urine of those affected attracted flies and ants, and they called it "honey urine" (3).

The word mellitus (honey sweet) was added by the British physician Thomas Willis in 1675 when he as the first European discovered the sweetness of urine in patients with diabetes (5). In 1776, Doctor Matthew Dobson from Manchester did experiments showing that sugar was present in both urine and blood of diabetic patients.

Another important man in the history of diabetes was the Frenchman Claude Bernard, who through experiments in the early 19th century discovered the role of the liver in glycogenesis. It was the German medical student, Paul Langerhans who first found the pancreatic islets cells in 1869, but did not know their function (2, 3, 6). Later, in 1893, the French histologist Gustave Laguesse named the islet cells "islets of Langerhans" after their discoverer (7, 8).

In 1889, German diabetologist Oscar Minkowski and pharmacist Joseph von Mering demonstrated that removal of the pancreas from a dog led to development of diabetes in

the dog. Insulin was discovered not long thereafter. It was the young physician, Frederick Banting, who thought it might be possible to isolate the internal secretions of the pancreas by ligating the pancreatic ducts to induce atrophy of the acinar cells and thereby minimize contamination of the tissue extract with digestive enzymes. Banting presented his suggestion to J.J.R. Macleod, a physiologist at the University of Toronto who provided Banting with a laboratory for the summer and some dogs for the experiments. Macleod also assigned Charles Best, a young student, to work as Banting's assistant for the summer. During the summer of 1921, Banting and Best made remarkable progress, and by fall they had isolated material from pancreas extracts that dramatically prolonged the life of dogs made diabetic by removal of the pancreas. In the winter of 1922, Banting and Best treated their first human patient, a 14-year old boy named Leonard Thompson, whose life was saved by the treatment (9).

After that, the Eli Lilly Company was brought in to collaborate in the production and manufacture of insulin. By 1923, insulin was available in quantities adequate for relatively widespread treatment of diabetes. In 1923, the Nobel Prize in Medicine was awarded to Banting and Macleod. To acknowledge Best's role in the discovery of insulin, Banting shared his prize with him (2).

History of gestational diabetes mellitus

Gestational diabetes mellitus (GDM) was first described in 1823 by the German physician Heinrich Bennewitz, who described thirst and polyuria in a pregnant woman. He considered that diabetes actually was a symptom of the pregnancy, since the symptoms and the glycosuria disappeared after pregnancy (10). Studies in the 1940s and 1950s showed that a lesser degree of maternal hyperglycaemia during pregnancy also was a risk for pregnancy outcome and increased perinatal mortality (11-13). The Belgian researcher J.P. Hoet published a study called "Carbohydrate Metabolism during Pregnancy" in French and was the first to use the term "metagestational diabetes". The paper was translated into English by doctor F.D.W. Lukens and published in Diabetes 1954 (14).

The modern term "gestational diabetes" was used by John B O'Sullivan in 1961 and is said to have been used instead of the more neutral "Carbohydrate Intolerance of Pregnancy", because the authorities thought women should take the diagnosis more seriously.

In 1964 John B. O'Sullivan performed a 100 gram 3-hour oral glucose tolerance test (OGTT) in 752 pregnant women during mainly the second or third trimester. From this material the first, second and third standard deviation (SD) upper limits for these glucose values were published, which were the first statistically based criteria for assessing the upper limit of glycaemic normality in pregnancy. The O'Sullivan criteria, published with statistician Claire Mahan, were the standard for diabetes detection in pregnancy for the next 40 years (15).

Jorge H. Mestman showed at about the same time an increased rate of perinatal mortality associated with abnormal glucose tolerance in southern California. The population consisted of more than 60% Latino women (16).

In October 1979, doctor Norbert Freinkel (representing the American Diabetes Association) and doctor John Josimovich (representing the American College of Obstetricians and Gynaecologists) met in Chicago at the First International Workshop Conference on Gestational Diabetes Mellitus. Experts from around the world attended this meeting and shared their clinical experience, research, and opinions about GDM. During this and the next coming International Workshop Conferences on GDM held in 1984 and 1990 a definition of GDM was established (17).

History of autoantibodies

Islet cell antibody (ICA) was the first discovered autoantibody against the pancreatic beta-cells, results published by GF Bottazzo in the Lancet 1974 (18). Richard Lendrum was another scientist who studied ICA at the same time period and he demonstrated that the prevalence of ICAs fell with increasing duration of the disease (19). ICA is analysed by immunofluorescence with human pancreas of blood type O as antigen (20). In 71-86% of patients with newly diagnosed type 1 diabetes, ICA are detected (21, 22) and the prevalence in the general background population (schoolchildren) is 0.9-2.8% (23, 24).

Insulin autoantibody (IAA) was discovered next (25), and is detected in 43-69% of type 1 diabetes patients. It can only be measured before exogenous insulin treatment has begun, since antibodies also form against exogenous insulin, which leads to a cross reaction (21, 26). The prevalence in the general background population (schoolchildren) is 0.9-3.0% (22, 24).

Glutamic acid decarboxylase (GAD) is an enzyme that catalyses the decarboxylation of glutamate to GABA and CO2 production. GAD exists in two isoforms, GAD67, **Figure 1**, and GAD65, **Figure 2**, with molecular weights of 67 and 65 kDa, respectively.

GAD67 and GAD65 are expressed in the central nervous system, where GABA is used as a neurotransmitter. GAD65 is also expressed in the pancreas. Autoantibodies against glutamic acid decarboxylase, GAD67, were found in patients with the rare neurological disease Stiff-man syndrome, and when GAD67 cross reacted with GAD65 this lead to the discovery of this type 1 diabetes specific autoantibody, GADA (26-29). The prevalence of GADA in the general background population (schoolchildren) is 0.5-3.0% (23, 24) and GADA are found in about 70% of patients with type 1 diabetes (21, 22).

Figure 1.
X-ray crystal structure of GAD67 (Wikimedia Commons).

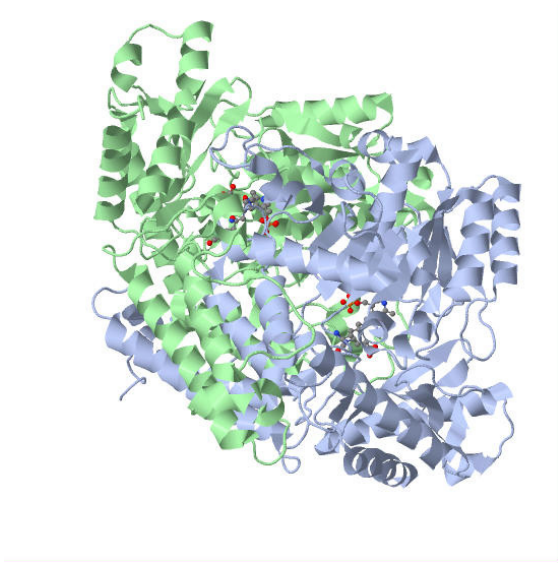
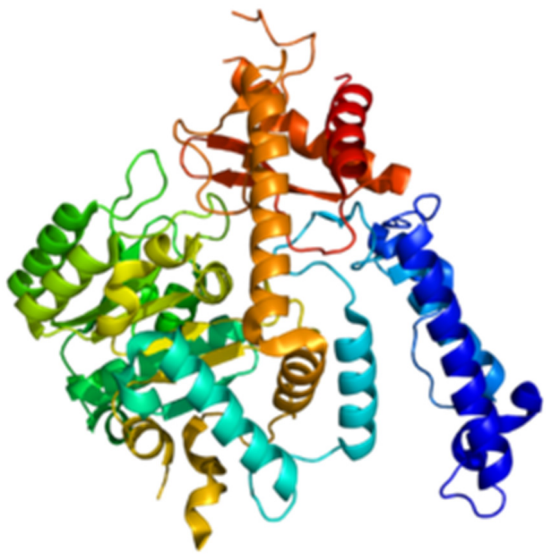


Figure 2.
X-ray crystal structure of GAD65 (Emw, Wikimedia Commons).



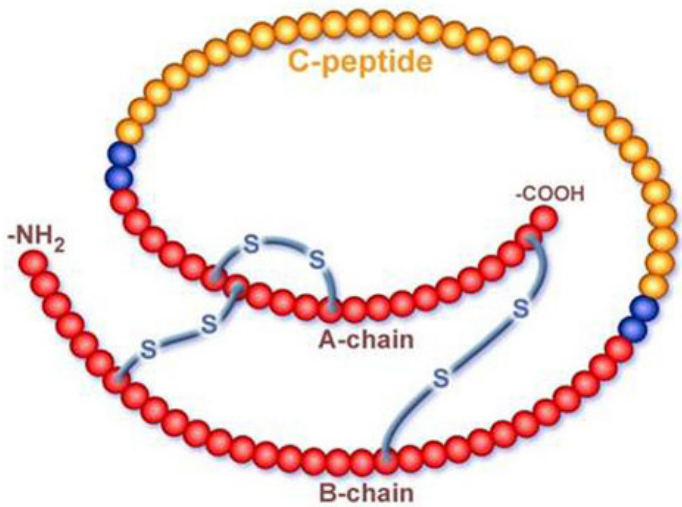
Another autoantibody in autoimmune diabetes is the tyrosine phosphatase antibody (IA-2A), against a trans-membrane protein in the beta-cells (30). IA-2A is detected in 59-80% of type 1 diabetes patients (31, 22) and in the general background population (schoolchildren) the prevalence of IA-2A is 0.6-2.4% (23, 24).

A new major diabetes auto-antigen was identified a few years ago, a member of the zinc transporter family (ZnT8), which is expressed in pancreatic alpha- and beta-cells. It is localized in the membrane of the insulin secretory granules and facilitates the accumulation of zinc from the cytoplasm in intracellular insulin containing vesicles, and plays a major role in providing zinc for insulin maturation and/or storage processes (32-35). Studies show that ZnT8A is a good complement to GADA and IA-2, in particular as a marker of adult-onset autoimmune diabetes (36). However, the role of ZnT8A as an autoimmune marker during GDM is less well studied.

History of C-peptide

C-peptide was first described in 1967 by D.F. Steiner and is a stable marker for endogenous insulin production. From the beta-cells, preproinsulin is secreted with an A-chain, C-peptide, a B-chain, and a signal sequence. The signal sequence is cut off, leaving proinsulin. Then the C-peptide is cut off, leaving the A-chain and B-chain to form insulin and both are secreted in equal amounts into the portal circulation (37), **Figure 3**. C-peptide assays are widely used for evaluation of the beta-cell reserve (38) and differential diagnosis between type 1 and type 2 diabetes (39). Compared to insulin measurements, determination of C-peptide is preferable; reflecting beta-cell production of insulin irrespective of treatment with exogenous insulin, and as insulin rapidly is eliminated from the circulation by the liver with an individual variation (40).

Figure 3.
Proinsulin consisting of an A-chain, C-peptide, and a B-chain. After C-peptide is cut off, the A-chain and B-chain form insulin.



Classification of diabetes mellitus

Type 1 diabetes

Type 1 diabetes is sometimes called insulin-dependent, immune-mediated or juvenile-onset diabetes. This form of diabetes is caused by a cellular mediated autoimmune destruction of the insulin producing beta-cells in the pancreas. The reason why this occurs is not fully understood and is related to multiple genetic predispositions and environmental factors.

Markers of the autoimmune process such as ICA, IAA, GADA and IA-2A are present in 85–90% of individuals at their onset of autoimmune diabetes (1, 41-43). There is also a strong association between type 1 diabetes and the human leukocyte antigen (HLA) region on chromosome 6p2 and the DQA and DQB genes (44-45). The disease can affect people of any age, but usually occurs in children or young adults and the progression of the disease is variable. Younger patients usually have a more rapid progression, often together with ketoacidosis (46). Patients with type 1 diabetes always need insulin treatment, since the majority of the beta-cells are destroyed. At present, type 1 diabetes cannot be prevented (1, 41-43).

For women with type 1 diabetes, pregnancy can lead to different complications. In a UK study, the perinatal mortality in babies of women with type 1 diabetes was 3.2% and the prevalence of major congenital anomalies was 4.8% (47). A study from the Netherlands showed congenital malformations in 8.8% (5.5% for major congenital malformations) and perinatal mortality in 2.8% of babies to women with type 1 diabetes (48).

Type 2 diabetes

Type 2 diabetes is sometimes called non-insulin dependent diabetes or adult-onset diabetes, and is characterized by relative insulin deficiency and insulin resistance, either of which may be the predominant feature. At least initially, and often through many years, these patients do not need insulin treatment. The diagnosis is more common among older people and overrepresented among obese patients. Type 2 diabetes can remain undetected for many years and is often incidentally discovered after associated complications or at regular health controls (1, 41, 49). By maintaining a healthy weight and being physically active, type 2 diabetes can be prevented, or at least delayed in many cases (50, 51).

As in type 1 diabetes, pregnancies with type 2 diabetes can lead to complications. In a UK study during 1990-2002, the rate of perinatal mortality was 2.5% and congenital malformation was 9.9% (52). Another large study from UK showed a perinatal mortality of 3.2% and that the prevalence of major congenital anomalies was 4.3% (47). When comparing pregnancy outcomes in type 1 and type 2 diabetes, some studies show almost

the same rate of malformation and mortality (47, 53, 54), or even higher rates (55) in type 2 than in type 1 diabetes.

Gestational diabetes mellitus

GDM was for many years defined as "any degree of glucose intolerance with onset or first recognition during pregnancy" (56, 57). Even though GDM often resolves after delivery, the definition applied whether or not the condition persisted after pregnancy. Therefore, it did not exclude the possibility that the glucose intolerance could have antedated or begun concomitantly with the pregnancy. Though the limitations of this definition were apparent for many years, the definition remained. Because the number of women with overweight, obesity and diabetes continue to increase, the number of pregnant women with undiagnosed type 2 diabetes has increased. Therefore, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommend that high risk women, where diabetes is found at their initial prenatal visit, receive the diagnosis overt diabetes instead of the GDM diagnosis (1).

GDM, depending on the population studied, affects 1-14% of all pregnant women (1). In Sweden 2% of pregnancies are complicated by GDM (58). GDM is often more common in populations with a high frequency of type 2 diabetes, such as India and China (59). It is well known that women with GDM have a substantial risk of developing type 2 diabetes later in life (60), but the risk of developing type 1 diabetes is also increased (61).

Other specific types of diabetes also exist, but will not be further discussed in this dissertation.

Epidemiology of diabetes mellitus

In the year 2000, the World Health Organization (WHO) estimated that there were 171 million people in the world with diabetes (62). The International Diabetes Federation (IDF) estimated in 2011 the number at 366 million (of which 183 million are undiagnosed) and in 2030 at total of 552 million people are expected to have diabetes (63). Diabetes is most common between 40-59 years of age and 80% of people with diabetes live in low–income and middle-income countries (63).

There is a more than 350-fold difference in the incidence among the 100 populations worldwide. The highest incidences of type 1 diabetes are found in Finland, Sardinia and Sweden (64-67). The lowest incidences of type 1 diabetes are found in China and Venezuela (67-69). The five countries with the greatest number of people with type 2 diabetes are India, China, USA, Indonesia and Japan (70-72).

In Sweden about 365 000 people have diabetes and 40 000 of them have type 1 diabetes (73). Worldwide, type 1 diabetes approximately accounts for 5-10% whereas type 2 diabetes accounts for approximately 90-95% of the total diabetes incidence (1). The American Diabetes Association (ADA) estimated the national costs in the USA of diabetes for 2002 at USD 132 billion dollars (74) and in 2011 the costs were USD 465 billion dollars (63).

Diagnostic criteria for diabetes mellitus

WHO has published guidelines for the diagnosis and classification of diabetes since 1965. The current guidelines were published in 2006 (75) and are listed in **Table 1** together with the diagnostic criteria for impaired glucose tolerance.

Table 1.
Diagnostic criteria in plasma glucose levels for diabetes mellitus and for impaired glucose tolerance, according to WHO.

	Venous plasma glucose (mmol/l)	**Capillary plasma glucose (mmol/l)**
Diabetes Mellitus		
Fasting[1]	≥7.0	≥7.0
2-hour OGTT[2]	≥11.1	≥12.2
Impaired glucose tolerance		
Fasting	≥6.1-6.9	≥6.1-6.9
2-hour OGTT	≥7.8- 11.0	≥8.9- 12.1

[1]After overnight fasting of eight hours
[2]OGGT=oral glucose tolerance test consisting of a 75 gram glucose solution

Diagnostic criteria for gestational diabetes mellitus

During the years there have been different screening methods and different criteria for diagnosis of GDM. Complications during pregnancy and the early postnatal period due to GDM for both mother and child are extensively studied.

The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study published in 2008, was the result of a large, multicentre, multinational observational study (25 000 pregnant women) that examined the relationship between maternal hyperglycaemia less severe than overt diabetes mellitus and adverse pregnancy outcomes. The study showed that the risk of large for gestational age infants, increased cord blood C-peptide levels (evidence of foetal hyperinsulinemia), neonatal hypoglycaemia, and caesarean delivery increased with the mother's glucose levels, even if they were below the value for GDM (76).

Since then, the IADPSG has come with new recommendations for the diagnosis and classification of hyperglycaemia during pregnancy. They recommend that all women without known diabetes undergo a 75 gram, 2-hour OGGT at 24-28 weeks of gestation. For GDM diagnosis at least one of the following plasma glucose values should be exceeded: Fasting: ≥5.1 mmol/l, 1-hour value of the OGGT: ≥10.0 mmol/l or 2-hour value of the OGGT ≥8.5 mmol/l (77). There is yet no evidence that identification and treatment of women based on these recommendations will lead to clinically significant improvements in maternal and neonatal outcomes, but it would lead to a significant increase in health care costs.

The WHO current guidelines for GDM were published in 1999 and are widely used worldwide. WHO also recommends a 75 gram 2-hour OGGT but with a 2-hour value of the OGGT ≥7.8 mmol/l (41).

The Diabetic Pregnancy Study Group of the European Association for the Study of Diabetes (EASD) also suggests a 75 gram 2-hour OGGT but with different diagnostic values (78). The 2-hour OGTT capillary plasma glucose value for defining GDM is >10.0 mmol/l, or >9.0 mmol/l for capillary blood glucose (used before 2004) (58). These criteria are used in Sweden and Denmark (58, 79). In our region in Sweden since around 1990, a 2-hour OGTT in the 28th gestational week is performed on every pregnant woman as a screening for GDM. Women with prior GDM and/or heredity for diabetes are tested already during the 12th gestational week (58).

Using HbA1c in general GDM screening instead of the OGTT has been studied, but is still controversial and can lead to misclassification (80). Because of changes during pregnancy, HbA1c decreases and normal reference intervals can therefore not be used (81). OGTT is still the gold standard when screening for GDM.

However, there is still today no universal recommendation for the ideal approach for screening and diagnosis of GDM.

Table 2 shows the different diagnostic criteria for GDM.

Table 2.
Diagnostic criteria in plasma glucose levels for gestational diabetes.

	IADPSG (mmol/l)	WHO (mmol/l)	EASD (mmol/l)
Gestational diabetes			
Fasting[1]	≥5.1		
1-hour OGTT[2]	≥10.0		
2-hour OGTT	≥8.5	≥7.8	≥10.0

[1]After overnight fasting of eight hours
[2]OGGT=oral glucose tolerance test consisting of a 75 gram glucose solution

Changes during pregnancy with gestational diabetes mellitus

Metabolism

In a pregnancy complicated by GDM, the same metabolic changes occur as in a normal pregnancy. During pregnancy, the mother's metabolism is adapted to supply nutrients to the foetus for its growth. Glucose is the main nutrient that crosses the placenta and constitutes the primary energy source for the foetus. Early during pregnancy basal plasma glucose, hepatic gluconeogenesis and insulin levels are unchanged (82). But during late pregnancy the mother develops basal hypoglycaemia, which is due to the high rate of placental transfer, despite reduced glucose consumption (because of insulin resistance) and enhanced gluconeogenesis (83). The placental transfer of glucose is carried out by facilitated diffusion by different glucose transporters (GLUT) and concentration dependent kinetics (84).

In the first half of pregnancy, there is storage of energy and nutrients due to maternal changes. The appetite of the mother is increased and the insulin sensitivity is normal or increased. This leads to an increase in the lipid store (82, 85). During the second half of pregnancy, the stored reserves are used for foetal and placental growth. The insulin resistance also increases during this time and leads to a decreased uptake of glucose by maternal tissues sensitive to insulin, such as muscle and adipose tissues (86).

Insulin resistance

The mechanisms behind insulin resistance induced by the pregnancy per se are still not fully understood. In pregnant rats (are believed to be similar to humans) the degradation of insulin by the placenta is increased, which leads to accelerated insulin removal (85). There are also different hormonal and metabolic changes during the second half of pregnancy which facilitate insulin resistance. One is the high plasma level of progesterone during the second part of pregnancy (88-90).

GDM is associated with both insulin resistance and an impaired insulin secretion (91-93). There is a lack of insulin during a period of time with high insulin needs, to compensate the insulin resistance that develops during the third trimester of pregnancy. In the maternal tissues where glucose uptake is insulin-dependent, the uptake is decreased because of the lack of insulin and postprandial hyperglycaemia develops. Since the maternal-placental-foetal transfer of glucose is concentration dependent, the hyperglycaemia of the mother leads to an increased placental transfer of glucose to the foetus. This leads to foetal hyperglycaemia and hyperinsulinism. Because insulin is one of the main growth factors for the foetus, the hyperinsulinism leads to macrosomia and can cause delivery complications such as shoulder dystocia (94).

The hyperinsulinism remains in the newborn after delivery and once the umbilical supply of glucose has disappeared, the risk of hypoglycaemia is increased. Early feeding of the newborn is important as well as the monitoring of their blood glucose levels, since untreated hypoglycaemia can lead to brain damage (95).

Future risk for the mother

Women with GDM have an increased risk of developing diabetes later in life. Studies have shown an incidence between 2.6-70% (60, 96). However, one has to remember that it is difficult to compare and evaluate risks for developing diabetes, since diagnostic tests and criteria vary. Studies have shown that women with insulin treatment during their GDM have a higher risk of developing overt diabetes, than women treated with diet only (97, 98). Other specific risk factors for development of diabetes after GDM are body mass index (BMI) >30 kg/m^2 and at least two pregnancies before the GDM pregnancy (98). Higher fasting blood glucose levels, higher OGTT 2-hour values and a higher OGTT glucose area under the curve, are strong predictors of later development of diabetes (99).

Because of the increasing prevalence of diabetes worldwide (100), early diagnosis and prevention is proving increasingly important. Since type 2 diabetes can be asymptomatic during at least 4-7 years before the clinical diagnosis, many patients already have developed micro- or macro-vascular complications at diagnosis (101, 102).

It is of uttermost importance that women with a prior GDM are offered appropriate follow-up and advised to lose weight after pregnancy (if they are overweight or obese), to maintain a healthy diet and exercise regularly. In preventing diabetes, life styles changes seem to be more effective than pharmalogical intervention (103).

All women with GDM are followed up at our Department of Endocrinology with an annual OGTT during the first two years postpartum, and with an additional OGTT at five years postpartum. If they do develop type 2 diabetes, they are retransferred to their Health Care Centre. In women who are autoantibody positive during their pregnancy, the first OGTT is performed already three months postpartum, and those who develop type 1 diabetes are followed up at our Department of Endocrinology.

Future risk for the child

Short term complications for the newborn after a pregnancy with GDM can be both metabolic and hematologic. Known complications are hypoglycaemia, hypocalcaemia, hypomagnesia, macrosomia, polycythaemia, hyperbilirubinemia and congenital malformations (104-108). Long term complications consist of an increased risk for overweight, obesity and the metabolic syndrome (obesity, insulin resistance, hypertension, dyslipidaemia and glucose intolerance) (109-111). Studies have also shown that children born to mothers with a prior GDM have an increased risk for deficient neurological and psychological development. The proposed mechanisms behind this are birth trauma (112) and prolonged severe hypoglycaemia (113).

It is of general belief that an intrauterine environment complicated by maternal diabetes increases the risk for overweight and obesity in the offspring (114-117). But overweight among women with GDM per se can also increase risk for overweight and obesity in their offspring (118-120). In 2011, a systematic review of the relationship between GDM and childhood obesity was published. A total of 192 articles were found concerning this topic, and 12 of them were thoroughly examined. The conclusion was that it is still impossible to distinguish between maternal obesity and GDM as the cause of a higher risk for overweight and obesity in the offspring (121). With the exception of the high birth weight, it is unclear at which age overweight starts to appear in children (122). Simultaneously, overweight and obesity are increasing rapidly among children in the world, and about 17.6 million children are estimated to be overweight (123). Among school-aged children around the world, 10% are estimated to be overweight and 25% of these children are obese (124). The prevalence of overweight is also increasing in European countries (125), including Sweden (126).

Data from the European Childhood Obesity Group show that during the last 20-30 years obesity has increased steadily in Europe (125, 127), especially in southern Europe (128, 129). In northern Europe the prevalence of overweight and obesity is still lower, with an overweight prevalence of 10–20%, compared to 20–35% in southern Europe (125). The

reasons for these differences are still not clear, but could perhaps consist of a combination of economic and social factors. Many children, especially adolescents, continue to be overweight and obese throughout their adulthood (130, 131).

Today, there is still no consensus regarding intervention in this group of women with GDM and their offspring, and more studies are needed on this topic.

Aims

- Determine how many women with GDM that have beta-cell specific autoantibody markers during pregnancy and follow these women after delivery to estimate the risk for later development of type 1 diabetes.

- Estimate the frequency of ZnT8A in patients with GDM and evaluate its importance as an autoimmune marker in GDM.

- Evaluate C-peptide levels in women with GDM as a predictor for future development of diabetes. Investigate the role of C-peptide in relation to other birth related factors.

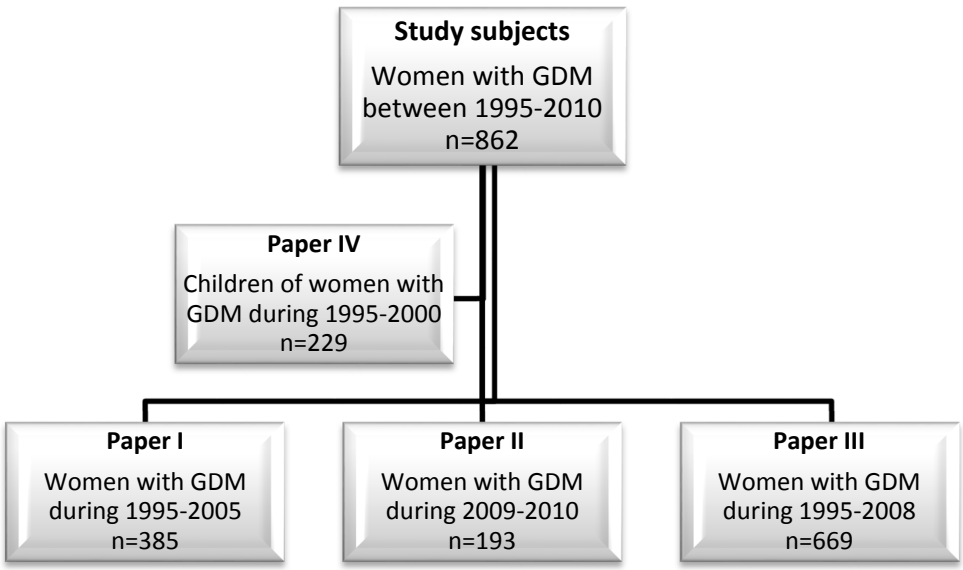
- Investigate the effects of maternal GDM on childhood height, weight and BMI compared to the age-specific reference values in Sweden. Compare the BMI of these children with that of their siblings born after non-GDM pregnancies.

Materials and Methods

Subjects

In this thesis, women diagnosed with GDM during 1995-2010 (n=862) in the district of Lund in Sweden have been studied, as well as the children of these women, illustrated in **Figure 4.**

Figure 4.
Schematic view of subjects in this thesis.



In our district, a 2-hour OGTT is performed in every pregnant woman in the 28th gestational week as a screening for GDM. Women with prior GDM and/or heredity for diabetes are tested already during the 12th gestational week. The 2-hour OGTT capillary plasma glucose value for defining GDM was ≥10.0 mmol/l, or ≥9.0 mmol/l for capillary blood glucose (58).

Paper I

In this paper, women who had GDM during 1995-2005 (n=385) were tested for the autoantibodies GAD and IA-2. There were 24 women (6.2%) with GDM that were positive for at least one autoantibody. Two control subjects who also had GDM, but without autoantibodies, were selected for each woman (n=48). The control subjects were matched for age ±5 years and year of delivery. The medical records from the two groups of women were examined and compared. Frequency of women who had developed diabetes was also noted. At follow-up, the women who were autoantibody positive during their pregnancy with GDM and had developed diabetes were asked to reanalyse GADA and IA-2A. If they had not developed diabetes at follow-up they also underwent a new OGGT.

Paper II

In this study, women who had GDM during 2009-2010 were investigated (n=193) and tested for GADA, IA-2A and ZnT8A. A total of 19 women (9.8%) were positive for at least one autoantibody. The women's medical records from their GDM pregnancy were also examined.

Paper III

Women who were diagnosed with GDM during 1995-2008 (n=669) were included in this study and tested for GADA and IA-2A. There were 34 women (5.1%) with GDM that were positive for at least one autoantibody and their medical records were examined regarding later development of diabetes. C-peptide levels were also measured in women with GDM during 2006-2008 (n=281) and the role of C-peptide for later development of diabetes and other birth related factors were studied. Three women had GDM twice during this time period and only their first pregnancy was used for analysis in this study.

Paper IV

Children born to women with GDM during 1995-2000, and their siblings born after pregnancies without GDM, were examined in this study. There were 204 pregnancies with GDM, corresponding to 189 women. Among these 189 women, 14 women chose not to participate in the study. Written consent to contact the children's Health Care Centre and their present school for data on height and weight measurements were obtained from the parents. The women were also asked to report their own and the children's fathers' present height and weight. Finally, 110 of 175 women (63%) chose to participate in the study. These women had in total given birth to 235 children, including three twin pregnancies, which meant 232 pregnancies. The six children from duplex pregnancies were excluded. In 151 of the pregnancies the women were diagnosed with GDM. The children were compared at ages 0, 0.5, 1, 1.5, 4, 5, 6, 7, 8, 10 and 12 years. Swedish population based reference values for height, weight (132) and the age-specific BMI references values for Swedish children (133, 134) were used for comparison. For the parents, the international BMI (kg/m^2) thresholds of ≥25 and ≥30 respectively were used for defining overweight and obesity (135).

Analyses

Islet cell antibodies (ICA)

ICA (**Paper I**) were analysed by a two-colour immunofluorescence method. Human pancreas of blood type 0 was used as antigen (20). The samples were diluted until negative. Thereafter, the highest positive titre for each sample was converted to Juvenile Diabetes Foundation units (JDF-U) according to a standard curve for the specific pancreas used. A cut-off equal or above 6 JDF-U, was considered positive. The sensitivity was 100% and the specificity 88% when tested in the International Diabetes Workshop (136).

Glutamic acid decarboxylase antibodies (GADA)

GADA (**Paper I-III**) were analysed in a radioimmunoprecipitation assay (137) with in vitro translated human GAD65 that was antigen labelled with ^{35}S-methionine. An index, calibrated to a positive and negative standard expressed the levels. GADA indexes <0.08 were defined as negative and represented values below the 97.5th percentile. The GADA assay had a sensitivity of 70% and a specificity of 100% when tested in the Diabetes Antibody Standardization Program (138). Since 2006 GADA are analysed using a commercial enzyme linked immunosorbent assay (ELISA) supplied by RSR® Ltd, Cardiff, UK and performed according to manufacturer's instructions. The cut-off levels for

positivity were 10 U/ml for GADA. The specificity was 94% and the sensitivity was 90% when tested in the Diabetes Antibody Standardization Program 2010 (unpublished data).

Tyrosine phosphatase antibodies (IA-2A)

IA-2A (**Paper I-III**) were also analysed in radioimmunoprecipitation assays (139) with in vitro translated human IA-2A that was antigen labelled with ^{35}S-methionine. An index, calibrated to a positive and negative standard, expressed the levels. IA-2A indexes <0.05 were defined as negative and represented values below the 97.5th percentile. The IA-2A assay had a sensitivity of 50% and a specificity of 100%, when tested in the Diabetes Antibody Standardization Program (138). Since 2006, assays for IA2A are analysed using ELISA supplied by RSR® Ltd, Cardiff, UK and performed according to manufacturer's instructions. The cut-off levels for positivity were 15 U/ml for IA-2A. The specificity for IA-2A was 100% and the sensitivity was 64% in the Diabetes Antibody Standardization Program 2010 (unpublished data).

Zink transporter 8 antibodies (ZnT8A)

ZnT8A (**Paper II**) were analysed using ELISA from RSR Ltd®, Cardiff, UK according to the manufacturer's instructions. The cut-off levels for positivity were 15 U/ml. The reported specificity was 99% and the sensitivity was 68% in the Diabetes Antibody Standardization Program 2010 (unpublished data).

C-peptide

C-peptide levels (**Paper II**) were analysed with a commercial ELISA from (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions. The detection limit of the assay was 15 pmol/l. The samples for C-peptide (**Paper III**) were analysed by radioimmunoassay, using a commercial kit (Euro-Diagnostica, Malmö, Sweden). The reference range (only defined for fasting condition) was 0.25-1.0 nmol/l. The detection limit of the assay was 0.13 nmol/l.

Statistical methods

A p-value of <0.05 was considered significant in all papers.

Paper I

Since the values were not normally distributed, the results are shown as median and interquartile range. Mann-Whitney U test is a non-parametric test which is used to investigate whether the values of a certain variable tend to be higher in one of two study groups, and was used for comparison of levels. For comparison of frequencies for categorical data, the Chi-square test was used and Fischer's exact test when working with low numbers. The frequencies are shown as numbers and percent. For the analyses, the programme Statistical Package for the Social Sciences for Mac (version 11.0) was used.

Paper II

D'Agostino-Pearson test was used to examine normal distribution of data. Mean ±SD is shown when normality was accepted. Moreover, median and interquartile range was used when normality was rejected. Spearman's rho (r_s) is a non-parametric measure of statistical dependence between two variables, and was used for testing correlations. Mann-Whitney U test was used to test for differences between groups. The software MedCalc® for Windows (version 12.1.4) was used for statistical analyses.

Paper III

Normal distribution was tested with D'Agostino-Pearson test. Results that were normally distributed are presented as mean ±SD. The T-test was used for comparison between groups. The non-parametric results are presented as median and range, and Mann-Whitney U test was used for comparison between groups. The frequencies are presented as numbers and percent, and were compared using Chi-square test. Correlations were tested using Spearman's rho (r_s) correlation test. A Kaplan-Meier Survival Analysis is a method of estimating time-to-event models in the presence of censored cases and was used to examine the time from GDM with autoantibodies until the development of type 1 diabetes. The programme Statistical Package for the Social Sciences for Windows (version 17.0) and MedCalc® for Windows (version 12.0.3.0) was used.

Paper IV

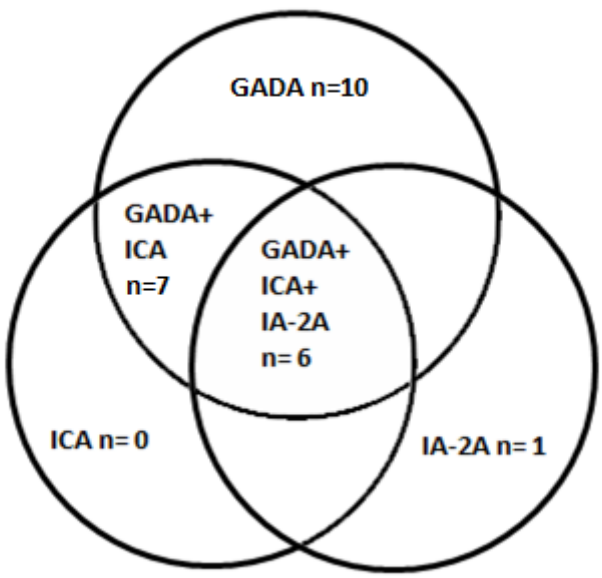
Normal distribution was tested with D'Agostino-Pearson test. Results are presented as mean ±SD, and the children's height (cm), weight (kg) and BMI (kg/m^2) were compared to Swedish reference values. Non-parametric results are presented as median and range. The T-test for two independent parametric samples was used for comparing height, weight and BMI between groups. The frequencies are presented as numbers and percent. The programme Statistical Package for the Social Sciences for Windows (version 17.0) and MedCalc® for Windows (version 12.0.3.0) was used for analyses.

Results

Paper I

There were 385 women with GDM between the years 1995-2005, and 24 women (6.2%) were autoantibody positive. Among these 24 autoantibody positive women 95.8% (23 of 24) were positive for GADA and 29.2% (7 of 24) were positive for IA-2A. Only 22 of 24 women had been tested for ICA, and 59.1% (13 of 22) were found positive for ICA. Positivity for at least two autoantibodies was found in 54.2% (13 of 24) of the women and 27.3% (6 of 22) women were found positive for all three autoantibodies. A schematic illustration of the number of autoantibodies among the 24 women is shown in **Figure 5**.

Figure 5.
Schematic view of autoantibodies among the 24 autoantibody positive women. Only 22 women were tested for ICA.



The medical records of the 24 autoantibody positive women were compared to the 48 control subjects who also had GDM but without autoantibodies. The results are shown in **Table 3.**

Table 3.
Characteristics for the autoantibody positive women with GDM and their control subjects.

	Antibody positive women with GDM (n=24)	Antibody negative women with GDM (n=48)	p-value
Age (years)[1]	29.5 (27.0-34.0)	30.0 (27.0-34.0)	NS
BMI (kg/m^2)[2]	24.5 (22.4-28.4)	25.4 (21.9-30.1)	NS
Heredity[3]	15 (62.5)	22 (45.8)	NS
Ethnicity			
Scandinavian	21 (87.5)	37 (77.1)	NS
Non-Scandinavian	3(12.5)	11 (22.9)	NS
OGTT value during pregnancy[4]	10.0 (9.4-12.0)	9.5 (9.1-10.4)	NS
GDM during previous pregnancy	8 of 19 (42.1)	9 of 42 (45.2)	NS
Insulin during pregnancy	14 (58.3)	18 (37.5)	NS
Birth weight of the child (g)	3430 (3170-3770)	3710 (3300-4080)	NS
Caesarean delivery	5 (28.8)	8 (16.7)	NS

Data are median (interquartile range) or numbers (%), NS=not significant

[1]Age of mother at time of pregnancy, when the autoantibodies were discovered

[2]Values are from the first trimester

[3]Family history of type 1 or type 2 diabetes among first or second-degree relatives

[4]During pregnancy week 12 or 28

Follow-up

At follow-up, significantly more women had developed diabetes among the autoantibody positive women compared to the autoantibody negative women (p=0.001). In all, 50% of the 24 autoantibody positive women had developed type 1 diabetes and none had developed type 2 diabetes. Impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) was found in 20.8%. Among the 48 control subjects, 12.5% had developed type 2 diabetes and none had developed type 1 diabetes. This is illustrated in **Figure 6** and **Figure 7.**

Figure 6.
Development of diabetes among the 24 autoantibody positive women with GDM during their pregnancy.

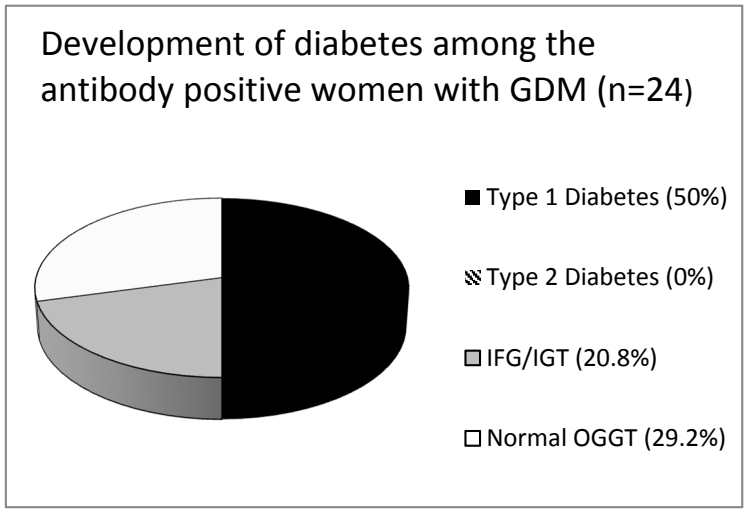
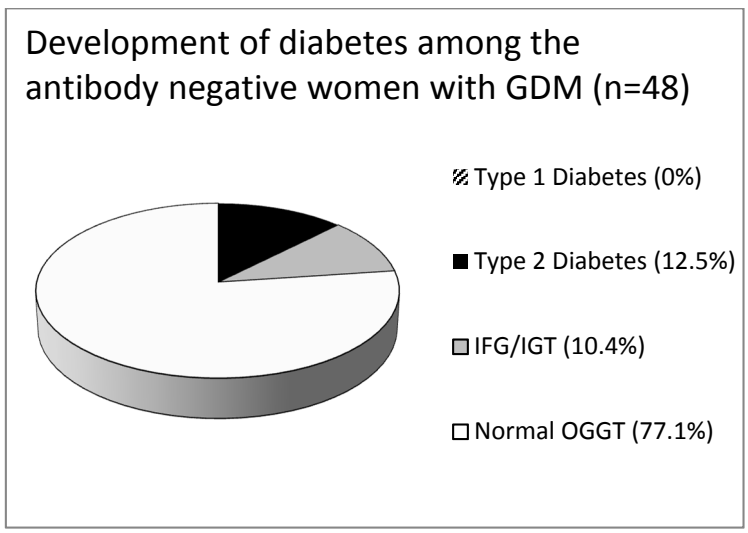


Figure 7.
Development of diabetes among the 48 autoantibody negative women with GDM during their pregnancy.

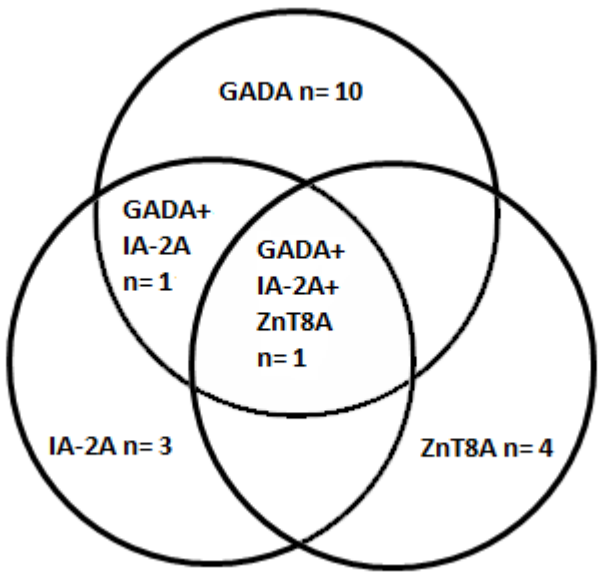


At follow-up among the 12 women who had developed type 1 diabetes after their GDM pregnancy, 6 women chose to reanalyse GADA and IA-2A. GADA persisted in 83.3% (5 of 6) and IA-2A in 33.5% (2 of 6) of these women. Among the 12 women who were not diagnosed with diabetes, 11 underwent a new OGTT and reanalysed GADA and IA-2A. GADA persisted in 81.8% (9 of 11), IA-2A in 18.2% (2 of 11) and 45.5% (5 of 11) of these women had disturbed glucose metabolism (IGT or IFG).

Paper II

Among the 193 women who had GDM during their pregnancy between the years 2009-2010, 7.8% (15 of 193) were positive for GADA and/or IA-2A. When adding ZnT8A, 9.8% (19 of 193) were positive for at least one autoantibody. GADA was found in 63.2% (12 of 19), ZnT8A in 26.3% (5 of 19) and IA-2A in 26.3% (5 of 19). This is shown in **Figure 8.**

Figure 8.
Schematic view of autoantibodies among the 19 autoantibody positive women.



C-peptide

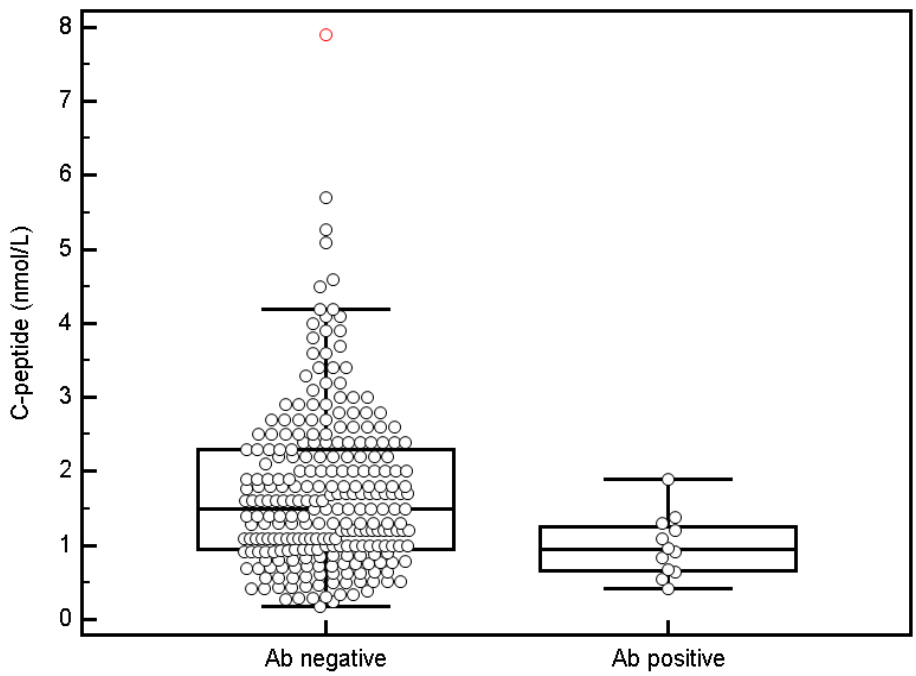
Median C-peptide levels did not differ between the group of autoantibody positive (n=19) and autoantibody negative (n=174) women. No statistically significant difference in median age was found between the group of autoantibody positive and autoantibody women. There was also no significant correlation found between C-peptide levels and GAD antibody titres or IA-2 antibody titres. However, there was a weak tendency towards high ZnT8A titres with low C-peptide levels (r_s=0.13; p=0.07).

Paper III

C-peptide in relation to later development of diabetes

C-peptide levels were compared in autoantibody negative women (n=261) and autoantibody positive women (n=12) during their pregnancy with GDM during 2006-2008 which is shown in **Figure 9**. C-peptide levels were missing from medical records in five cases. The median C-peptide levels were significantly higher in the group without autoantibodies 1.5 (0.2-7.9) than in the group with autoantibodies 0.9 (0.4-1.9) (p=0.007).

Figure 9.
Comparison of C-peptide levels in autoantibody (Ab) negative women (n=261) and autoantibody positive women (n=12) during their pregnancy with GDM.



Significantly more women had developed diabetes among the autoantibody positive group (5 of 12) compared to the autoantibody negative group (21 of 266) during 2006-2008 (p=<0.001). There was, however, no significant difference in C-peptide levels between women who later developed diabetes and those who did not.

C-peptide correlations in women with GDM

C-peptide levels correlated significantly with the women's first weight during pregnancy (r_s=0.3; p=0.0003). When comparing age, first weight of the mother during pregnancy and birth weight of the newborn between the autoantibody positive and autoantibody negative women with GDM during 2006-2008, there was no significant difference found, **Table 4.**

Table 4.
Comparison between autoantibody positive and autoantibody negative women with GDM.

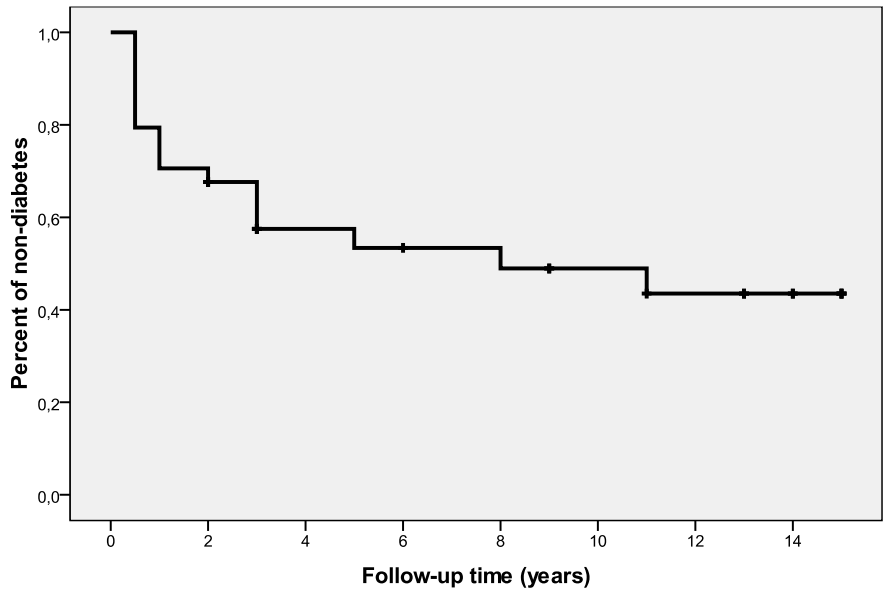
	Antibody positive women with GDM (n=12)	**Antibody negative women with GDM (n=266)**	**p-value**
Age (years)[1]	33.5 (20.0-42.0)	33.0 (17.0-44.0)	NS
First weight during pregnancy (kg)[2]	56.5 (48.0-105.0) n=8	68.0 (44.4-150.0) n=196	NS
Birth weight of the child (g)	3473.1 ±519.3 n=8	3408.7 ±541.1 n=213	NS

Data are median (range) or mean ±SD, NS=not significant
[1]Age of mother during the pregnancy
[2]Values are from the first trimester

Follow-up of the autoantibody positive women with GDM

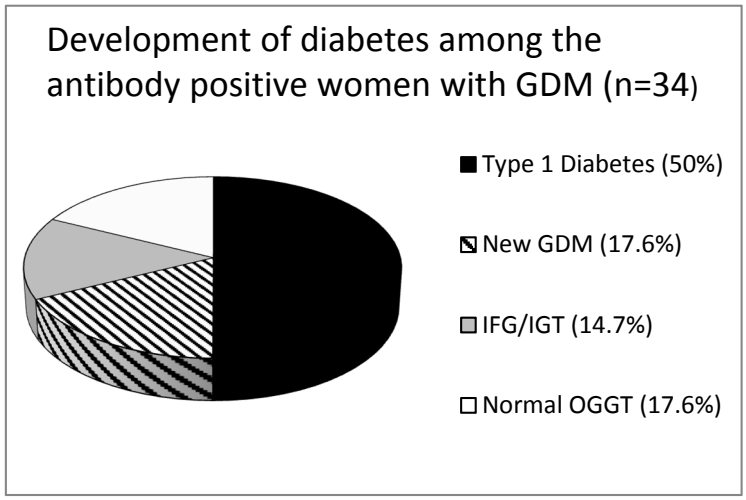
There were 34 women (5.1%) who were positive for at least one antibody and their medical records were examined regarding later development of type 1 diabetes. The follow-up time was between 2 and 15 years, with a median time of 9 years. Of the 34 women, 94.1% (32 of 34) were positive for GADA and 29.4% (10 of 34) were positive for IA-2A. In **Figure 10**, a Kaplan-Meier Survival Analysis shows the time from GDM diagnosis until development of type 1 diabetes.

Figure 10.
Kaplan-Meier Survival Analyses that shows time from GDM diagnosis until development of diabetes in the 34 autoantibody positive women.



At follow-up, 17 (50%) had developed type 1 diabetes and 5 of the 34 women had disturbed glucose metabolism (IGT or IFG). In addition, 6 of the 34 were pregnant again with GDM and positive autoantibodies. The data is shown in **Figure 11**.

Figure 11.
Development of diabetes among the 34 autoantibody positive women with GDM during their pregnancy.



Paper IV

When comparing the height in male children, where the mother had GDM during her pregnancy, to Swedish reference values, height was significantly greater at birth, **Figure 12**. For female children where the mother had GDM during her pregnancy, height was significantly grosser at birth, ages 0.5-5 years and ages 7-10 years compared to Swedish reference values, **Figure 13**.

Figure 12.
Height was significantly greater at birth for male children where the mother had GDM, compared to Swedish reference values.

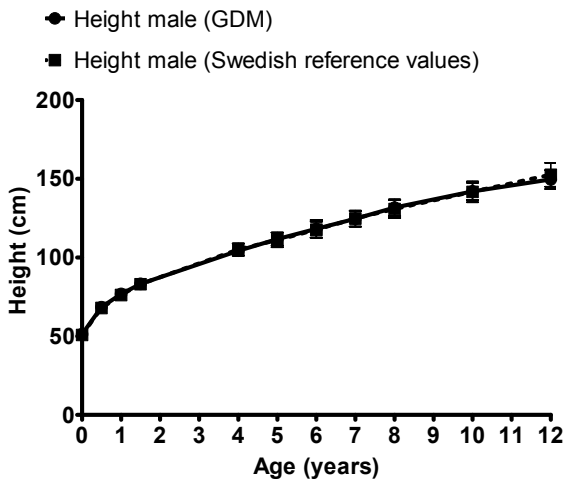
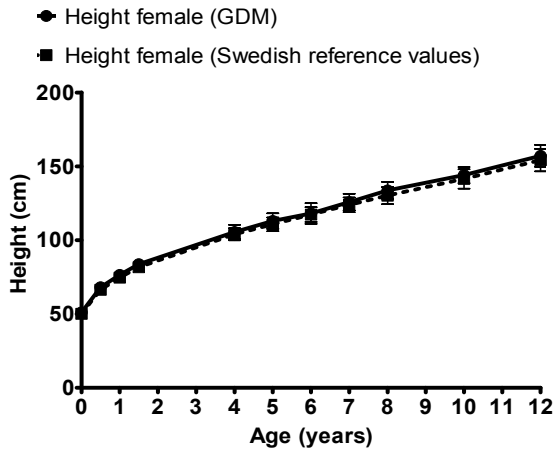


Figure 13.
Height was significantly greater at birth and ages 0.5-5 years and at ages 7-10 years for female children where the mother had GDM, compared to Swedish reference values.



Weight of male children where the mother had GDM during her pregnancy was significantly lower at age 1.5 year and higher at birth and ages 8-10 years, compared to Swedish reference values, **Figure 14**. When comparing weight for female children where the mother had GDM during her pregnancy, to Swedish reference weight values, weight was significantly higher at birth, age 0.5 year and at ages 4-12 years, **Figure 15**.

Figure 14.
Weight was significantly lower at age 1.5 year, higher at birth and ages 8-10 years for male children where the mother had GDM, compared to Swedish reference values.

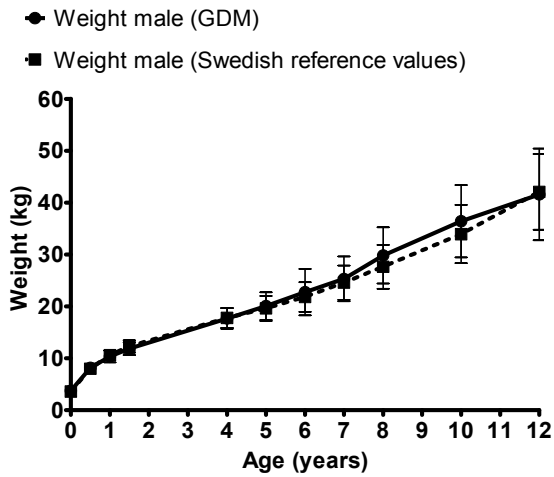
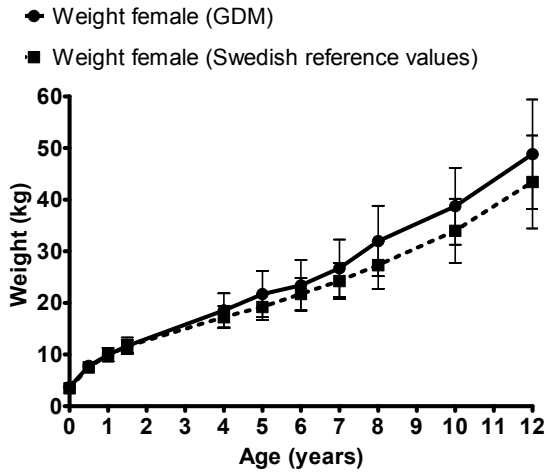


Figure 15.
Weight was significantly higher at birth, age 0.5 year and ages 4-12 years for female children where the mother had GDM, compared to Swedish reference values.



BMI of male children where the mother had GDM during pregnancy was significantly lower at ages 1-1.5 years and significantly higher at ages 7-10 years, compared to Swedish reference values, **Figure 16.** For female children where the mother had GDM during pregnancy, BMI was significantly lower at ages 1-1.5 years and significantly higher at birth and ages 4-12 years, compared to Swedish reference values, **Figure 17**.

Figure 16.
BMI was significantly lower at ages 1-1.5 years and significantly higher at ages 7-10 years for male children where the mother had GDM, compared to Swedish reference values.

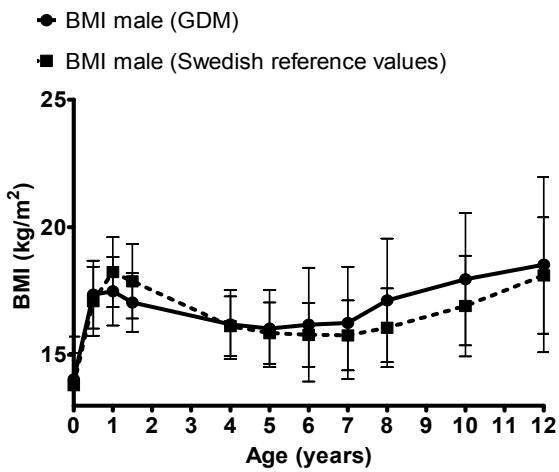
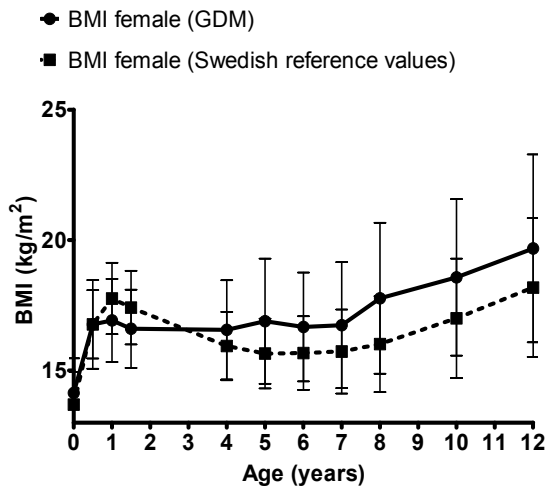


Figure 17.
BMI was significantly lower at ages 1-1.5 years and significantly higher at birth and ages 4-12 years for female children where the mother had GDM, compared to Swedish reference values.



When comparing the BMI of male children, where the mother had GDM during her pregnancy, to their siblings born after a non-GDM pregnancy, there was no significant difference between the two groups, **Figure 18**. For the female children, BMI was significantly lower at age 6 months compared to their siblings born after a non-GDM pregnancy (p=0.04), **Figure 19**.

Figure 18.
Comparison between the BMI of male children where the mother had GDM during pregnancy, and their male siblings born after a non-GDM pregnancy. There was no significant difference between the two groups.

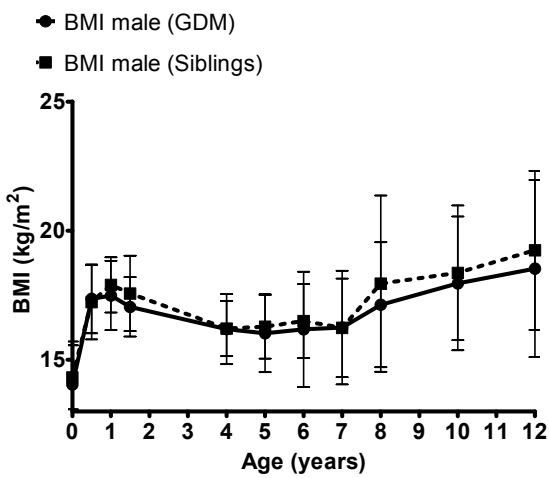
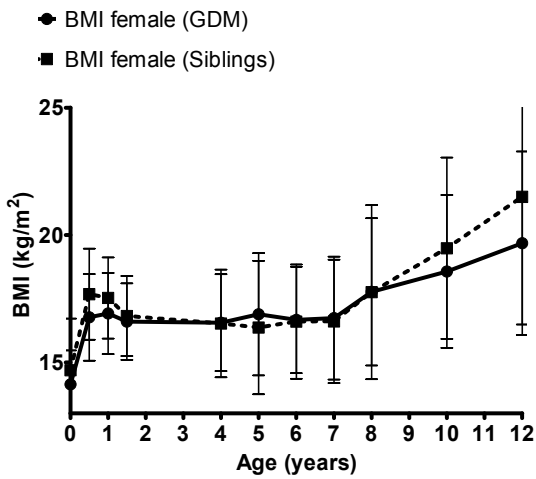


Figure 19.
Comparison between the BMI of female children where the mother had GDM during pregnancy, and their female siblings born after a non-GDM pregnancy. BMI was significantly lower at age 6 months compared to their siblings.



BMI of all male children, delivered by women who had had GDM at any of her pregnancies, was significantly lower at ages 1-1.5 years and significantly higher at birth and ages 6-10 years, compared to Swedish reference values, **Figure 20.** BMI of all female children, delivered by women who had had GDM at any of her pregnancies, was significantly lower at ages 1-1.5 years and significantly higher at birth and ages 4-12 years, compared to Swedish reference values, **Figure 21**.

Figure 20.
BMI was significantly lower at ages 1-1.5 years and significantly higher at birth and ages 6-10 years for male children where the mother had at least one pregnancy with GDM, compared to Swedish reference values.

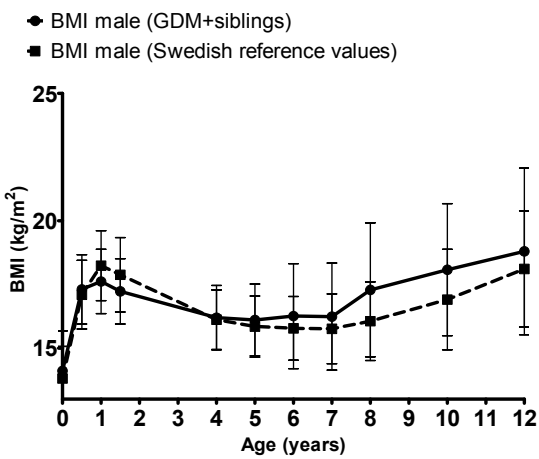
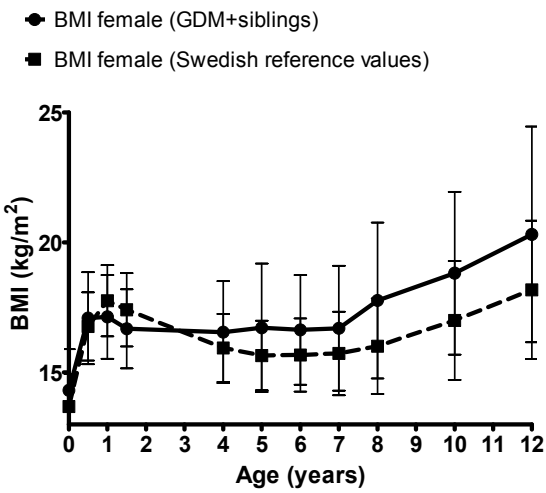


Figure 21.
BMI was significantly lower at ages 1-1.5 years and significantly higher at birth and ages 4-12 years for female children where the mother had at least one pregnancy with GDM, compared to Swedish reference values.



Data for the mothers and fathers are presented in **Table 5.**

Table 5.
Data for the parents at follow-up.

	Mothers (n=110)	**Fathers (n=110)**
First BMI during pregnancy (kg/m²)[1]	24.8 (17.6-48.9) (n=95)	NT
Present age 2011[2]	46 (34-59) (n=110)	48 (34-71) (n=101)
Present weight (kg)	69.0 (51-160) (n=105)	89.0 (55.0-122.0) (n=91)
Present height (m)	1.65 ±6.6 (n=109)	1.80 ±7.0 (n=93)
Present BMI (kg/m²)	25.4 (18.3-59.5) (n=105)	26.5 (18.6-38.1) (n=90)

Data are median (range) or mean ±SD, NT=not tested
[1]Values are from the first trimester
[2]Age of parents at follow-up

Discussion

Paper I-III

In our studies (**Paper 1-Paper III**), 5-10% of all women with GDM had beta-cell specific autoantibodies during pregnancy, that are characteristic of autoimmune type 1 diabetes. It is known that autoantibodies against beta-cells can be present for months and years before the clinical symptoms of diabetes shows (140, 141), which is often when 70% of the beta-cell function is lost (140). The increased insulin resistance during pregnancy leads to an increased demand on the remaining and affected cells. A pregnancy could therefore uncover an early stage of type 1 diabetes but be interpreted as just GDM. Autoantibody measurement during GDM, for predicting development of type 1 diabetes later in life, has been investigated in a number of studies for presence of ICA (142-146) or GADA (144, 146-148). The prognostic value of ZnT8A (**Paper II**) has not been studied before.

Frequency of ICA during pregnancy with GDM has been 1-15% (145, 146 152-160) in studies using the same standard method as in our own study (**Paper I**). For GADA, frequency during pregnancy with GDM has been reported between 0-13% (146, 148, 153-157, 159-165). In our studies (**Paper I-Paper III**), the frequency of GADA was 5-6%.

Follow-up time in our studies (**Paper 1, Paper III**) for the women with GDM, to estimate the risk for later development of type 1 diabetes, varied between 6 months and 15 years, which is a long time for this kind of study. At follow-up, 50% had developed type 1 diabetes in both studies, and many of these women developed type 1 diabetes within the first years after their GDM pregnancy with autoantibodies. There was also an additional 15-21% of the autoantibody positive women who had IFG or IGT at follow-up (**Paper I, Paper III**) and since GADA positivity can precede and also persist after the diagnosis of type 1 diabetes (144) it is not improbable that even more of these women might develop type 1 diabetes with time. When we reanalysed autoantibodies in 50% of the 12 women that had developed type 1 diabetes (**Paper I**), 83.3% (5 of 6) were positive for GADA and 33.5% (2 of 6) for IA-2A. Among the other 12 women who were not diagnosed with diabetes, GADA and IA-2A were reanalysed in 11 women and GADA persisted in 81.8% and IA-2A in 18.2% (2 of 11).

When ICA were measured during pregnancy with GDM in a Danish study, 75% of these women developed type 1 diabetes later in life (143). In a Finnish case-control study of women with GDM, 4.6% (20 women) developed type 1 diabetes and during pregnancy,

66% of these 20 women were positive for ICA, 56% for GADA and 38% for IA-2A (146). When investigating diabetes development in autoantibody positive women with GDM, 10.6% (32 women) of all women in a German study had antibodies against GAD, IA-2A or both during their pregnancy. Postpartum, 31 women developed type 1 diabetes and 47% progressed to type 1 diabetes within one year after delivery (98).

We have recommended screening for GAD autoantibodies in patients with gestational diabetes, as this has shown to be the most frequently found autoantibody in our studies (**Paper I-Paper III**). It is important to find these women early to prevent onset of diabetes with ketoacidosis, which potentially could be life-threatening (167-169).

Whether measurement of other autoantibodies than GADA can add any prognostic information in GDM women is still open to discussion. When we added the analysis of ZnT8A among the GDM autoantibody positive women (**Paper II**), the incidence of autoantibody positivity increased from 8% to 10%. A limitation was that we did not have the data for how many of the ZnT8A positive women that developed manifest type 1 diabetes after their GDM pregnancy. This follow-up data could be valuable proof in evaluating the importance of ZnT8A as independent marker for autoimmunity.

ZnT8A have been associated with a fast progression towards diabetes (within 5 years) in young first-degree relatives of patients with type 1 diabetes (170, 171) and ZnT8A has also proved to be a useful additional risk marker in people with low genetic risk of diabetes and older individuals (172). Future research on ZnT8A as a predictor in GDM of type 1 diabetes development post-partum is of great priority.

In the Better Diabetes Diagnosis study where 3165 patients with newly diagnosed type 1 diabetes participated, ZnT8A was found in 65% of the patients and in 3.4% as the only autoantibody. With the exception of children under two years of age, the prevalence of ZnT8A was independent of age (173). ZnT8A has also been reported in the same range as IA-2A in latent autoimmune diabetes in adults (LADA) (36).

It would be interesting to analyse ZnT8A in autoantibody negative pregnant women without diabetes. Since ZnT8A autoantibody was only recently identified, such data would be of major importance when interpreting ZnT8 antibody titres in women with GDM.

The clinical use of C-peptide among women with GDM, as a predictor for future development of diabetes was also explored (**Paper III**). Unfortunately, C-peptide did not discriminate between women who at follow-up developed diabetes and those women who did not. C-peptide levels were not able to predict future development of diabetes in the woman. Clinical usefulness of C-peptide was therefore very limited and should not be used to replace the testing of GADA. Levels of C-peptide correlated significantly with the women's first weight during pregnancy, but otherwise no correlations were found. A limitation of this study was the lack of C-peptide data on patients diagnosed with GDM before 2006.

There is a physiological increase in insulin resistance that occurs in all women during the second half of pregnancy, because of increased blood levels of different hormones (174, 175). Some studies have also shown that women with GDM are more insulin resistant than women without diabetes (176-177) which could be due to defective insulin secretion as well as defective insulin action.

Insulin resistance in the peripheral tissue could maybe have been preferable to measure instead of C-peptide levels. This could have been done by analysis of p-glucose and p-insulin in fasting samples for estimation of HOMA (homeostasis model assessment) index (178).

Key points in Paper 1, Paper II and Paper III

Women who have had GDM and autoantibodies during their pregnancy should be offered a structured programme for follow-up during several years postpartum, since the progression to manifest diabetes in this group of women, is higher than in other risk groups. We further recommend that GAD analyses should be performed in all women with GDM, since a frequency of 5-8% is not negligible and the risk for type 1 diabetes is at least 50% among women with autoantibodies during GDM. By adding ZnT8, the number of autoantibody-positive patients increased to 10%. Commercial GADA ELISAs are easy to obtain and manage, and the cost is relatively low.

Paper IV

In this study, children born to women with GDM during pregnancy, and their siblings born after non-GDM pregnancies were studied. Height, weight and BMI were compared to Swedish age-specific reference values. Strengths of this investigation were that there is a general screening for GDM in the district of Lund in Sweden, which gives a representative sample of women. The children were followed from birth up to 12 years of age, which is a long time for this kind of study. Since the data regarding height and weight of the children was measured in Health Care Centres and at schools, the validity was considered to be high. Swedish reference values, which were used as comparisons in this study, are based on a large representative sample of the population.

We also collected present height and weight data from the mothers and fathers, which is important when considering environmental influence of life style and dietary habits in the family.

Among children born after a GDM pregnancy, there was a significant difference in BMI compared to Swedish reference values. For boys, the BMI was higher at ages 7-10 and for girls at birth and ages 4-12 compared to Swedish reference values.

When separating measurements of height and weight, we could observe a discrepancy in the relation between the rates of longitudinal increase. Height increased significantly faster than weight in girls, and non-significantly in boys during the first years of life in the group where the mother had GDM during at least one of her pregnancies. This leads to a BMI equal or below normal in offspring younger than four years of age, despite a larger increase of both weight and height compared to Swedish reference values.

We included only children born 1995-2000, because we did not have a general OGTT screening among pregnant women before 1995. There have been studies where the children have been followed for a longer period of time and in a Danish follow-up study the offspring were between 18-27 years old. Their mothers had either diet only treated GDM or type 1 diabetes during their pregnancy. Risk of overweight was doubled in offspring of women with diet only treated GDM and the risk of the metabolic syndrome was increased 4-fold compared to the background population (179). This suggests that overweight and obesity among children born to women with a prior GDM will continue through adulthood.

Even if the relationship between maternal GDM and overweight and obesity among their children previously has been investigated (180-186), there is still no consensus concerning postnatal care and follow-up of these families. In our study, a large proportion of the children developed overweight and obesity. Previous studies have shown a higher frequency of overweight in offspring of mothers with a prior GDM in populations with both high (109) and low risk of GDM (187), even if some studies have shown only modest (188) or no association (189). High birth weight and future weight development can not only be explained by hyperglycaemia during pregnancy with diabetes as tight metabolic control

during pregnancy still can lead to foetal macrosomia (76, 190-192). One also has to remember that during GDM the hyperglycaemia often is not that severe (118, 193). In a randomized controlled trial from the Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS), comparing routine care to an intervention group in mild GDM, 199 mothers participated. The effect of GDM on the BMI of the children at ages 4 to 5 years was studied, and the result showed no significant difference in BMI between the two groups (194).

Maternal overweight and obesity are important risk factors for GDM and can per se lead to overweight among the offspring (119, 120, 195-197). But the first median BMI during the 110 women's GDM pregnancy in our study was normal, 24.8 (17.6-48.9 n=95).

To investigate other factors that could play a role in the association between GDM and offspring overweight, we collected data on height and weight of siblings, born after non-GDM pregnancies. Since most women only had one child during this period, the siblings formed a separate group for comparison, so that the whole material could be used. Our results showed that the same growth pattern was found in siblings born after non-GDM pregnancies, which suggests the importance of environmental influence such as life style and dietary habits in the family as risk factors for overweight and obesity. However, the influence of genetic factors was not tested in this study. This could have been important information, since the genetic heritability of individual BMI has been shown to be around 70% (198).

BMI is often presented in centiles, meaning that the reached BMI value is given for a determined percent of children, which will exclude the effect of outliers and non-parametric distribution. The latest BMI centile curves for Swedish children were published in 2000 (133).

BMI is also often presented with Z-score. Z-score is of interest to assess if an individual subject is normal for his/her age by a quantification of how far from average the measured value is and is based on the following calculation: ((measured value-average value in the reference population)/standard deviation in the reference population). A Z-score from -1 to 1 will include 67% of the reference population (=1 SD), while a Z-score from -2 to 2 will include 95% (=2 SD).

BMI values at birth are normally distributed but later on BMI show a positive skewed distribution over age, due to the fact that overweight is more common than underweight. The median values for BMI will therefore be smaller than the mean values. With the statistic Box transformation it is possible to force a non-parametric curve into a normal distribution using the formula $Y=(X^{\beta}-1)/\beta$ (134).

Karlberg et al has constructed a formula for the beta value, giving the best fitting normal curve for BMI, by a third degree polynomial logarithm ($\beta=0.031179-0.289503*Age+0.008617*Age^2+0.000221*Age^3$) for boys and ($\beta=0.10848-0.563978*Age-0.052448*Age^2-0.00143*Age^3$) for girls. After Box transformation the smoothed mean and SD can be calculated before transformed back to the original scale (134).

Transformed BMI values are useful when individual children are compared to reference values. In our study we have compared groups of children and the transformation is therefore not that important, since the positive skewness will be present in both groups.

To further investigate the environmental influence on the children's growth patterns, height and weight of the mothers and fathers were collected at follow-up. The data from the parents was self-reported and therefore of lower validity, but more likely to be underestimated than the reverse. We found that median BMI was 25.4 (18.3-59.5 n=105) for the mothers and 26.5 (18.6-38.1 n=90) for the fathers which both are over the limit for overweight (135). Several studies have shown associations between the BMI of parents and the BMI of their children (199, 200).

Key points in Paper IV

Children born to mothers with GDM have a higher risk for overweight and obesity. BMI for boys was higher at 7-10 years of age and for girls at birth and 4-12 years of age compared to Swedish reference values. Similar BMI pattern was found in their siblings born after a non-GDM pregnancy. Present BMI of the mothers and fathers also showed a high frequency of overweight and obesity. These findings suggest that life style habits in the families rather than only prepregnancy BMI and/or the intrauterine environment that causes overweight and obesity in the offspring. However, a shared genetic predisposition to large body size cannot be ruled out.

Conclusions

- Between 5-8% of all women with GDM have beta-cell specific autoantibodies during their pregnancy and at least 50% of these women develop type 1 diabetes later in life. GAD was the most frequent autoantibody and GAD analyses should therefore be performed in all women with GDM.

- When adding ZnT8A as an autoimmune marker in GDM, the number of autoantibody positive women increased by 2%.

- C-peptide analyses did not add any valuable information to women with GDM for development of either for type 1 or type 2 diabetes, and should therefore not be undertaken by routine.

- Children to women with a prior GDM have a high risk for overweight and obesity. This is thought to be due to life style habits in the family rather than prenatal factors, even if genetic factors could not be tested in this study. The similar BMI pattern was found in siblings and strongly suggests that environmental factors are of importance. Early life style intervention is very important in these families to prevent manifest overweight and obesity among these children.

Future research

It would be of great interest to perform a new follow-up in five years of time of the women with autoantibodies during their pregnancy, to investigate if more women had developed type 1 diabetes. The data for how many of the ZnT8A positive women that developed manifest type 1 diabetes after their GDM pregnancy are under working progress, but the calculations are not finalised yet. The medical journals of women with GDM between 1995-2010 have been thoroughly examined and we are planning new study designs from this material. We are also planning to collect data on the height and weight of children born to mothers with prior GDM after 2000.

Svensk sammanfattning

Graviditetsdiabetes är diabetes som uppkommer eller först upptäcks under en graviditet. Mellan 1-14 % av alla gravida kvinnor runt om i världen drabbas av graviditetsdiabetes och i Sverige är det ungefär 2 %. I Sverige behandlas graviditetsdiabetes med kostråd och om detta inte räcker lägger man till insulinbehandling. Kända riskfaktorer för att utveckla graviditetsdiabetes är övervikt och fetma, som är växande problem runt om i världen. Fetma och övervikt åtföljs även av en rad hälsoproblem, som ökad risk för hjärt- och kärlsjukdomar. Graviditetsdiabetes ökar även risken för att föda stora barn, kejsarsnitt samt förlossningsskador. Det finns dock inte så många studier som är gjorda på långtidsprognos samt eventuella framtida risker för barn vars mammor haft graviditetsdiabetes.

Kvinnor som haft graviditetsdiabetes löper även ökad risk att utveckla diabetes efter sin graviditet. Det vanligaste är att utveckla typ 2 diabetes, som kan bero på att förmågan att producera insulin är nedsatt och/eller att känsligheten för insulin i muskel- och fettceller är nedsatt. I första hand behandlas typ 2 diabetes med kostråd, motion, viktnedgång samt tabletter men ibland behövs även tillägg av insulinbehandling.

Det finns även kvinnor som haft graviditetsdiabetes och som utvecklar typ 1 diabetes istället. Typ 1 diabetes är en autoimmun sjukdom, vilket innebär att man tror att kroppens eget immunsystem har rubbats så att det angriper och förstör de insulinproducerande cellerna i bukspottkörteln. Det beror på att det bildas s.k. antikroppar mot dessa celler vilket gör att immunförsvaret attackerar de insulinproducerande cellerna. Man kan mäta nivåerna av dessa antikroppar med blodprover, och det finns flera olika sorters antikroppar vid typ 1 diabetes. Man vet fortfarande inte vad som sätter igång denna process som leder till att kroppen inte längre kan tillverka sitt eget insulin. Bristen på insulin leder i sin tur till att sockret stannar kvar i blodet och blodsockernivån stiger. Typ 1 diabetes behandlas alltid med insulin. För att mäta hur mycket egen insulinproduktion man har kvar i kroppen används C-peptid, som är en biprodukt från insulin.

Studiedesign

Alla kvinnor i Lunds sjukvårdsdistrikt genomgår en 2-timmars glukosbelastning under vecka 28 av sin graviditet som screening för graviditetsdiabetes. De som har haft graviditetsdiabetes tidigare eller har ärftlighet för diabetes genomgår glukosbelastningen redan under vecka 12 av sin graviditet. Kvinnorna kommer fastande till sin mödravårdscentral och får dricka en lösning med 75 gram glukos och efter två timmar mäts deras blodsocker i fingret. Ett blodsocker på ≥10,0 mmol/l räknas som graviditetsdiabetes. De kvinnor som haft graviditetsdiabetes mellan åren 1995-2010 samt deras barn är studerade i denna avhandling.

Metod

Denna avhandling består av fyra delarbeten:

I **Arbete I-III** undersöktes kvinnor som haft graviditetsdiabetes mellan 1995-2010. Syftet var att se hur många av dessa kvinnor som haft antikroppar mot de insulinproducerande cellerna i bukspottskörteln under sin graviditet. De antikroppar som undersöktes i dessa studier heter GADA, IA-2A samt ZnT8A. Därefter följdes dessa kvinnor för att se hur många som faktiskt utvecklade typ 1 diabetes efter sin graviditet. Betydelsen av C-peptid nivån hos kvinnor med graviditetsdiabetes för att senare utveckla diabetes undersöktes också.

I **Arbete IV** undersöktes barnen till kvinnor som haft graviditetsdiabetes mellan 1995-2000. Deras längd- och viktkurvor samlades in från BVC och skolor. Syftet var att jämföra deras body mass index (BMI=kg/m^2) med Sveriges referensvärden för pojkar och flickor i samma åldrar samt mot BMI hos deras syskon, födda efter en normal graviditet. Åldrarna som studerades var från födseln upp till 12 års ålder.

Resultat

I **Arbete I-III** var det mellan 5-8 % av alla kvinnor med graviditetsdiabetes som hade antikropparna GADA och IA-2A under sin graviditet. Av dessa kvinnor som haft antikroppar under sin graviditetsdiabetes utvecklade 50 % typ 1 diabetes senare i livet. Dessutom var det många som hade förhöjda blodsockervärden efter sin graviditet, och en del som även hade graviditetsdiabetes vid nästa graviditet. När även förekomst av antikroppen ZnT8A undersöktes, var ytterligare 2 % av kvinnorna med graviditetsdiabetes positiva för denna antikropp. Den vanligast förekommande antikroppen var dock GADA. C-peptid nivåerna hos kvinnorna med graviditetsdiabetes hade ingen betydelse för utveckling av vare sig typ 1 eller typ 2 diabetes efter graviditeten.

I **Arbete IV** var BMI hos pojkar vars mamma haft graviditetsdiabetes högre vid ålder 7-10 år jämfört med Sveriges referensvärden. För flickor var BMI högre vid födseln samt ålder 4-12 år jämfört med Sveriges referensvärden. Samma BMI mönster fanns hos deras syskon födda efter en normal graviditet.

Slutsats

Arbete I-III: Kvinnor som haft graviditetsdiabetes löper en ökad risk att utveckla typ 1 diabetes (minst 50 %) om de haft antikroppar under sin graviditet. GADA analyser bör göras på alla kvinnor med graviditetsdiabetes, och det är därför viktigt att följa kvinnor som haft antikroppar för att tidigt upptäcka typ 1 diabetes.

Arbete IV: Barn till kvinnor som haft graviditetsdiabetes löper en ökad risk att utveckla övervikt och fetma. Detta tros i första hand bero på livsstilsfaktorer, eftersom deras syskon födda efter en normal graviditet hade samma BMI mönster. Därför är det viktigt att följa dessa familjer och tidigt ge livsstilsråd avseende kost och fysisk aktivitet, för att förhindra uppkomst av övervikt och fetma.

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