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INSULIN RESISTANCE IN CHILDREN AND ADOLESCENTS; MECHANISMS AND CLINICAL EFFECTS

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INSULIN RESISTANCE IN CHILDREN AND ADOLESCENTS; MECHANISMS AND CLINICAL EFFECTS

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To Linus and Jonatan

ABSTRACT

Background: Insulin resistance is a condition in which insulin fails to achieve an appropriate response in different target tissues. It is associated with obesity and is one of the main culprits in the development of type 2 diabetes. An early sign of disturbed glucose-insulin homeostasis is impaired fasting glucose (IFG) where glucose is elevated in the fasting state. The American Diabetes Association (ADA) and the International Society for Pediatric Diabetes (ISPAD) suggest 5.6 mmol/L as a cut-off level for IFG while the World Health Organization supports 6.1 mmol/L. Impaired glucose tolerance (IGT) is defined as elevated glucose after a 2-hour glucose tolerance test, which is also a sign of disturbed glucose-insulin homeostasis. IFG and/or IGT, collectively prediabetes, are associated with a significantly elevated risk of the development of type 2 diabetes in adults, but the consequences of the prediabetic condition in children are not as evident. In Sweden the prevalence of type 2 diabetes in youth has been low despite increasing overweight and obesity. However, the exact present prevalence of type 2 diabetes among youth is not currently known. Early-onset type 2 diabetes is associated with a high morbidity already at a young age and seems to be more aggressive than with early-onset type 1 diabetes.

Aim: The aims of this thesis were to investigate the pathogenesis of prediabetes in obese children, to examine the prevalence of prediabetes and type 2 diabetes among severely obese adolescents, and to estimate the occurrence of complications related to early-onset type 2 diabetes compared to type 1 diabetes of the same duration of disease.

Method: Studies I and II in this thesis contain data from the Swedish Childhood Obesity Register (BORIS), which is a national quality register for obesity treatment in childhood and adolescence. Fasting glucose and glucose levels after an oral glucose tolerance test (OGTT) were determined to define normal glucose tolerance or prediabetic stage. A frequently sampled intravenous glucose tolerance test was used to study acute insulin response (AIR), insulin sensitivity (Si), and disposition index (DI) in children with obesity. Study III contains data from the National Diabetes Register (NDR) and the pediatric diabetes register (SWEDIABKIDS); regarding adolescents and young adults with type 1 and 2 diabetes. Studies I and II are cross-sectional observational studies and Study III is a longitudinal, retrospective cohort study.

Results: Among severely obese children the prevalence of isolated IFG_{ADA} was 35.8 %, isolated IGT was 6% and the combined IFG and IGT prevalence was 14.2%. Combined IFG/IGT was associated with significantly lower AIR compared with subjects who had normal glucose metabolism ($p<0.05$) and DI was the major determinant of 2-h OGTT glucose levels ($\beta=-0.49$, $p=0.0126$). Comparing IFG_{ADA} and IFG_{WHO} in obese children, only IFG_{WHO} was associated with a lower AIR and DI ($p<0.001$). In total 1413 adolescents and young adults were diagnosed and registered in NDR with type 2 diabetes between 1994 and 2014. Compared with individuals with type 1 diabetes with an equivalent diabetes duration, those with early-onset type 2 diabetes had a significantly higher risk of developing microalbuminuria with a hazard ratio (HR) of 3.32 (95% CI 2.86-3.85, $P < .001$), and also of retinopathy with an HR of 1.17 (95% CI 1.06-1.30, P 0.04). These differences occurred despite lower HbA1c levels among individuals with type 2 diabetes than among those with type 1 diabetes.

Conclusion: The prevalence of prediabetes was very high among adolescents with severe obesity. IFG_{WHO} and a combination of IFG_{ADA} and IGT is significantly associated with disturbed glucose metabolism. Although both type 1 and 2 diabetes were associated with complications, the prevalence of complications and comorbidities is significantly higher among those with early-onset type 2 diabetes than among those with type 1 diabetes. Our results confirm the findings of previous studies, that type 2 diabetes is a severe disease when young individuals are affected, and an active treatment with a widened focus on cardiometabolic risk factors is required to reduce the risk.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Bakgrund: Insulinresistens är ett tillstånd där insulin i normala nivåer inte kan reglera blodsockernivåerna i olika vävnader, det är tydligt associerat med fetma och är en bidragande faktor i utvecklingen av typ 2 diabetes. Ett tidigt tecken på att glukos- och insulin regleringen är ur balans är när en individ utvecklar förhöjda faste blodsocker (IFG). Det pågår en debatt om vilket gränsvärde som skall använda för IFG, medan den amerikanska diabetes associationen (ADA) och internationella föreningen för pediatrisk diabetes (ISPAD) föreslår 5.6 mmol/L som gränsvärde för IFG, så förordar Världs Hälsoorganisationen (WHO) att använda 6.1 mmol/L. Nedsatt glukostolerans (IGT) är definierat som ett förhöjt blodsocker efter en per oral glukosbelastning och är också ett tecken på störd glukos- och insulinreglering. Det finns tecken på att dessa tillstånd har olika ursprungsmekanismer, men i vilken utsträckning är oklart. Båda dessa tillstånd är hos vuxna individer associerade med en ökad risk att utveckla diabetes typ 2, men konsekvenserna av att ha IFG och/eller IGT som barn är inte lika tydliga. Förekomsten av typ 2 diabetes hos unga har tidigare varit låg i Sverige, trots att förekomsten av övervikt och fetma ökar, men den aktuella förekomsten av typ 2 diabetes hos unga idag är inte känd. Typ 2 diabetes som debuterar i unga år är kopplat till hög sjuklighet, och verkar vara mer aggressiv än typ 1 diabetes.
Syfte: Syftet med denna avhandling är att undersöka utvecklingen och orsakerna till rubbad glukosreglering hos barn med fetma, förekomsten av förhöjda blodsocker och typ 2 diabetes hos ungdomar med uttalad fetma samt att uppskatta förekomsten av diabetesrelaterade komplikationer hos individer med tidigt debuterande typ 2 diabetes jämfört med individer med typ 1 diabetes.
Metod och material: Studie I och II är baserad på data från det svenska barnobesitas registret (BORIS). BORIS är ett nationellt kvalitetsregister för behandling av fetma hos barn och ungdomar. Faste blodsocker samt blodsockernivåer efter en oral glukosbelastning (OGTT) diagnostiserar individens glukostoleransnivå. Ett intravenöst glukostoleranstest gjordes för att bedöma insulinfrisättning (AIR), insulinkänslighet (Si) samt dispositions index (DI) hos barn med fetma. Studie III är baserad på data från det Nationella Diabetes Registret (NDR); dels från barnregistret (SWEDIABKIDS) samt från vuxenregistret (NDR) med data från ungdomar samt unga vuxna med typ 1 samt 2 diabetes. Studie I och II är observationella tvärsnittsstudier, Studie III är en longitudinell, retrospektiv kohortstudie.
Resultat: Bland barn med uttalad fetma är förekomsten av IFG enligt ADA 35,8%, IGT 6% och kombinerad IFG och IGT 14,2 %. De individer som hade kombinerad IFG/IGT hade lägre insulinfrisättning jämfört med de som hade normal glukostolerans. Vid jämförelse mellan olika gränsnivåer av IFG, så var det bara IFG enligt WHO som var tydligt associerat med lägre insulinfrisättning och dispositionsindex. Totalt blev 1413 ungdomar och unga vuxna med typ 2 diabetes diagnostiserade och registrerade i NDR/SWEDIABKIDS mellan 1994–2014. Jämfört med unga individer med typ 1 diabetes, så hade de med tidig typ 2 diabetes debut signifikant högre risk att utveckla njur- och ögonkomplikationer. Trots att individerna med typ 2 diabetes hade lägre långtidsblodsocker (HbA1c), så hade de högre förekomst av komplikationer jämfört med individerna med typ 1 diabetes.
Slutsats: Förekomsten av förhöjda blodsocker var hög hos barn med uttalad fetma. IFG enligt WHO liksom en kombination IFG/IGT hos barn med fetma är tydligt kopplat till påverkad glukos- och insulin reglering, och detta kan vara en faktor som påverkar risken att utveckla typ 2 diabetes. Både typ 1 och 2 diabetes är associerat med komplikationer, men risken verkar vara högre vid typ 2 diabetes. Våra resultat stöder tidigare studier som påvisat att tidigt debuterande typ 2 diabetes är en allvarlig sjukdom och behöver aktiv behandling med fokus på samtidigt förekommande sjukdomar och kardiovaskulära riskfaktorer.

LIST OF SCIENTIFIC PAPERS

I. **High prevalence of prediabetes in a Swedish cohort of severely obese children**
Ek AE, Rössner SM, Hagman E, Marcus C.
Pediatric Diabetes 2015 Mar; 16:117-128

II. **Insulin function in obese children within the low and high ranges of impaired fasting glycemia.**
Hagman E, Ek AE, Marcus C
Pediatric Diabetes 2019 Mar;20:160-165

III. **Microalbuminuria and retinopathy in adolescents and young adults with type 1 and 2 diabetes.**
Ek AE, Samuelsson U, Jansson A, Carlsson C, Elimam A, Marcus C.
Pediatric Diabetes 2020 Nov;21:1310-1321

CONTENTS

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AIR	Acute Insulin Response
BMI	Body Mass Index
BMI SDS	Body Mass Index Standard Deviation Score
BORIS	The Swedish Childhood Obesity Register
CVD	Cardiovascular Disease
CRF	Cardiorespiratory fitness
DI	Disposition Index
DQA-B	Specific HLA-gene A-B belonging to the major histocompatibility complex II
eNOS	Endothelial Nitric Oxide Synthase
FFA	Free Fatty Acids
FPG	Fasting Plasma Glucose
FOXO	FOrkhead boX O transcription factor
GCK	Glucokinase
GIP	Glucose dependent insulinotropic polypeptide
GLP-1	Glucagon like peptide-1
GLUT	Glucose Transporter
GSK3	Glycogen synthase kinase 3
HbA1c	Glycated hemoglobin
HEC	Hyperinsulinemic-euglycemic clamp
HLA	Human Leukocyte Antigen
HNF1-α	Hepatocyte Nuclear Factor 1-alfa
HOMA	Homeostasis model assessment
HOMA-IR	HOMA-derived index of insulin resistance
HOMA%B	HOMA-derived index of beta cell function
HOMA%S	HOMA-derived index of insulin sensitivity
HR	Hazard Ratio
IFG	Impaired Fasting Glucose Tolerance
iIFG	Isolated Fasting Glucose Tolerance
IFG_{ADA}	IFG according to American Diabetes Association
IFG_{WHO}	IFG according to World Health Organisation
IGT	Impaired Glucose Tolerance

IGF-1,2	Insulin Like Growth Factor-1, 2
IKK	IκB kinase
IOTF	International Obesity Task Force
IMCL	Intramyocellular Lipid
INSR	Insulin Receptor
IVGTT	Intravenous Glucose Tolerance Test
IRS	Insulin Receptor Substrates
ISPAD	International Society for Pediatric and Adolescent Diabetes
JNK	c-Jun N-terminal kinase
KCNJ11	Potassium inwardly rectifying channel, subfamily J, member 11
LADA	Latent Autoimmune Diabetes of Adulthood
NEFA	Non Esterified Fatty Acids
MODY	Maturity Onset of Diabetes in the Young
mTOR	Mammalian Target of Rapamycin
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic SteatoHepatitis
NDR	National Diabetes Register
NFG	Normal Fasting Glucose
NFkB	Nuclear factor kappa B
OGTT	Oral Glucose Tolerance Test
PDK-1	Phosphatidylinositol dependent kinase
PKB	Protein kinase B, Akt pathway
PI3K	Phosphonositide 3-kinase
PPARγ	Peroxisome proliferator-activated receptor γ
Si	Insulin sensitivity measured by fs-IVGTT
SD	Standard Deviation
TCF7L2	Transcription Factor 7 Like 2
TBC1D1/TBC1D4	TBC Domain family 1, 4
WHO	World Health Organization

1 GENERAL INTRODUCTION

The emergence of obesity among children and adolescents during the last 30 years has brought new diseases, earlier only present in adults, into the field of pediatrics. There is overwhelming evidence of the risk of obesity, and this thesis follows a trail from the early risk factors associated with obesity in children to type 2 diabetes in youth.

Diabetes is a chronic metabolic disease characterized by elevated levels of glucose and is commonly divided into two categories. Type 1 diabetes is the dominant type of diabetes in children, with an immunological destruction of the insulin-producing β-cells leading to insulin deficiency. Type 2 diabetes is by far the most prevalent diabetes type in adults. It is associated with obesity and insulin resistance and has lately also appeared in children. The treatment and management of these two disorders differs considerably and poses new challenges to pediatric diabetes specialist teams, who are currently more familiar with the management of type 1 diabetes. There is an increased risk of micro- and macrovascular complications in the heart, kidney, eyes, and nervous system in both diabetes types. However, in recent years there have been increasing reports that the metabolic consequences associated with early-onset type 2 diabetes seem to be more severe than those associated with type 1 diabetes. There is also emerging evidence that early-onset type 2 diabetes is associated with comorbidities and complications at the time of diagnosis and seems to be more aggressive disease than later-onset type 2 diabetes.

The theme in this thesis circles around insulin resistance: an insensitivity toward the effects of insulin which is believed to be one of the key players in the development of obesity complications such as type 2 diabetes. Many obese children will be obese as adults and remain under the burden of obesity for a long time unless lifestyle changes are made. Hence, they have an increased risk of complications associated with insulin resistance, both as adolescents and young adults. Besides being a pathologic reaction to obesity, insulin resistance increases during puberty in both healthy and obese children. It is a dynamic interplay between insulin secretion and insulin resistance, depending on the demand in different tissues and conditions, making insulin resistance a volatile phenomenon. Extensive research into insulin resistance and insulin secretion in both adults and children has been carried out in the recent decades, which has led to an enormous increase in the knowledge in of type 2 diabetes development. Not all adult studies are directly applicable to the growing child, as the risk of being obese as a child does not equal being obese as an adult. Not all obese children have the same risk of developing complications, and the cause of this is unclear.

This thesis provides some insight into the factors causing prediabetes in severe obesity in childhood, the occurrence of early-onset type 2 diabetes in Sweden, and the prevalence of diabetes related complications in both type 1 and 2 diabetes in youth.

2 BACKGROUND

2.1 OBESITY IN CHILDREN AND ADULTS

2.1.1 Definition of obesity

Definitions of overweight and obesity are different for children and adults, depending on growth patterns in the child. The standard classification of overweight and obesity in adults is based on body mass index (BMI), and is defined as a person's weight in kilograms divided by their height in meters squared (kg/m^2). Overweight is defined by as BMI ≥25 and obesity as BMI ≥30, according to the World Health Organization (WHO) [1]. BMI classification does not distinguish between fat mass and fat free mass, varies with height at certain ages, and can also vary in different populations, which can lead to misclassification. However, it is a simple and widely used measure of adiposity.

As BMI in children varies considerably with age, height, and to a certain degree gender, specific BMI cut-offs for obesity are used in children. Different definitions and national references in regard to childhood weight patterns have been used in previous studies, making assessments of prevalence of childhood obesity difficult. In 2002 the International Obesity Task Force (IOTF) implemented the most commonly used international classification of childhood obesity, based on Cole's age- and gender-specific cut-off points corresponding to the adult criteria of a BMI of 25 for overweight (ISO-BMI 25) and 30 for obesity (ISO-BMI 30) [2]. An extended international reference was created in 2012 in an effort to make comparisons between different populations easier and facilitate division into SD scores and centiles [3].

The Body Mass Index Standard Deviation Score (BMI SDS) is often used as a measure of relative weight in children, aged between 2 and 18 years of age. It is calculated based on the weight, height, age, and sex, using a reference population. In this thesis we use a Swedish and an international reference population [3, 4]. In many other countries, for example the USA, growth and weight are commonly described in percentiles instead of BMI SDS, with overweight and obesity in childhood being defined as a BMI above the 85^{th} and 95^{th} percentile for children of the same age and sex, using national reference growth charts [5].

2.1.2 Prevalence of obesity

2.1.2.1 Children

The rates of overweight and obesity continue to grow among children worldwide. The WHO reports that, since 1975, the prevalence of overweight or obese children between 5 and 19 years of age increased from 4 % to 18 % globally, and the rise is evident in both developed and non-developed countries [6]. The prevalence of obesity is difficult to assess, since not all children, adolescents, or adults with obesity seek health care. In Sweden children are routinely measured in primary health care and at school at certain ages but reports on weight in older children are often self-assessments, which makes assessment of the prevalence more complicated.

In Sweden we can see the same pattern of increasing weight among children: the prevalence of overweight and obesity in children and adolescents has doubled over the past 30 years, and the risk of overweight and obesity increases with age. A national health report on self-reported childhood weight patterns in Sweden revealed an increase from 7% to 15%

overweight among 11–15-year-old school children between 1989 and 2018, with a corresponding increase in obesity from 0.8% to 4% [7, 8]. In a recent report dated 2019, based on data from the WHO Childhood Obesity Surveillance Initiative, the overall prevalence of overweight among Swedish primary school children was 17%, of whom 6% were obese and 1% had severe obesity [7, 9].

In 2018 a survey of Swedish national data was conducted to estimate the prevalence of overweight and obesity in four-year-old children found that 9.4% were overweight and 2.3 % were obese [10]. In the Stockholm region in 2020 the prevalence of overweight and obesity were 8.4% and 1.8 % respectively among four-year-old children [11]. The overweight and obesity rates differ substantially in different regions in Sweden and also in different municipalities in Stockholm, in the case of overweight ranging between 4.6% and 18.2 % in certain areas [11].

The rates of obesity are higher in southern Europe than in Sweden, with a very high prevalence (40–47%) of overweight and obesity among children in countries such as Greece, Spain, and Italy and a high rate of severe obesity, as seen in Figure 1 [9]. In the USA the overall prevalence of obesity, although high among the adolescents (20.5%), has reached a plateau, however, the prevalence of severe obesity has continued to increase [12].

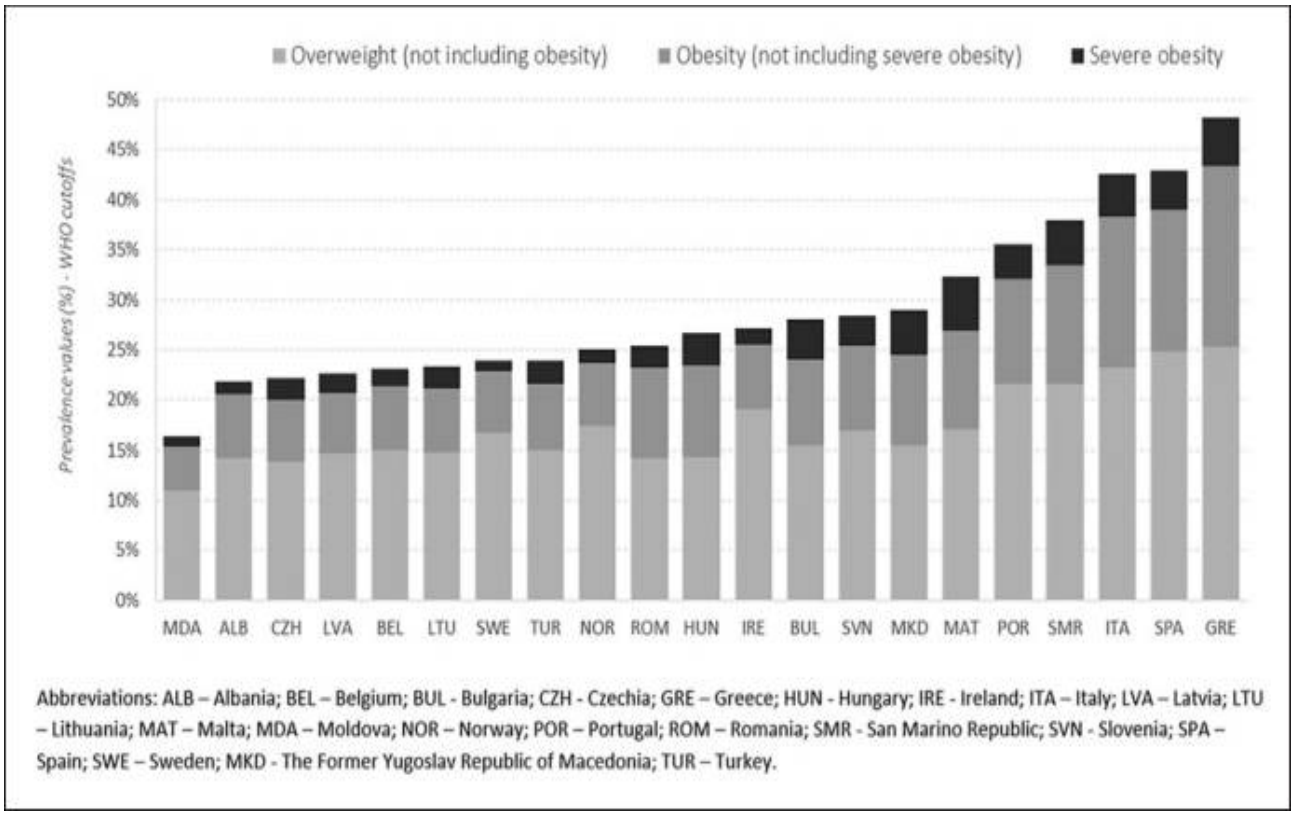


Figure 1. *Prevalence by country of overweight, obesity, and severe obesity among in children aged 6-9 based on WHO definitions in 21 European countries. Spinelli et al. 2019 Obesity Facts.*

2.1.2.2 Adults

According to the WHO's report in 2016, more than 1.9 billion adults worldwide were overweight or obese, with prevalence rates of obesity varying across countries and regions [13]. In a large national health survey in 2020 carried out in Sweden, Hälsa på lika Villkor, the self-reported prevalence of overweight or obesity was 52%, with a higher prevalence among men (57%) than in women (46%) [7]. As in children, BMI increases with age, and there are large variations in the prevalence of overweight and obesity in adults according to region and evident sociodemographic differences.

2.1.2.3 Causes of obesity

The underlying cause of obesity is, in a simplified version, an imbalance between calorie intake and the calories used. There is an evident genetic susceptibility, but also psychosocial, endocrine, environmental, and nutritional factors are also among the components that contributes to the development of the overweight/obese condition. Society, the availability of healthcare, and life conditions are also important factors in the development of childhood obesity.

2.1.3 Some consequences of obesity

Obesity affects both children and adults and has several social and health-related consequences. Obesity-related disease can be detected at an early age, and obesity in all age groups accounts for over 70 % of premature deaths worldwide according to the WHO, as being one of the most important risk factors for cardiovascular diseases, cancer and diabetes mellitus [13, 14]. The risk of obesity has also become even more apparent during the Covid-19 pandemic, with an increased risk of severe disease and increased mortality [15, 16].

Obesity in childhood often continues into adulthood [17], so children with obesity are at risk for a long time. As obesity is becoming more prevalent in children, there is significant risk of both present comorbidity and the development of future disease in children with obesity. Children with obesity are more likely to have many psychosocial consequences related to obesity such as a lower self-esteem, lower quality of life, depression, and a high prevalence of comorbidity with neuropsychiatric disorders [18-20]. It is also more common for a child with obesity to have asthma [21], obstructive sleep apnea, and musculoskeletal disorders [22-24].

Even before comorbidity is present, signs of endothelial dysfunction and cardiovascular risk factors can be detected among children with obesity [25-27]. Like adults with obesity, children with obesity can have several comorbidities, such as hypertension, prediabetes, dyslipidemia, elevated liver enzymes and non-alcoholic fatty liver disease [26, 28-31].

Obesity is the most important risk factor for type 2 diabetes in adults [32, 33], and during recent years evidence has emerged that a greater risk is associated with early-onset type 2 diabetes compared with a later-onset of type 2 diabetes [34]. The earlier the onset of type 2 diabetes, the greater loss of life years, and there is a higher morbidity among those who are younger at diagnosis [34]. Both obesity and type 2 diabetes are associated with insulin resistance, which is one of the main abnormalities in disturbed glucose regulation.

2.2 GLUCOSE REGULATION

2.2.1 Normal glucose homeostasis

Normal glucose homeostasis in the feeding state is regulated by insulin, an endocrine hormone with the ability to "open the door" to glucose to act as energy, primarily in skeletal muscle, adipose tissue, and the liver. Blood glucose levels are regulated by an intricate communication between endocrine and neurological factors in a feed-back loop system both in the fed state and in periods of fasting. Fasting glucose is normally maintained at between 3.9 and 5.6 mmol/L [35], and the increase after a meal rarely exceeds 3 mmol/L in young and healthy individuals [36]. Low blood glucose is especially dangerous for the brain cells, and glucose transport to brain cells is therefore not dependent on insulin, instead glucose is transported by facilitated diffusion through the cell membrane. To secure sufficient glucose for brain cells, there are several other mechanisms to elevate blood glucose. In the fasting state the insulin counterpart hormones; glucagon, cortisol, growth hormone (GH), and the catecholamines adrenaline and noradrenaline are involved in glucose homeostasis. All these hormones raise blood sugar levels through different effects.

2.2.2 Insulin and insulin signaling

Insulin is an anabolic and regulatory hormone, discovered by Frederick Banting and Charles Best in 1921, for which they received the Nobel Prize in 1923. Insulin is synthesized in the β-cells in the islets of Langerhans of the pancreas and has a regulatory effect on blood glucose levels, mainly by promoting the entry of glucose into cells, especially skeletal muscle. It is the only hormone with a glucose lowering capacity [37, 38]. The pharmacological half-life is short, approximately 5-8 minutes [39]. Since the discovery of insulin, extensive research has been conducted to understand the intricate insulin signaling pathways, providing increasing knowledge about the different complex regulations of carbohydrate, protein, and lipid metabolism. The process of understanding these biologic patterns is ongoing and offers possibilities of understanding the pathogenesis of type 2 diabetes and finding new potential treatment strategies.

Elevated levels of blood glucose trigger pancreatic insulin release; upon binding to the insulin receptor (INSR) insulin promotes glucose uptake and glycogen storage. Virtually all cells in the body express INSRs, but the effect on glucose homeostasis is exerted mainly in skeletal muscle, white adipocytes, and hepatocytes. When insulin binds to receptors in these tissues, a signaling phosphorylation cascade is activated, promoting glucose uptake and a variety of metabolic actions. Insulin binds preferentially to INSR , but insulin-like growth factor-1 and 2 (IGF-1, IGF-2) can also bind to INSR with a reduced affinity [40]. Insulin action is mainly on glucose uptake but also affects lipid and protein metabolism, while IGF-1 and IGF-2 primarily promote cell differentiation and growth [41].

The INSR is composed of two extracellular α-subunits, which binds insulin, and two transmembrane β-subunits which contains tyrosine kinase [42]. Two isoforms of the INSR, A and B; where binding to the B isoform is more specific to insulin binding and is highly expressed in liver, muscle, and adipose tissue [40]. The A isoform of INSR is predominantly expressed in fetal tissues and in the brain and has comparable affinity for both insulin and IGF-2 [43].

The activation of INSR leads to a cascade of cellular phosphorylation by activating tyrosine kinase in the β-subunits [25]. This initiates metabolic signaling by recruiting INSR substrates (IRS 1-6), which leads to further downstream activation [44-47]. Among the most important

steps in this cascade of events are the subsequent activation of phosphoinositide 3-kinase (PI3K), leading to the activation of phosphatidylinositol dependent kinase (PDK-1), mammalian target of rapamycin (mTOR) complex, and subsequently the serine/threonine kinase Akt pathway (also known as protein kinase B, PKB) [48-50]. These are necessary steps in the insulin signaling cascade to promote the translocation of glucose transporters (GLUT) to the cell membrane [47, 51, 52], and a critical pathway to link the IRS proteins to the metabolic actions of insulin. Transport of glucose over the cell membrane is facilitated by the GLUT family members, and several different GLUT transporters have been identified. The most important in glucose homeostasis are GLUT 1-4 [53].

The Akt/PKB family of proteins consists of three different isoforms of serine/threonine protein kinases. The Akt 2 isoform is the most abundant form in insulin-sensitive tissues and appear to play a role in mediating insulin action on metabolism [54]. Activation of Akt complex allows the activation of many downstream targets besides glucose uptake: glycogen synthesis by glycogen synthase kinase 3 (GSK3), and protein and fat synthesis through the mTOR complex, which regulates a network of genes controlling metabolism, protein synthesis, and cell growth [55].

Additionally, it seems that Akt is involved in the activation of the transcription factor family Forkhead box O transcription factor (FOXO), which controls lipogenic and gluconeogenic genes [56, 57] and also affects other transcription factors and survival proteins [55, 58, 59]. Insulin also has a role in cell differentiation by activating non-INSR substrates, independently of the Akt pathway, for example heterotrimeric G protein, SOS, RAS, MAPK factors [48].

An overview of the myriad of activities in the INSR region, with cascade phosphorylation´s and allosteric reactions, is shown in Figure 2.

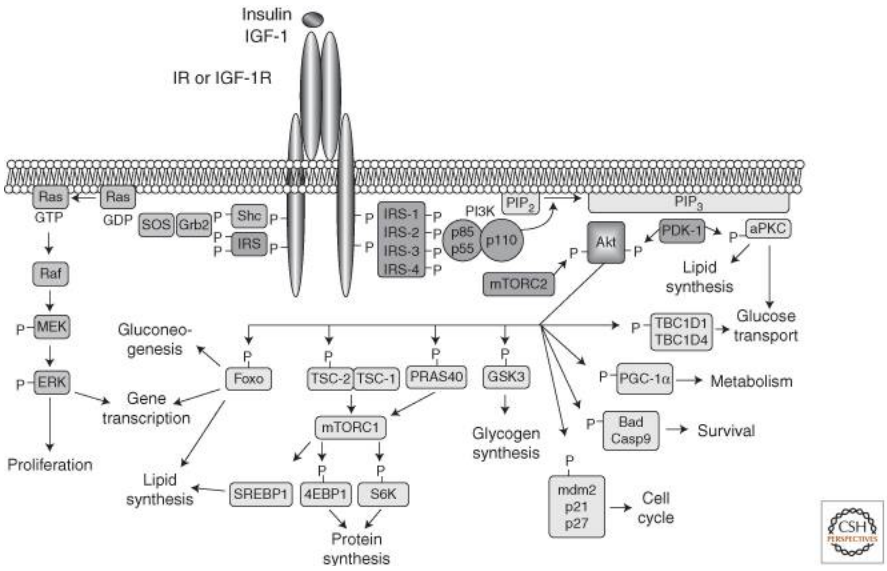


Figure 2. *Insulin and IGF-1-signaling pathways. J Boucher et al. 2014. Cold Spring Harbour Perspectives in Biology*

2.2.3 Major insulin actions in different tissues

2.2.3.1 Insulin action in skeletal muscle

Skeletal muscle is an energy-consuming tissue. It stores glucose as glycogen when glucose is abundant, for later use. Most of the postprandial glucose uptake in humans (approximately 60-80%) is made in skeletal muscle, and proper insulin action is important to maintain glucose homeostasis [60-62]. The effect of insulin in skeletal muscle myocytes is mainly the promotion of glucose uptake, through the translocation of GLUT 4 to the cell membrane. Physical activity also increases the translocation of GLUT 4, hence also glucose uptake, independently of insulin [63]. GLUT 1 transporters are responsible for basal glucose uptake and are not expressed to a great extent in adults but probably more in infants and young children.

The primary INSR substrate in skeletal muscle appears to be IRS-1 and the most important part seems to be Akt 2, since studies in mice have observed that mice lacking Akt 2 develop insulin resistance [64, 65]. Akt phosphorylates several proteins involved in glucose uptake in the myocytes. Among the best characterized are TBC1D1/TBC1D4, which seem to have an effect of the translocation of GLUT 4 to the cell membrane [66].

2.2.3.2 Insulin action in adipocytes

The white adipocytes are cells responsible for the storage of lipids and mobilization of energy by releasing fatty acids. In adipose tissue insulin enhances glucose uptake through increased GLUT 4 translocation to the cell surface by stimulation of the INSR complex and increased lipogenesis; thereby, insulin regulates the secretion of free fatty acids (FFA) in the blood stream. White adipose tissue (WAT) accounts for only <5 % of glucose uptake after a meal [67].

2.2.3.3 Insulin action in hepatocytes

Insulin is released from the pancreas into the portal vein, so the liver is exposed to higher insulin levels than the general circulation [68]. Glucose uptake in the liver is not directly dependent on insulin; rather, it is mainly dependent on the glucose gradient and facilitated by GLUT 2 [69, 70]. The main effect of insulin on the hepatocytes is the activation of glycogen synthesis, lipogenesis and protein synthesis. Both the direct and indirect action of insulin leads to suppressed gluconeogenesis as an effect of postprandial insulin [71-73], however, the mechanisms are not clear. The major isoforms of IRS in hepatocytes are IRS-1 and 2 [74]. Although glucose transport in the hepatocytes is mainly independent of insulin, insulin stimulates glycogen synthesis through the cascade of phosphorylation and dephosphorylation in a similar way as the signaling cascade in skeletal muscle [75, 76]. Insulin also facilitates glucokinase translocation, which is necessary for hepatic glucose regulation [77]. The pathway of hepatic insulin signaling appears to diversify distal to Akt activation, involving substrates regulating glycogen synthesis and mTOR activity, leading to lipogenesis and protein synthesis [57].

2.3 INSULIN RESISTANCE

2.3.1 Pathophysiology of insulin resistance

In 1939 the first notion of insulin resistance came from Himsworth who pointed out that diabetes should be subdivided into two categories "according to which of these disorders predominates into insulin-sensitive and insulin-insensitive types". Reaven and colleagues further explored this proposition and suggested in the Banting Lecture in 1988 that insulin resistance was a link between obesity, hypertension, dyslipidemia and type 2 diabetes in a description of the metabolic syndrome [78]. Insulin resistance is defined as a failure in target tissues to respond to insulin, and the mechanisms underlying is both on the level of the whole body and on cellular level in different tissues [33, 79]. Obesity contributes to the development of insulin resistance in both adults and children and is thought to be one of the major factors in obesity leading to type 2 diabetes. In children adiposity is one of the most important determinants of insulin resistance irrespective of age, ethnicity, and gender [80, 81].

Although extremely rare, a few conditions with mutations in the insulin receptor gene exist and are associated with insulin resistance in childhood. These conditions have varying degrees of severity, ranging from mild to severe phenotypes such as Insulin Resistance syndrome type A, Rabson-Mendenhall syndrome, and Leprechaunism. Severe insulin resistance, in for example Leprechaunism, results in a variety of characteristics such as growth deficiency, hyper-glycemia and other endocrine abnormalities [82, 83]. A milder type of insulin resistance occurs at different stages of life, with variations in insulin resistance during puberty, pregnancy, and ageing [84-86].

Normal glucose regulation is maintained by a feedback loop involving insulin secretion from the islet β-cells and insulin-sensitive tissues. When insulin sensitivity declines, the insulin secretion increases to keep glucose levels in the normal range. When the β-cells cannot secrete enough insulin to compensate for the insulin resistance, the glucose homeostasis is disturbed and progresses to prediabetes and type 2 diabetes [87, 88]. Sophisticated communication is taking place between different tissues, such as skeletal muscle, liver and adipose tissue, and the severity of insulin resistance may vary between organs. It is proposed that impaired insulin secretion from the β-cells in the pancreas affects the brain, liver, skeletal muscle and adipocytes in the form of increasing weight, increased insulin resistance and higher levels of non-esterified fatty acids (NEFA) [33]. The resulting increases of blood glucose and NEFA levels further stress the β-cells, causing a phenomenon referred to as glucolipotoxicity [89, 90].

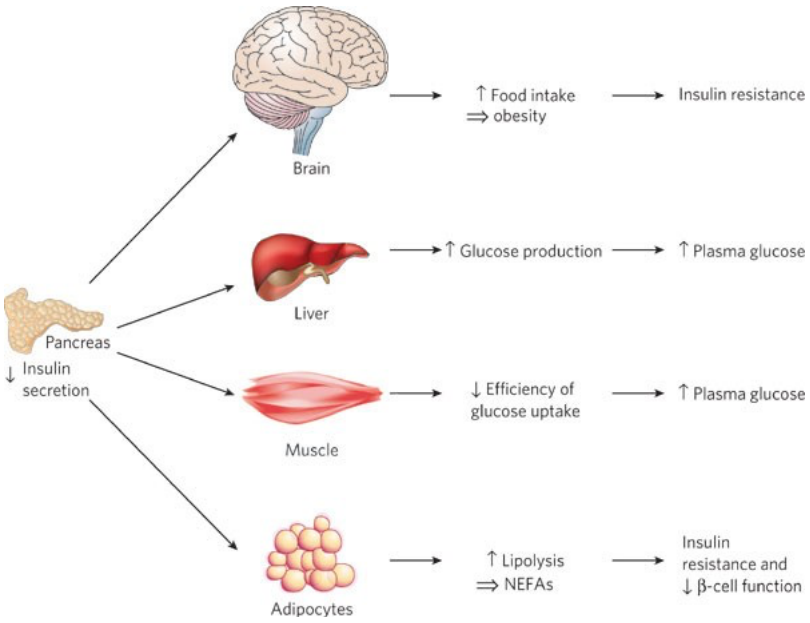


Figure 3. *Mechanism linking obesity to insulin resistance and type 2 diabetes. Kahn SE et al. Nature 2006.*

The causes of insulin resistance are not fully known, but the effects are enormous and many of the alterations work in concert with each other. Insulin and IGF-1 signaling are constricted by several mechanisms; uncontrolled signaling would lead to severe alternations in metabolism and also have negative effects on cell differentiation and cell growth. On the other hand, too much inhibition of these regulatory functions leads to a cascade of adverse events and can play a role in the development of insulin resistance. A diverse group of bioactive factors and molecules are proposed as potential mediators in impairing insulin sensitivity such as hyperinsulinemia per se, low grade inflammation, defects in insulin signaling pathways, lipid metabolites, endoplasmic reticulum stress, oxidative stress, and mitochondrial dysfunction.

2.3.2 Molecular mechanisms behind insulin resistance

The molecular mechanisms in the development of insulin resistance are not easily investigated nor fully understood, possibly due to the intricate nature of the pathways and the fact that many co-exist and interact with each other. A description of all the pathways is not feasible in this thesis. However, some of the cellular and molecular mechanisms underlying insulin resistance in specific tissues are described below.

2.3.2.1 Skeletal muscle insulin resistance

Physiological and molecular studies have observed that the translocation of GLUT 4 is defective in insulin resistance. It seems the most proximal levels of insulin signaling are affected, namely INSR, the IRS-family, PI3K and Akt [91, 92]. Defects in phosphorylation of the IRS substrates are associated with reduced insulin signaling activity, for example phosphorylation of IRS-1 at serine 307, and by some kinases such as IκB kinase (IKK), nuclear factor kappa B (NFkB), and c-Jun N-terminal kinase (JNK) [93]. Some evidence exists that low-grade inflammation is specifically related to muscle insulin resistance and not

hepatic insulin resistance [94], but this association is not fully understood. The inflammatory pathways seem to affect insulin sensitivity in particular by the NF-κB cascade [95, 96], but many other different pathways have also been investigated. The alterations with phosphorylation of the AKT complex, are associated with insulin resistance, and there is also evidence of effects on endothelial dysfunction through endothelial-derived nitric oxide synthetase (eNOS) [97-99].

Impaired insulin signaling in skeletal muscle is also believed to be affected by increased fatty acid intermediates, via impaired IRS-1 phosphorylation and subsequent lower GLUT 4 translocation to the myocyte membrane surface [100-102]. The accumulation of intramyocellular lipid content (IMCL) in skeletal muscle and its metabolites diacylglycerol (DAG) is proposed to be the potential mediator in the development of insulin resistance. There is some evidence that DAG affects PKC, one of the mediators in the INSR complex, and the activation thereby results in an cascade of events leading to a blockage of the Akt pathway, causing impaired insulin signaling [103] [60]. Physical activity mainly targets muscle insulin sensitivity, by promoting GLUT 4 translocation to the cell membrane, thus facilitating glucose uptake [104].

2.3.2.2 Adipose tissue insulin resistance

Visceral adipose tissue and adipose tissue dysfunction, rather than the overweight or obese condition per se, are proposed to be associated with insulin resistance in both adults and children [105, 106]. Adipose tissue dysfunction is characterized by adipocyte hypertrophy rather than increased adipocyte number and is associated with systemic inflammation with altered levels of adipokines, chemokines and macrophage infiltration [107]. The activation of macrophages and subsequent released of chemokines such as TNF-α and interleukin-6 (IL-6) are associated with obesity and correlated with the risk of type 2 diabetes [108-111]. TNF-α affects the insulin signaling pathways through phosphorylation of IRS, which leads to a reduced glucose uptake, and this process is associated with an increased transcription of inflammatory genes via NFκB and JNK [112, 113].

Adipocyte dysfunction is proposed to be an essential event in the development of insulin resistance, increasing the inflammatory responses and FFA delivery to skeletal muscle and liver, promoting development of insulin resistance in those tissues [114, 115]. The specific molecular defects in adipocyte insulin resistance in humans are not well known; most studies have focused on the endocrine and autocrine functions in white adipocytes.

2.3.2.3 Hepatic insulin resistance

Insulin directly and indirectly promotes hepatic glucose uptake, in combination with hyperglycemia. This also involves suppression of gluconeogenesis and glycogenolysis and the activation of glycogen synthesis. In hepatic insulin resistance the effect of insulin's ability to suppress gluconeogenesis is diminished, causing fasting hyperglycemia and also increased lipid synthesis [116-118].

Studies in knockout mouse models have described that changes in insulin signaling in the Akt complex leads to insulin resistance and impaired glucose tolerance (IGT), in liver as well as in skeletal muscle and adipose tissue [119, 120]. Besides exerting effects on glucose metabolism in the liver, insulin also regulates lipid metabolism by promoting lipogenesis. Individuals with insulin resistance should according to this assumption have a decreased lipid synthesis in the liver, which is seen in genetic mice models with a knockout of the INSR

where a decreased lipid synthesis is evident [121-123]. However, in the insulin-resistant state there is often an association with increased lipogenesis and hepatic steatosis suggesting that hepatic insulin resistance is not selective but maybe more a part of an integrated biological system.

Overnutrition and the subsequent insulin resistance and increasing insulin levels has effects on skeletal muscle, adipose tissue, hepatocytes, β-cells in pancreas, and the brain as summarized in Figure 4.

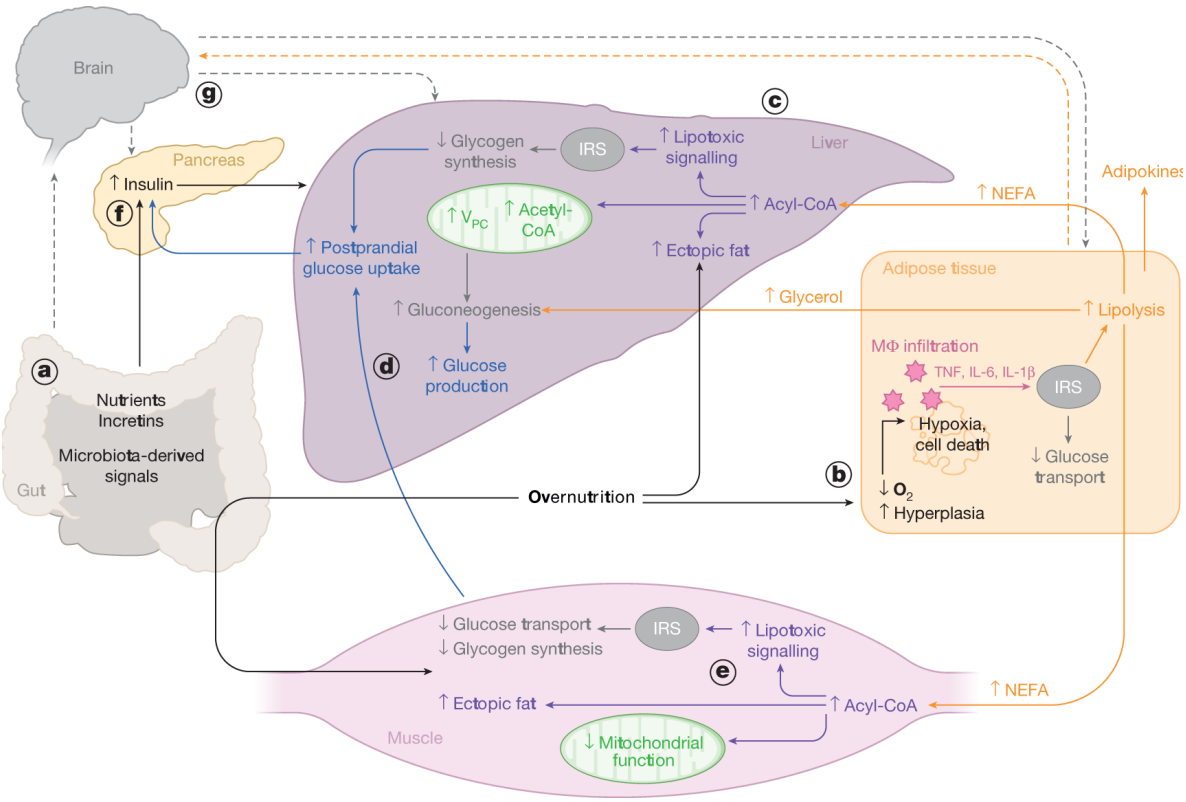


Figure 4. *The integrative biology of type 2 diabetes. Roden et al. Nature Dec 2019.*

2.3.3 The gut

2.3.3.1 Incretin effect

Oral glucose induces insulin secretion, in spite of equally elevated blood glucose levels, more than intravenous glucose [124]. This phenomenon is called the incretin effect and is mediated by the gut-derived hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like polypeptide (GLP-1)[125]. GIP is released from enteroendocrine K cells, which resides mainly in the duodenum and upper jejunum and mediates most of the incretin effect in healthy individuals [126, 127]. GLP-1 is produced by enteroendocrine L cells, residing mostly in the mucosa in the ileum and colon [128]. In both adults and adolescents with prediabetes and type 2 diabetes, a reduced incretin effect can be seen [129-134]. Patients with type 2 diabetes have a reduced β-cell mass [135], and it is hypothesized that this reduction in β-cell mass is a contributor to the diminished incretin response. This hypothesis is supported by findings that such patients have lost their GIP-mediated insulin response [136]. The transcription factor 7 like 2 (TCF7L2) gene variant is associated with an

increased risk of prediabetes and type 2 diabetes, possibly through impaired hepatic insulin sensitivity and β-cell function [137, 138]. There are indications that a reduced incretin effect is associated with the TCF7L2 gene variant, in both healthy and obese adults as well as in adolescents [139, 140], not because of reduced secretion of GLP-1 and GIP, but rather due to the effect of TCF7L2 on the sensitivity of the β-cell to incretins. Incretin-based drugs are widely used in adults with type 2 diabetes, with positive effects on glycemic control and BMI and are also of beneficial effect in patients with kidney and heart failure [125].

2.3.3.2 Gut microbiota

In 1908, the Nobel Prize for Medicine was awarded to Elia Metchnikoff and Paul Erlich who were the first to pay attention to human gut flora. Having observed that mountain farmers who consumed fermented milk lived longer, the recipients formulated the oral bacteriotherapy theory [141]. The intestinal microbiota plays a part in the metabolism, partly by having an effect on the immune system but also through neuroendocrine signaling [142, 143]. An altered gut microbiota has been proposed as a driver of the inflammation associated with insulin resistance and the development of type 2 diabetes [144, 145], but the mechanisms are largely unknown.

2.3.4 Genetic background

The relationship between obesity and insulin resistance exists regardless of ethnicity [146]. However, the relation between adiposity and insulin resistance varies across ethnic groups [147, 148]. Ethnic differences appear to exist in the relationship between insulin sensitivity and insulin response in cohorts with normal glucose tolerance as presented in Figure 5, making some ethnic populations more susceptible to diabetes [149]. A longitudinal study on insulin resistance and insulin secretion in normal-weight children and adolescents, revealed that both insulin sensitivity and insulin secretion diminish transiently during puberty; however, only the African Americans in this cohort of children had a low disposition index (DI), which might reflect a higher risk of developing type 2 diabetes [150].

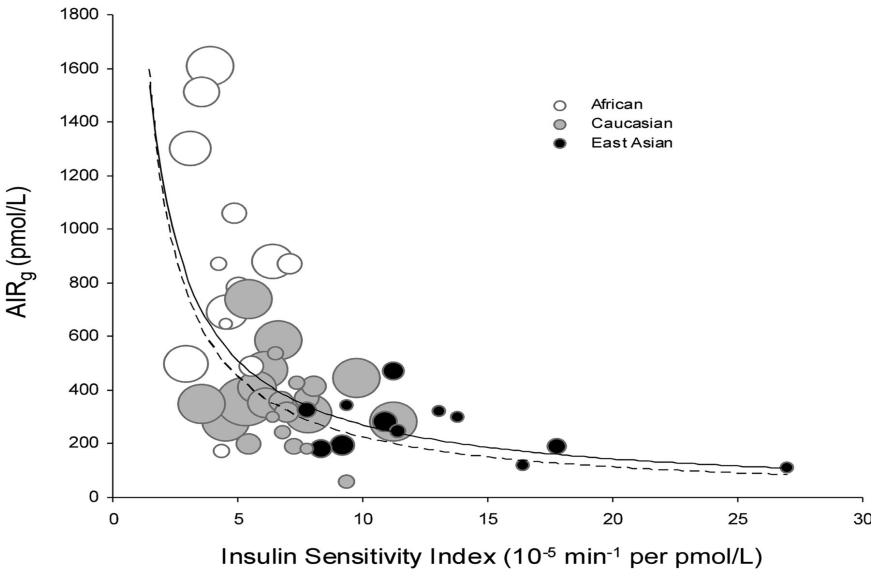


Figure 5. *Ethnic differences in the relationship between insulin sensitivity and insulin response. Kodama et al. Diabetes Care June 2013.*

Genetic background is of importance in the development of both type 1 and 2 diabetes, which has led to an extensive search for the gene variants associated with diabetes during the past decade. In type 1 diabetes genes in the human leukocyte antigen (HLA) region confer 50 % of the genetic risk of T1D [151, 152], and more than 60 non-HLA risk variants have been identified [153-155].

Finding genetic markers for the early detection of prediabetes or diabetes has been proposed as a tool to identify individuals at high risk, but only 10 % of the type 2 heritability is explained by gene variants [156, 157]. While obesity is the strongest predictor of type 2 diabetes, heritability is also a predictor, varying from 26% to 69 % depending on age at onset [158-160]. The first discovered type 2 risk alleles were three genetic variants in KNJ11, PPARy, and TCF7L2 [161]. Over recent decades, over 400 risk gene variants associated with type 2 diabetes risk have been discovered through extensive research by large-scale genetic studies [162].

2.3.5 Puberty

In healthy children the insulin sensitivity decreases with the onset of puberty, and recovers by the end of puberty, and insulin secretion increases to compensate for the higher insulin resistance [84, 163-165]. IGF-1 levels follow the rise and fall in insulin resistance during puberty, suggesting an effect of the GH/IGF-1 axis on pubertal insulin resistance [166].

2.3.6 Physical activity

A sedentary lifestyle is clearly associated with increased insulin resistance and obesity as well as an increased risk of death due to any cause [167]. The level of physical activity decreases during the transition from childhood to adolescence [168], and there is evidence that physically active youth remain active in adulthood [169]. A recent randomized study proved the positive effects of increased physical activity in different modalities in increasing insulin sensitivity and reducing ectopic fat in obese adolescents [170].

2.3.7 Diet

Many trials on different diets have been conducted during the last decades, with different compositions, with conflicting results on how to achieve the most successful weight management. The diversity of results is due to a great number of causes, such as variations in the duration of trial, type of diet, difficulties in adherence to dietary advice, and also the heterogeneity of trial settings. It is evident that changes in dietary patterns are extremely difficult to achieve and maintain: a recent metanalysis of 121 randomized trials in obese adults enrolled on different diets showed that all diets (low carbohydrate, moderate macronutrients and low-fat diet) had similar effects on weight loss and improvement in cardiovascular risk factors after six months and that all these effects had disappeared after twelve months [171]. Studies in lifestyle alterations and diet in obese children have shown that a very-low-carbohydrate diet, a reduced intake of carbohydrates limited to 20-50 g/day or 5-15 % of total calories, have proven more positive results on short-term weight loss and improved insulin levels and insulin resistance than the traditional low-fat diet [172-174]. Studies of which dietary pattern is beneficial for children with obesity are limited, but it appears that a reduction in carbohydrate intake, may be beneficial in reducing risk factors for type 2 diabetes in youth with obesity [174, 175]. However, another study presented positive

effects on weight independent of type of diet, which was interpreted as showing that any structured diet program can have positive effects [176].

2.4 MEASUREMENTS OF INSULIN RESISTANCE AND INSULIN SECRETION

Many methods are available for the estimation of insulin resistance, from simple fasting blood measures to elaborate protocols involving intravenous infusion and repeated testing. All methods are approximations of insulin resistance in a multicompartment system, the human body, and several different methodologies and models are used to create indexes of insulin resistance. A wide range of cut-off values to define insulin resistance exists, which makes comparison between studies and populations complicated. A few studies have used arbitrary cut-off levels from population estimates, but there is no uniform categorical definition of insulin resistance.

2.4.1 Direct measures of insulin resistance

2.4.1.1 Hyperinsulinemic-Euglycemic Clamp

The gold standard to determine insulin resistance in vivo was developed by DeFronzo et al [177]. This technique assesses whole-body and tissue insulin sensitivity and estimates whole-body glucose disposal under steady-state conditions. In the fasting state the glucose production, mainly from the liver, is equal to glucose uptake in peripheral tissues. Insulin suppresses hepatic glucose production and stimulates glucose uptake. The clamp technique uses this by creating a steady-state level of exogenous insulin infusion suppressing endogenous hepatic glucose production, while the plasma glucose concentration is held constant at normal glucose levels in a one-compartment model. The rate of variable glucose infusion necessary to maintain normal glucose levels, "clamp", provides a measure of the effect of insulin on glucose production and utilization. Subjects who require a higher amount of glucose infusion to remain euglycemic are more insulin-sensitive, giving a measure of insulin-stimulated glucose disposal (M) and insulin sensitivity (M/I), where I is steady-state insulin concentration) [177, 178]. This technique requires infusion of both insulin and glucose via two intravenous lines, and frequent blood sampling to control the hyper-insulinemic and euglycemic state.

2.4.2 Indirect measures of insulin resistance

2.4.2.1 Fs-IVGTT minimal model

The Frequently Sampled Intravenous Glucose Tolerance Test (fs-IVGTT) was developed by Bergman et al in 1979 [179]. It is a validated method to assess insulin sensitivity in both adults and in children [180, 181]. Fs-IVGTT data are most commonly analyzed in a minimal model assessment [182], in which the glucose and insulin data are entered in the MINMOD computer program generating an insulin sensitivity index (Si), acute insulin response (AIR) and glucose effectiveness (Sg).

The fs-IVGTT is performed after an overnight fast, and the subjects receive an intravenous catheter in each arm. Baseline blood samples of insulin and glucose are drawn, and at time 0 minutes an intravenous bolus of glucose is given. Frequent sampling of glucose and insulin concentrations is performed every minute during the first 10 minutes to measure the acute (first-phase) insulin response and used to derive the late (second-) phase response, and

measurements of insulin and glucose continued throughout the test. Insulin is administered at 20 minutes, a given amount of insulin per kg body weight as an intravenous bolus, and measurements of glucose and insulin are taken over a period of 180 minutes. An overview of the test is displayed in Figure 6.

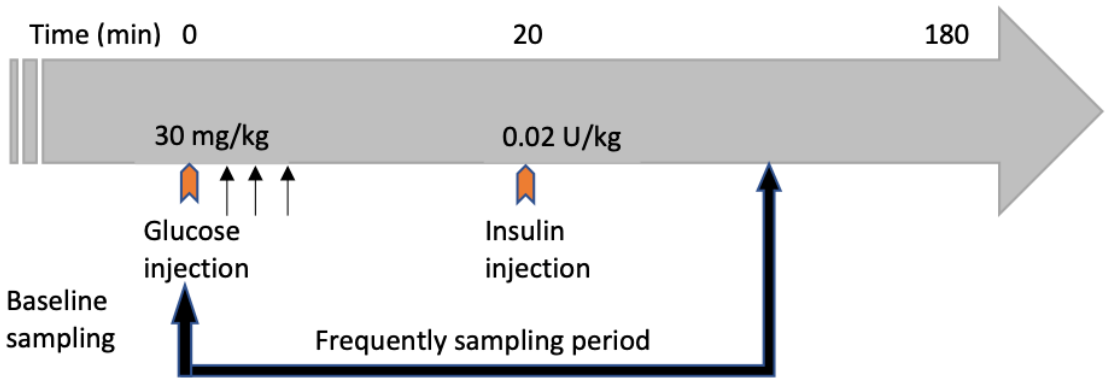


Figure 6. *Schematic display of the fs-IVGTT test. Initially, a repeated baseline sampling of glucose and insulin is performed; thereafter an intravenous glucose bolus is given. A period of frequent sampling period of glucose and insulin levels follows, and after 20 minutes an intravenous bolus of insulin is given. Sampling is performed frequently during the initial phase, and thereafter more spaced out over the 180-minute period of the test.*

The minimal model is a mathematical model investigating glucose and insulin kinetics by two differential equations assuming two compartments: dynamics of glucose uptake after an external stimulus and assuming that insulin is the driving function; and in a separate, remote compartment in which the dynamics of insulin release in response to glucose is assumed, where glucose drives the function. Si is calculated from two of these model parameters and is defined as the fractional glucose disappearance per insulin concentration unit [179, 182].

$$Differential\ eqation\ 1 = \frac{dG(t)}{dt} = -G(t) \times (Sg + X(t)) + Gb \times SG(0) = G0$$

$$Differential\ equation\ 2 = \frac{dX(t)}{dt} = -P2 \times X(t) + P3 \times F(t) X(0) = 0$$

$$F(t) = 0\ if\ I(t) \leq Ib$$
$$else$$
$$I(t) - I$$

Explanation of the equations: *G(t) is plasma glucose at time t. Sg is glucose effectiveness, X(t) is insulin action at time t. Gb is the basal glucose concentration. I(t) is the plasma insulin concentration at time t. Ib is the basal insulin concentration. F(t) is a function that represents the elevation of plasma insulin above basal insulin. P2 describes the removal rate of insulin from the interstitial space. P3 describes the movement of insulin to the interstitial space.*

An advantage of the fs-IVGTT is that it is easier to perform than the clamp-method since it does not rely on steady-state conditions, so constant adjustments of infusions are not needed.

The index of insulin sensitivity, Si, is comparable to clamp-derived insulin sensitivity measures in healthy subjects but displays slightly weaker associations in insulin-resistant populations [180, 183-185]. As insulin resistance varies, the endogenous hepatic glucose production may contribute to the variation in Si [186]. Another disadvantage is that when β-cell dysfunction is severely affected, the first-phase insulin response can be very low or unmeasurable [187, 188], although the insulin-modified protocol was intended to overcome this [182].

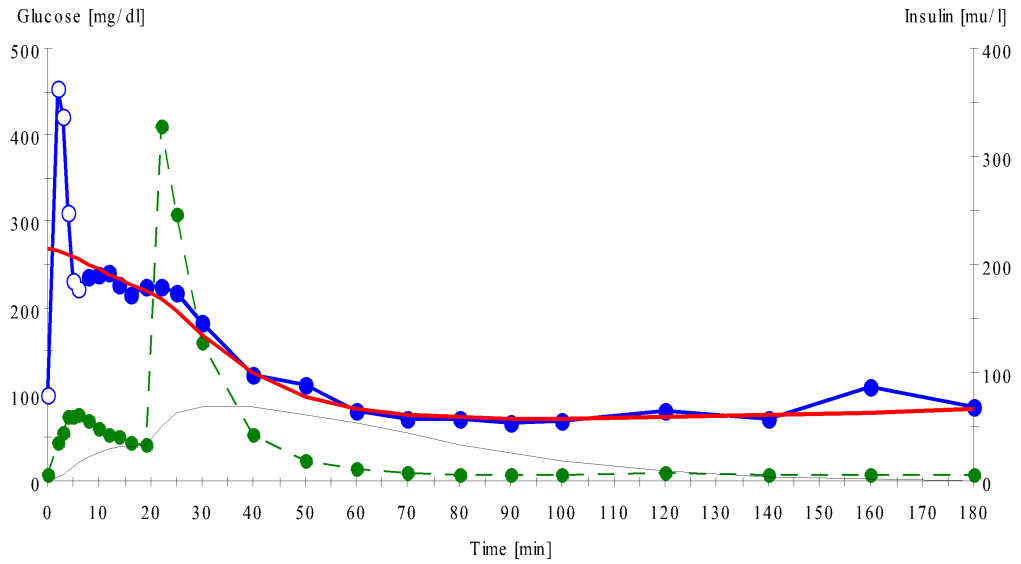


Figure 7. *Results of an fs-IVTT investigation with minimal modeling in MINMOD Millennium. Green dots represent insulin levels, and the peak after 20 min is displays the AIR to the glucose bolus. Blue dots represent glucose levels.*

2.4.2.2 Oral Glucose Tolerance Test

The Oral Glucose Tolerance Test (OGTT) is most often used as a test to determine glucose tolerance but can also be used to assess β-cell function and insulin resistance. The insulin, glucose and C-peptide levels can be measured after glucose ingestion, to calculate the early insulin and insulinogenic response [189, 190]. The surrogate markers of insulin secretion and insulin resistance derived from these modified OGTTs are, for example, SI index-Matsuda, Insulin Sensitivity Index [191, 192]. Extended OGTT models can also be used, with the simultaneous use of surrogate measures such as HOMA%S as well as minimal modeling techniques [193, 194].

2.4.3 Simple measures of insulin action and sensitivity

Homeostasis model assessment (HOMA) was developed in 1985 by Matthews et al. and is used to quantify insulin resistance and β-cell function from basal fasting glucose and insulin concentrations [195]. The HOMA model is robust in clinical and epidemiological studies when only fasting levels of glucose and insulin are available, and correlates well with insulin sensitivity determined by the euglycemic clamp [196-198]. Several other methods are used, but HOMA-IR is the most widely used surrogate measure and seems to be a reliable measure in children as well [198, 199].

A brief summary of methods defining insulin resistance is displayed in Table 1.

Table 1. Measures of insulin resistance and insulin secretion

	Method	**Measure**	
Direct	Hyperinsulinemic Euglycemic Clamp	Insulin sensitivity (M/I)	Steady state Gold standard
Indirect	Fs-IVGTT with minimal modeling	Insulin sensitivity (Si) Acute insulin response (AIR) Glucose effectiveness (Sg) Disposition index	Dynamic data Two-compartment assumption
	Oral glucose tolerance test (OGTT)	Determining glucose tolerance	Physiologic conditions
Simple surrogate measures	HOMA-IR HOMA%B HOMA%S QUICKI	HOMA-IR=(fasting insulin (μU/mL) x fasting glucose (mmol/l)/22.5 QUICKI= 1/log (fasting insulin μU/mL)+fasting glucose (mg/dL)	Based on fasting levels of insulin, c-peptide and glucose
Surrogates derived from dynamic tests	OGTT Mixed Meal Tolerance -Matsuda index -Stumvoll index -Gutt index	Insulin sensitivity ($ISI_{Matsuda}$), insulin secretion, DI ($ISI_{Stumvoll}$, ISI)	Dynamic data Correlates well with clamp

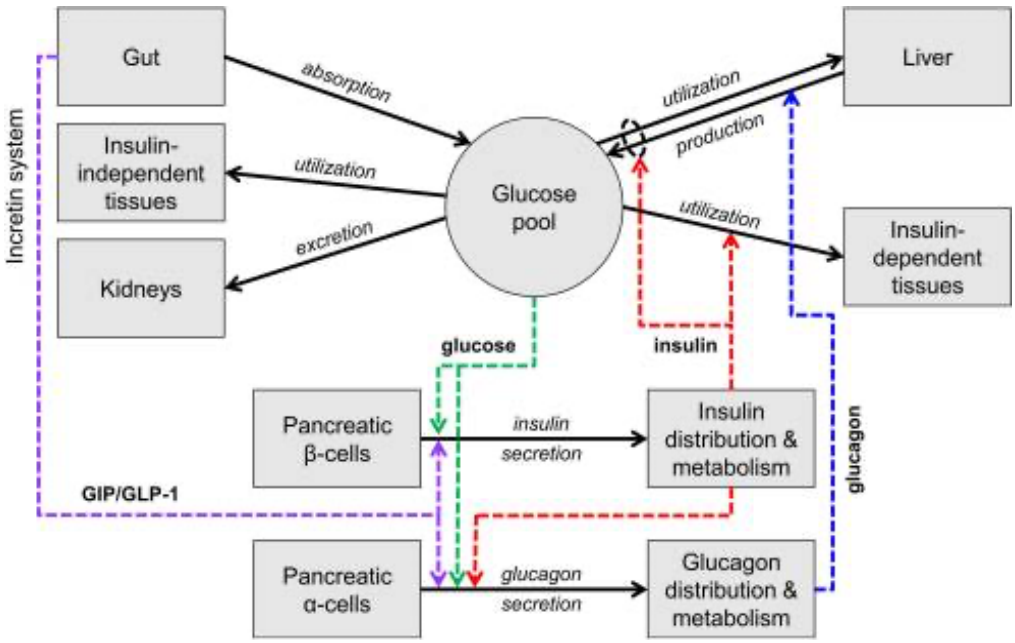


Figure 8. *To assess insulin resistance, different mathematical models of glucose and insulin fluxes have been created, all of which are assumptions of reality. This is a scheme of the main mechanisms in glucose homeostasis. The colored dashed arrows represent control signals that regulate glucose fluxes of insulin and glucagon secretion. Mathematical modeling of Glucose homeostasis. Mari et al. Frontiers in Physiology Nov 2020.*

2.5 PREDIABETES

Impaired fasting glucose (IFG) and IGT are intermediate stages in the development from normal glucose tolerance to diabetes. There is some evidence that IFG and IGT represent different and distinct phenotypes [200]. Studies of tissue-specific insulin-resistant phenotypes are rare, possibly since measures are not easily taken when multiple simultaneous biological processes are at play at the same time. IFG is characterized by a more pronounced hepatic insulin resistance and a measure of glucose disturbance in the basal state [201]. IGT is characterized by a peripheral or skeletal muscle insulin resistance [201, 202]. In individuals with more pronounced muscle insulin resistance, an increased inflammatory gene expression has been noted, and plasma markers of low-grade inflammation are increased [94].

2.5.1 Diagnosis of prediabetes

The cut-off level for diagnosing IFG has been widely debated. The American Diabetes Association (ADA) guidelines were changed in 2003, when the cut-off level was lowered from plasma glucose 6.1 mmol/L to 5.6 mmol/L (referred to as IFG_{ADA} in this thesis) to increase the sensitivity of testing to identify individuals at risk of type 2 diabetes. WHO guidelines categorize IFG as a fasting blood glucose level of 6.1-6.9 mmol/L (referred to as IFG_{WHO} in this thesis). IGT is diagnosed after a two-hour OGTT glucose load if glucose is 7.8-<11.1 mmol/L.

2.5.2 Epidemiology of prediabetes

Depending on the cut-off level of glucose used and the population studied, the prevalence of prediabetes varies widely. However, 7.3% of the global population were estimated to have prediabetes in 2017 [203]. In a recent population-based study in healthy adolescents aged 12–18 years in the USA, 18% had prediabetes, among them IFG_{ADA} was the most prevalent type of glucose dysregulation, and the prevalence was higher among males and associated with obesity [204]. In a comparison between Swedish and German obese children, the prevalence of IFG_{ADA} was much higher among the Swedish children than the German cohort (17.1% vs. 5.7%) [205].

2.6 DIABETES

2.6.1 Diagnosis of diabetes

Diabetes is a disease characterized by elevated blood glucose, and the diagnostic criteria are based on plasma blood glucose and dependent on the presence or absence of symptoms. Diabetes is diagnosed in both children and adults by a combination of classic symptoms and a random plasma glucose ≥11.1 mmol/L, fasting plasma glucose ≥ 7.0 mmol/L, or a plasma glucose ≥ 11.1 mmol/L two hours after an OGTT. The International Society for Pediatric and Adolescent Diabetes (ISPAD) 2018 guidelines also suggest the use of HbA1c as an aid in diagnosing diabetes, and a HbA1c level >48 mmol/mol indicates diabetes [206].

Diabetes in young people usually present with symptoms such as polyuria, polydipsia and weight loss. The initial presentation can vary among patients: some present with symptoms and are clinically stable and others present with severe symptoms and ketoacidosis. Assessing diabetes type can sometimes depend on the circumstances present at the time of diagnosis, since not all patients present with symptoms and the clinical picture can be very diverse. Some individuals with newly diagnosed diabetes cannot be easily categorized since the etiology of diabetes is heterogenous, although the majority of all diabetes cases can be classified into two categories of diabetes, as further discussed below.

2.6.2 Diabetes classification

2.6.2.1 Type 1 diabetes

Type 1 diabetes is the most common type of diabetes among children and adolescents, and it is considered to be an autoimmune disease. It is characterized by insulin deficiency, caused by an immunologic destruction of the insulin-producing β-cells in the pancreas. In 85-90 % of these individuals autoantibodies to GAD, as well as the tyrosine phosphatases IAA, IA2 or Znt8 autoantibodies, are present at diagnosis [207]. Type 1 diabetes has a strong association with the HLA system, with a linkage to DQA and DQB genes [208]. These patients are also predisposed for other autoimmune mediated diseases such as for example coeliac disease and Hashimoto´s disease.

2.6.2.2 Type 2 diabetes

Type 2 diabetes is caused by a combination of insulin resistance and the failure of the β-cells to produce sufficient insulin to maintain normal glucose levels and is associated with overweight and obesity.

2.6.2.3 MODY

There are several forms of inherited diabetes, they are referred to as maturity-onset of the diabetes in the young (MODY). They are inherited in an autosomal dominant pattern and are usually detected at an early age and accounts for 1-4 % of pediatric diabetes patients [209]. Several variants are known, the most common form is caused by a mutation in HNF-1 alfa (MODY 3) in chromosome 12, which is responsive to sulfonylurea treatment [210, 211]. A mutation in the glucokinase gene in chromosome 7 (MODY 2) leads to elevated glucose levels, where the glucose sensor is set to high and leads to elevated "normal" levels of glucose. It is often randomly discovered and does not need any treatment [212, 213].

2.6.2.4 LADA

Latent autoimmune diabetes (LADA) in adults, represents <10 % of all diabetes in adults. LADA initially presents as type 2 diabetes and becomes increasingly more similar to type 1 diabetes. It is associated with the presence of glutamic acid decarboxylase autoantibodies (GAD), and in time insulin treatment is needed [214].

2.6.2.5 Other types of diabetes

Although other diabetes types are rare in children, they can be a part of other diseases such as cystic fibrosis, metabolic or mitochondrial diseases or drug-induced diabetes, for example during cancer treatment. A few other endocrine disorders are associated with diabetes, for example polycystic ovary syndrome, Cushing´s syndrome, GH-producing tumors, and autoimmune polyendocrine syndromes. Genetic syndromes can also be associated with diabetes, for example Downs, Laurence-Moon-Biedl, and Prader-Willi syndromes.

2.6.3 Epidemiology of diabetes

2.6.3.1 Prevalence of type 1 diabetes

The incidence of type 1 diabetes is increasing. There is evidence suggesting that the trend in incidence varies in across countries, and it seems the incidence has shifted to a peak at a younger age [215, 216]. The highest incidence is in Finland, with an incidence of 60 cases per 100,000 children per year [217]. In Sweden the incidence is 43 per 100, 000 children per year and the peak incidence is 10–14 years [218, 219].

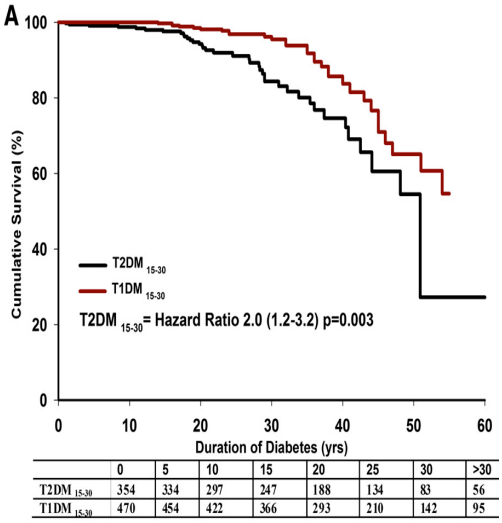
2.6.3.2 Prevalence of type 2 diabetes

The prevalence of type 2 diabetes among children and adolescents is increasing in several parts of the world. Large variations in the prevalence and incidence are apparent, with higher rates among certain ethnicities and populations. In the USA the overall incidence of type 2 diabetes in youth has increased from 9 to 12.5 cases per 100,000 youth per year from 2002-2003 to 2011-2012, and among Pima Indians 330 per 100,000 person-years [215, 220, 221]. The European countries have the lowest incidence rates and prevalence of early-onset type 2 diabetes; Germany, for example, has a low prevalence rate of 2.42 per 100,000 youth under 20 years of age [222, 223].

2.7 COMPLICATIONS ASSOCIATED WITH TYPE 2 DIABETES IN YOUTH

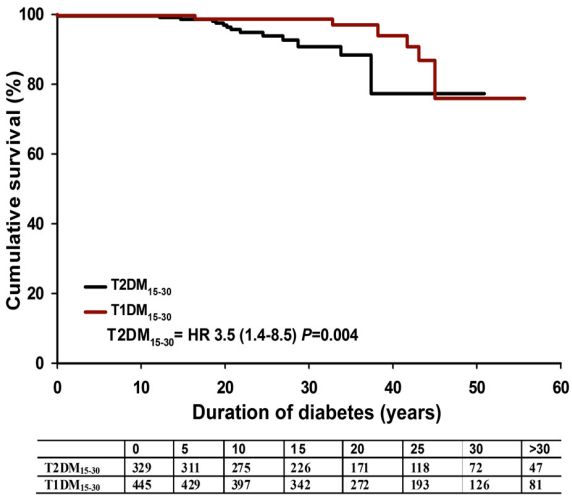
Individuals with early-onset of diabetes type 2 diabetes often present with complications such as hypertension, microalbuminuria, and dyslipidemia at the time of diabetes diagnosis [224]. Both type 1 and 2 diabetes have a similar pattern of complications, with an increased risk of micro-and macrovascular complications [224-226]. Individuals with type 2 diabetes have an excess risk of death of cardiovascular or any cause, and the risk is higher at a younger age and also associated with worse glycemic control and the presence of renal complications [227]. It appears that the risk of developing complications is higher in youth with type 2 diabetes, and there is also faster progression than seen in type 1 diabetes of similar duration [34, 224, 225, 228]. Obese youth with type 2 diabetes continue to have an adverse risk profile for several years after diagnosis, which implies that early-onset diabetes type 2 is more pathogenic than a later-onset [224]. In a cohort of individuals with type 1 and 2 diabetes and a diabetes onset between 15 and 30 years of age, early-onset type 2 diabetes was associated to a higher morbidity and mortality in cardiovascular disease as shown in Figure 9 [229] .

Survival



	0	5	10	15	20	25	30	>30
T2DM 15-30	354	334	297	247	188	134	83	56
T1DM 15-30	470	454	422	366	293	210	142	95

Cardiovascular death



	0	5	10	15	20	25	30	>30
T2DM15-30	329	311	275	226	171	118	72	47
T1DM15-30	445	429	397	342	272	193	126	81

Figure 9. *Long-term complications and mortality in youth-onset diabetes: Type 2 diabetes is more hazardous and lethal than type 1 diabetes. Constantino, M et al. Diabetes Care 2013.*

2.7.1 Kidney

Like adults, adolescents with type 2 diabetes have higher systolic blood pressure and the presence of microalbuminuria and dyslipidemia at an early stage, sometimes even present at diagnosis [230]. Glucose toxicity, even below the threshold for diabetes diagnosis, is associated with the presence of microalbuminuria in obese youth [231], suggesting that only moderately elevated blood glucose has an effect on the microvasculature in the kidney. Diabetes-related kidney disease is one of the most frequent complication of both type 1 and 2 diabetes and is the most common cause of end-stage renal disease [232].

It has been proposed that microalbuminuria I the result of increased vascular leakage due to endothelial dysfunction, but the mechanisms have not been established. In adult's microalbuminuria is predictive of cardiovascular disease [233], but the risk in youth is not clear.

Inflammatory pathways are proposed as central mechanisms in diabetic kidney disease, and serum amyloid A (SAA) increases in inflammatory states. Both studies in humans and models in mice show an increase of SAA in the blood and produced in the kidneys [234], and it seems that podocytes exposed to SAA in the kidney increases NF-κB activity, activating numerous inflammatory responses [234].

2.7.2 Retinopathy

The overall prevalence of diabetes retinopathy is 35% among people with diabetes worldwide, although diagnostic and therapeutic improvements in recent years have led to a decline in both any retinopathy and sight-threatening stages [235, 236]. Population-based studies from Europe reveal that, among individuals with newly-diagnosed type 2 diabetes, retinopathy was detected by screening in more than 13% [237].

The major determinant of diabetic retinopathy is chronic hyperglycemia [238], but the level of glycemia (HbA1c) is not the only factor of importance. The variation in risk is also dependent on other factors such as sex, genetics, disease duration, blood pressure, and lipids [238-241]. It also seems that type of diabetes, not just HbA1c, is a factor in the risk of developing retinopathy.

The pathophysiology underlying diabetes-related retinopathy is complex. It has been proposed that it is a mainly microvascular disease and that pericytes (small cells lining micro vessels), function as the primary trigger of vascular damage in the retina [242, 243]. The origin of pericytes is not fully understood; they are of a heterogenous background, evolving from the neural crest, mesenchymal stem cells or the bone marrow. Their role in the pathophysiology in the development of vasoregression (the formation of avascular capillaries) is unclear. Recent studies have proposed an intricate pathophysiologic process involving angiogenesis, oxidative stress, activation of glia cells, reactive metabolites and also development of neurodegeneration in the retina [244-247].

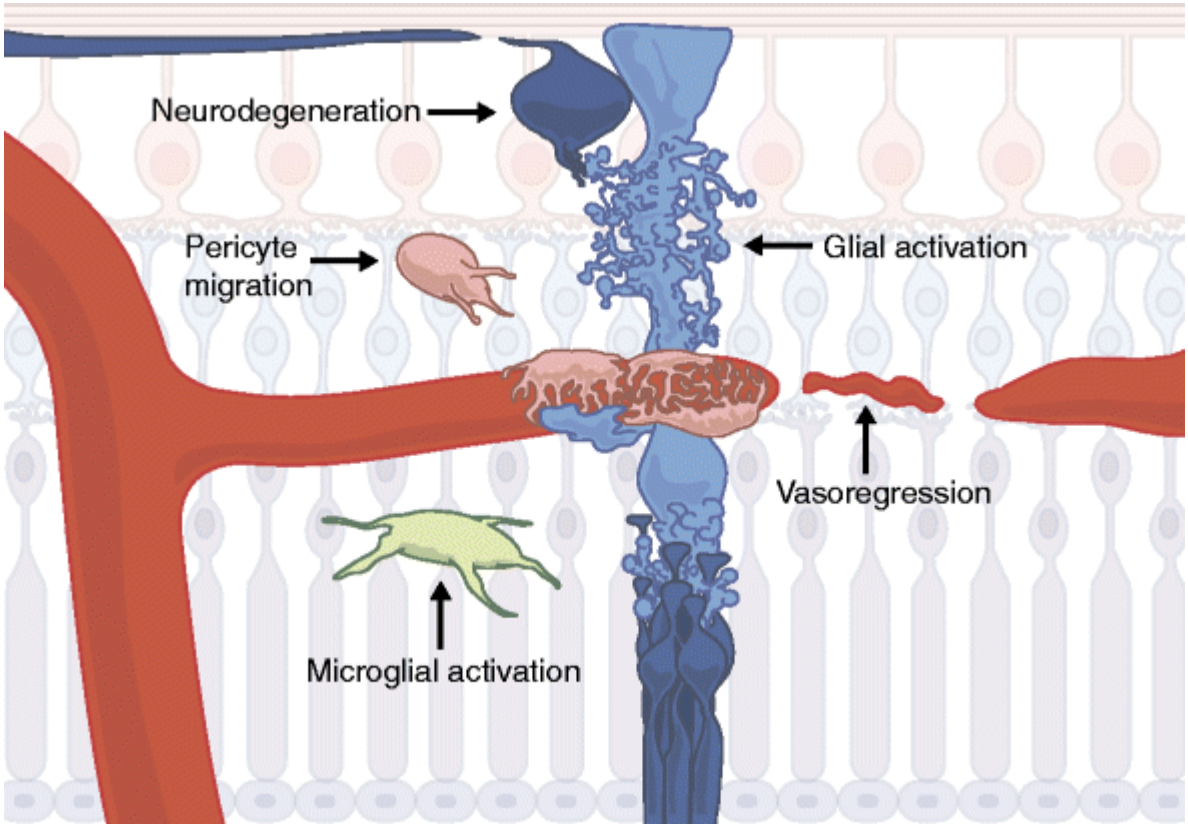


Figure 10. *Schematic summary of hyperglycemia-induced changes of the neurovascular unit in the Wistar rat STZ-induced model. Diabetic retinopathy: Hyperglycemia, oxidative stress and beyond. Diabetologia Hammes et al. 2017.*

2.8 TREATMENT OF YOUTH-ONSET TYPE 2 DIABETES

Pediatric specialist teams are mainly familiar with type 1 diabetes, and management of type 2 diabetes in children has other specific requirements. There are several differences between the two, and knowledge of their different pathophysiology and disease development is essential. In type 1 diabetes treatment, the education of children and family focuses on the underlying insulin deficiency, goals to have glucose in target and to minimize acute and chronic complications to diabetes. Type 2 diabetes is associated with obesity and obesity-related comorbidities, lack of physical activity, and frequent psychosocial and neuropsychiatric difficulties, all of which are all factors that influence the management and treatment in early-onset type 2 diabetes. Type 2 diabetes is usually diagnosed in adolescence, in association with puberty when parental influence and supervision is lessening. Hence, adolescents with a chronic disease must take greater responsibility for managing the disease, and healthcare professionals must educate them, and find individual and achievable goals.

Commonly, other family members have obesity or type 2 diabetes, so treatment goals and lifestyle changes often need to be directed to the whole family. Unlike in type 1 diabetes, comorbidities are sometimes present early such as fatty liver disease, dyslipidemia, hypertension and sleep apnea [228, 248, 249]. The central element of treatment is directed towards achieving lifestyle changes, but pharmacologic treatment is also given. The four main pillars of management are listed below and serve as a short summary of treatment according to ISPAD guidelines [250].

1) Lifestyle changes are recommended at diagnosis and includes diabetes education and advice concerning behavioral changes, covering physical activity and nutritional aspects.
2) Initial pharmacologic treatment includes metformin and insulin alone, depending on the first clinical presentation and level of glycemia.
3) Self glucose monitoring.
4) Assessment of comorbidities, such as presence of microalbuminuria, dyslipidemia, blood pressure, retinopathy, or elevated liver enzymes (NALFD, NASH).

2.8.1 Other pharmacologic therapies

Many other therapies are approved for adults with type 2 diabetes; however, in youth (<18 years old) only insulin, metformin, and recently the GLP-1 agonist (Liraglutide) have been approved as pharmacologic treatments [251]. Metformin has for long been the approved and mostly used pharmacological treatment for type 2 diabetes in youth, and insulin is added to metformin treatment if glucose and metabolic control is not achieved with metformin only.

Other treatment options are needed in youth, since neither insulin or metformin can affect the progressive deterioration of β-cell failure in IGT and type 2 diabetes [252], and insulin can lead to further weight gain. Liraglutide dosing and safety in youth with obesity has been assessed [253], and the use of Liraglutide in obese youth led to a significantly greater reduction in BMI SDS than placebo and conventional lifestyle therapy, although it is associated with a higher frequency of gastrointestinal adverse events [254]. In obese youth with type 2 diabetes adding Liraglutide to treatment with insulin or metformin improved glycemic control but was also associated with gastrointestinal adverse events [251]. A PPAR-γ inhibitor, rosiglitazone, which mainly increases insulin sensitivity, has in the TODAY study been used as an additional therapy to metformin and they demonstrated that a combination therapy improved the durability of glycemic control with no increased rate of adverse events [255]. However, studies on the efficacy and long-term safety of more diverse pharmacological options in early-onset type 2 diabetes have not yet been reported.

2.8.2 Metabolic surgery

Metabolic surgery may be considered for the treatment of severely obese adolescents with a BMI >35 mg/m2 with type 2 diabetes. Metabolic surgery includes Roux-en-Y gastric by-pass, vertical sleeve gastrectomy, and laparoscopic adjustable gastric banding. Bariatric surgery has positive effects on body weight in morbidly obese adult and adolescent patients, and it improves glucose homeostasis, and cardiovascular risk factors [256-258]. Although improvements in health are obvious, there are indications that adolescents have a higher rate of reoperations and complications from surgery than adults [259, 260].

3 AIMS

3.1 GENERAL AIMS

This thesis aims to investigate factors of importance in the development and clinical significance of disturbed glucose tolerance in obese children and adolescents.

3.2 SPECIFIC AIMS

- To quantify the prevalence of pre-diabetes and silent type 2 diabetes in a cohort of severely obese children and investigate the mechanisms of importance for impaired fasting glucose, impaired glucose tolerance and type 2 diabetes.

- To investigate associations between different groups of glucose tolerance in relation to insulin resistance, insulin secretion, disposition index, cardiorespiratory fitness and puberty.

- To examine factors regulating glucose homeostasis in the non-diabetic range cross-sectionally in obese children and adolescents.

- To examine the clinical traits and treatment in children and adolescents diagnosed with T2D in Sweden.

- To compare the frequency of complications related to diabetes among children, adolescents and young adults with type 1 and 2 diabetes.

4 METHODS

4.1 STUDY DESIGN AND POPULATION

An overview of the design and population of each of the studies is presented in Table 2.

Table 2 *. Study cohorts in the thesis*.

Study	Design	Population	Recruitment of population
Study I	Cross-sectional cohort	134 obese children who underwent OGTT and fs-IVGTT	Patients enrolled at National Center of Childhood Obesity between 2002-2007 and registered in the BORIS database.
Study II	Cross-sectional cohort	333 obese children who underwent fs-IVGTT	Patients enrolled at National Center of Childhood Obesity between 1997-2008
Study III	Retrospective cohort/case-control	1413 children and young adults with type 2 diabetes and 3748 age, sex, and diabetes onset-matched children and adults with type 1diabetes	Patients registered in SWE/NDR with type 2 and 1 diabetes diagnosis aged 10-25 years at onset during the years 1996-2014

Studies I and II in this thesis contain data from the Swedish childhood obesity register – Barn Obesitas Registret I Sverige (BORIS). BORIS is a national quality register for obesity treatment in childhood and adolescence and was initiated in 2005 [261, 262]. In Sweden children with obesity are treated in outpatient clinics and a specialized center for children and adolescents: the National Childhood Obesity Centre at Karolinska University Hospital, Huddinge. The BORIS register contains data from both outpatient clinics and the National Childhood Obesity Center. The register contains data on weight development and the obesity treatment registered for pediatric patients in Sweden.
BORIS has been supported by the Swedish National Board of Health and Welfare and the Swedish Association of Local Authorities and Regions.

Study III in this thesis contains data from the National Diabetes Register in Sweden (NDR). NDR is a national quality register for diabetes treatment in both children, adolescents and adults [263, 264]. It was initiated in 1996 and was initially a separate register for adults with diabetes in Sweden. The childhood diabetes register was initiated in 2000 and was named SWEDIABKIDS (SWE) [265]. The two functioned as separated registers until 2019, when they merged into one national diabetes register for both children and adults with diabetes. NDR is supported by the Swedish National Board of Health and Welfare and the Swedish Association of Local Authorities and Regions.

4.1.1 Study I

The aim of Study I was to investigate the prevalence of i-IFG, i-IGT, combined IFG/IGT and silent type 2 diabetes in a cohort of severely obese children and adolescents. It is a cross-sectional observational study which is based on data retrieved from the BORIS register. All study participants were consecutively investigated concerning clinical risk factors associated with obesity within a year of enrolment at the National Childhood Obesity Center. Inclusion criteria were being classified as obese according to IOTF at the enrolment at the clinic, children who had been investigated with OGTT and the absence of underlying chronic disease. Subjects who had diabetes prior to investigation, as well as medication that alters glucose or lipid regulation were excluded. The total number of patients included in Study I was n=134, aged 6.2–18.3 years. They had been referred to the National Childhood Obesity Center at Karolinska University Hospital between 2002 and 2007 from all regions of Sweden but mostly the Stockholm area. The cohort consisted of a population of several different ethnicities from an urban setting, representing a mixed Swedish population.

4.1.2 Study II

The objective in Study II was to investigate how insulin and glucose homeostasis in children and adolescents with obesity varies within different fasting glucose levels within the non-diabetic range. This cross-sectional cohort study examined subjects from BORIS who were children between 10 and 18 years of age, had obesity according to IOTF, and had undergone an intravenous glucose tolerance test at the National Center of Childhood Obesity between 1997 and 2008. Exclusion criteria were incomplete tests, treatment with metformin, and fasting glucose levels in the diabetic range (≥7.0 mml/L). In total, 333 obese children and adolescents were included, as shown in Flow chart presented in Figure 11, below.

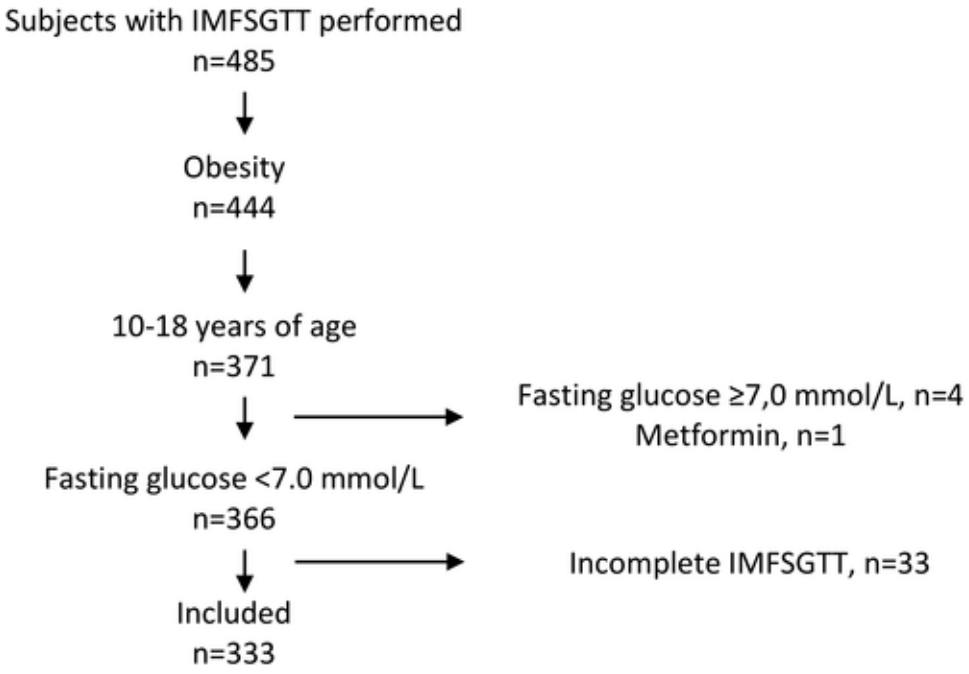


Figure 11. *Flow chart of Study II. IMFSGTT=Insulin Modified Frequently Sampled Glucose Tolerance Test*

4.1.3 Study III

Study III was designed to estimate the incidence of reported cases of type 2 diabetes among adolescents and adults in Sweden and to evaluate the risk of developing complications among patients with early-onset type 2 diabetes relative to that among patients with type 1 diabetes. In this retrospective cohort study, we used data from SWE and NDR from 1994 until 2014. All adolescents and young adults aged 10-25 years at onset of type 2 diabetes were identified. At baseline, defined as the first entry in the SWE or NDR, each patient with type 2 diabetes was matched for age, sex, and year-of-onset with approximately four individuals with type 1 diabetes who were randomly selected from SWE and NDR. Figure 12 shows the flowchart of analyses in Study III.

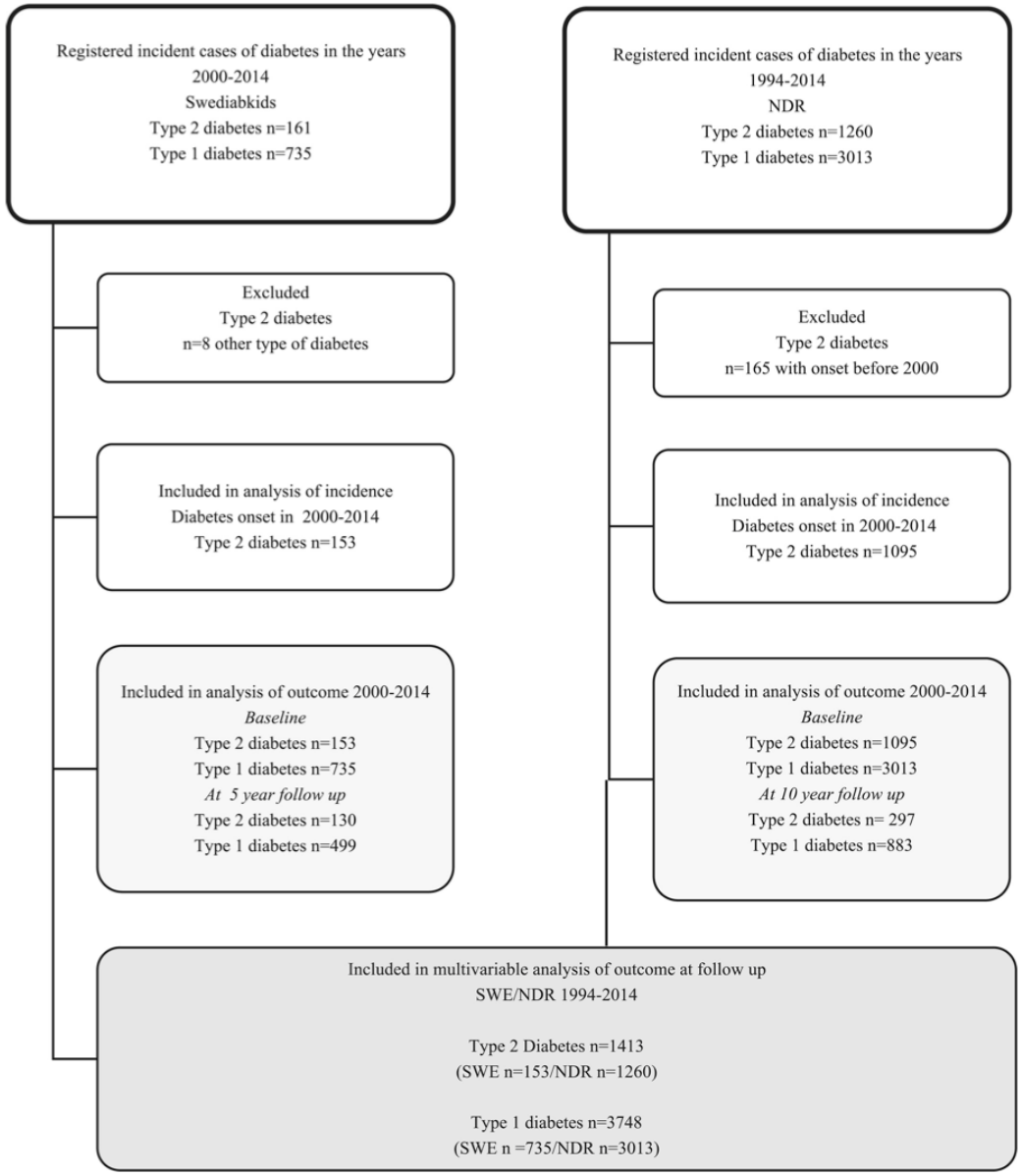


Figure 12. *Flowchart of analyses in Study III*

4.2 MEASUREMENTS AND DEFINITIONS

4.2.1 Obesity and degree of obesity

In all studies, children and adolescents were defined as obese according to the International Obesity Task Force (IOTF) [2], taking into account their age, gender, and BMI. For the young adults in Study III, overweight was defined as a BMI ≥25, and obesity was defined as a BMI ≥30. The degree of obesity in the children and adolescents in Studies I and II was calculated using a BMI age-and sex-dependent standard deviation score (BMI SDS) using a Swedish reference population [266], and in Study II an international reference was used [3]. The degree of obesity in adults in Study III was calculated in BMI.

4.2.2 Anthropometric measurements

All measurements in Study I and II were carried out by trained nurses in the same setting in the clinic. Weight and height were measured, and BMI was calculated as kg/m^2. Body composition was measured by dual X-ray absorptiometry (DEXA) using a total body scanner (Lunar Prodigy Radiation, Madison, USA).

Pubertal status in Studies I and II was assessed by a pediatric endocrinologist using the five-stage Tanner criteria for pubertal development. Genital development in boys, where patients with testicular volume <4 cc was considered prepubertal, and breast development in girls were used for classification into pubertal groups .

In Study III weight and height were measured at the visits to primary care and hospital outpatient clinics by trained nurses and physicians. Patient data were continuously reported directly or by annual via electronic patient clinical records into the SWE or NDR.

4.2.3 Laboratory analysis/biochemistry

In Studies I and II the blood samples were obtained after an overnight fast for the measurement of P-glucose, fs-insulin, c-peptide and Hba1c in study I and II. The samples were analyzed at the certified laboratory at the Department of Clinical Chemistry, Karolinska University Hospital, Huddinge, Sweden. P-glucose was analyzed with the glucose dehydrogenase method, Hemocue AB, Ängelholm, Sweden. Fasting serum insulin was analyzed using a radioimmunoassay, RIA 100 (Pharmacia Diagnostics AB, Uppsala, Sweden) or electrochemiluminescence immunoassay (ECLIA, Elecsys, Roche Diagnostics Scandinavia, Bromma, Sweden).
C-peptide was analyzed using electrochemiluminescence (ECLIA, Elecsys, Roche Diagnostics Scandinavia, Bromma, Sweden).

The blood samples in Study III were capillary blood samples, analyzed with Bayer/Siemens DCA-2000 or using a local laboratory. All laboratory methods used in Sweden are standardized through the External Quality Assurance in Laboratory Medicine in Sweden (EQUALIS). The data on HbA1c is presented as HbA1c Mono S (%) and also converted to the IFCC (International Federation of Clinical Chemistry) reference method and presented as IFCC (mmol/mol).

4.2.4 Definition of impaired fasting glycemia (Studies I-II)

Study I used the ADA (2003) [267] guidelines to define isolated IFG as between 5.6 and 6.9 mmol/L. In Study II both the ADA definition and WHO definition were used [268]. The subjects were divided into three groups based on their fasting glucose level: up to 5.5 mmol/L corresponding to normal fasting glucose (NFG), 5.6 to 6.0 mmol/L corresponding to the exclusive interval for IFG provided by ADA, and 6.1 to 6.9 mmol/L corresponding to IFG according to WHO.

4.2.5 Definition of impaired glucose tolerance (Study I)

The ADA definition of IGT was used in Study I, defined as 2-h post glucose level of between 7.8 and 11.0 mmol/L [267]. Combined IGT was defined as 2-h post OGTT glucose between 7.8 and 11.0 mmol/L and the presence of IFG between 5.6 and 6.9 mmol/L.

4.2.6 Classification of diabetes (Studies I-III)

In Studies I-II type 2 diabetes was defined as a fasting glucose level of 7.0 mmol/L at two occasions or 2-h post OGTT glucose level of 11.1 mmol/L or above according to the ADA criteria [267]. Diabetes type is defined in Study III by the reporting physician; in Sweden the WHO diagnostic criteria are used to diagnose diabetes [268], and aided by determination of C-peptide, autoantibodies and fasting levels of insulin which are part of standard care in Sweden [269, 270] .

4.2.7 Measurements of glucose tolerance

The OGTT was used in Study I. It was performed at approximately 08:00 hours in the morning after an overnight fast from 24:00 at the hospital. Baseline venous fasting values for insulin, glucose and c-peptide were drawn before the administration of 1.75 g glucose per kg bodyweight orally, to a maximum of 75 gram. Blood samples for insulin, glucose, and c-peptide were drawn after 120 min.

4.2.8 Measurement of insulin resistance, insulin secretion and disposition index

In Studies I and II, the fs-IVGTT was used to determine Si, AIR and DI. The fs-IVGTT was performed at 08:00 a.m., following an overnight fast. A peripheral intravenous catheter was inserted in each arm. Three fasting baseline samples for glucose and insulin were drawn at times -15, -10 and -5 minutes. At time 0 minutes, 0.3 grams of glucose per kg body weight was administered for one minute. At time 20 minutes, 0.02 U insulin per kg bodyweight was administered as a bolus dose (Actrapid, NovoNordisk Scandinavia AB, Malmö, Sweden). Blood samples for the determination of glucose and insulin were drawn at times -15, -10, -5, 0, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 24, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes. Si and AIR were calculated from the glucose and insulin values using the minimal model computer program developed by Bergman et al. (MINMOD Millennium 2003) [179]. DI was calculated as the product of Si multiplied by AIR. Glucose was analyzed with a bedside instrument (Hemocue AB, Ängelholm, Sweden) and insulin was analyzed at the Karolinska University Hospital Laboratory, Sweden).

HOMA-IR was calculated using the following formula: (fS-insulin(μU/mL) x p-glucose (mmol/L))/22.5 [195].

4.2.9 Physical fitness

In Study I cardiorespiratory fitness was assessed by submaximal bicycle ergometer test according to Åstrand and Rhyming. Absolute VO_2 max (L/min) was estimated from the measured heart rate and work load using the nomogram provided by Åstrand and Rhyming [271]. The submaximal bicycle ergometer test is validated in pediatric populations [272, 273]. Relative VO_2 (mL/kg/min) max was calculated from the absolute VO_2 max and the measured body weight (kg) and fat free mass (FFM) from the DEXA results.

4.2.10 Microalbuminuria

Microalbuminuria was defined as two positive results out of three samples obtained within one year in Study III, measured with the use of the urinary albumin-to-creatinine ratio (ACR)>3.5 mg/mmol (in SI units which corresponds to >30 mg/g) in a spot urine collection [274, 275].

4.2.11 Retinopathy

In Study III retinal status was assessed locally, that is, through fundus photography performed by an ophthalmologist and reported to the register. Retinopathy was categorized as “yes” or “no”, and stage of retinopathy was not reported in the registry.

4.2.12 Hypertension

Hypertension was defined as blood pressure levels in the 95th or greater centile for age (<18 years), 140/90 mmHg (>18 years), or documented use of antihypertensive therapy [276].

4.3 STATISTICAL ANALYSES

The statistical methods used in this thesis are presented in Table 3.

Table 3. *Statistical methods used in each study.*

Method	Study I	Study II	Study III
Descriptive statistics	x	x	x
T-test independent samples			x
Mann-Whitney U test	x		x
Chi square test	x	x	x
Roc Curve analysis			x
Kaplan Meier curve			x
Log rank test			x
Cox regression			x
Anova, one-way	x	x	
General linear model	x	x	

4.3.1 Main analyses

4.3.2 Study I

The association between the Si, AIR, DI, HOMA-IR and relative VO_2 max in different groups of glucose tolerance was analyzed. Potential confounding effects of sex, puberty and CRF were tested.

4.3.3 Study II

The association between insulin levels, Si, AIR, and DI in different glucose levels in the non-diabetic range was analyzed. The associations were adjusted for sex, age, migrant background, and degree of obesity.

4.3.4 Study III

The frequencies of reported patients with type 2 diabetes were tested by Chi square test per period of three years, and the difference in frequencies was also tested by sex.
The association between the two outcomes of microalbuminuria and retinopathy, with the variables BMI, systolic and diastolic blood pressure, and HbA1c was analyzed with a sensitivity analysis, namely a ROC curve. Sequentially-adjusted models for the associations of diabetes type and each outcome were used to determine which risk factors contributed to the higher risk. The base model was adjusted for age, sex, and diabetes duration. The additional risk factors of BMI, HBA1c, and blood pressure were added to the model in a Cox proportional hazards model. To investigate the risk of developing microalbuminuria or retinopathy, a Kaplan Meier curve was constructed to display the probability of outcome-free survival at the means of covariates age, BMI, HbA1c, and blood pressure.

4.3.5 Common statistical procedures

Outcome variables were tested for normality, through visual inspection of histograms or the Shapiro Wilks test. All tests were two-sided, and p-values <0.05 were regarded as statistically significant. Statistical analyses in Study I were performed with STATISTICA version 8.1 (Statsoft Inc, Tulsa, Oklahoma, USA). In Study II the statistical analyses were performed with SAS statistical software (SAS, version 9.4, Cary, North Carolina, USA) and in Study III IBM SPSS Statistics 23.0 and 25.0 for MacOS (SPSS inc, Chicago, Illinois, USA) was used.

4.3.6 Ethical approval

All ethical approval was granted by the Stockholm Regional Ethical Review Board:
-Research within the BORIS register: 166/02, 2005/784-31/1-4, 2014/381-31/5
-Research within the NDR/SWE register: 2014/1777-31/1

5 RESULTS

5.1 PREVALENCE OF IMPAIRED FASTING GLYCEMIA, IMPAIRED GLUCOSE TOLERANCE AND TYPE 2 DIABETES (STUDY I, II AND III

In the cohort of severely obese children and adolescents within Study I (n=134), the prevalence of isolated IFG was 35.8 %, 6 % had isolated IGT and 14.2 % had a combination of IFG and IGT according to ADA criteria. Cases of prediabetes were found in all ages and pubertal stages, and the prevalence of isolated IFG_{ADA} was observed earlier and more frequently among males. In Study II, the prevalence of IFG_{WHO} was 6.3%, and the prevalence of IFG_{ADA} was 19.5% among the obese children.

No cases of silent type 2 diabetes were found among the severely obese children and adolescents in Study I. In Study III, in the cohort of diabetes patients from NDR and SWE, a total of 1413 individuals with type 2 diabetes with an onset of diabetes at 10-25 years of age was found. As shown in Figure 13, 1095 individuals with type 2 diabetes were reported, with an increasing number between 2000 and 2014.

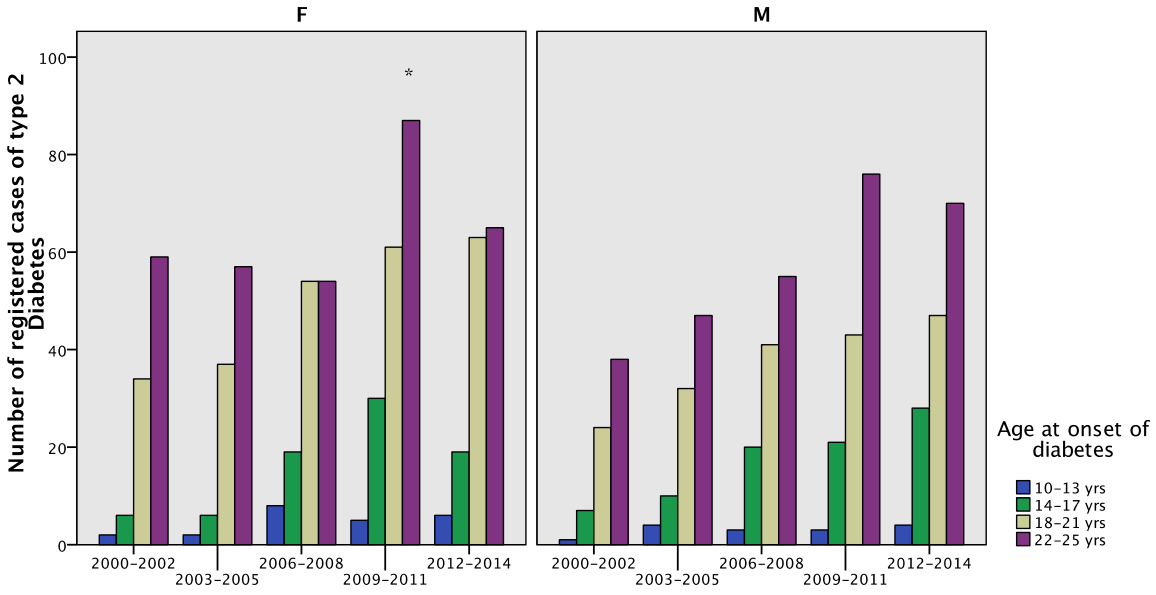


Figure 13. *Frequencies of reported cases of type 2 diabetes in SWE and NDR, with an age of onset of 10-25 years, between 2000 and 2014. Females and males are displayed in separate histograms, and the age at onset of diabetes is displayed in separate boxes. The column proportions differ significantly, with an increasing number of reported cases (p=0.043)* among females 14-17 and 22-25 years of age at onset of diabetes (p=0.034)*; differences in frequencies are tested with Pearson Chi-Square.*

5.2 FACTORS ASSOCIATED WITH IMPAIRED FASTING GLYCEMIA, IMPAIRED GLUCOSE TOLERANCE

Study I did not reveal any differences in age, BMI SDS, total fat mass, abdominal fat mass, or CRF in the different groups of glucose metabolism. The individuals with combined IGT had significantly higher 2-h OGTT levels of insulin and AIR than the individuals with normal glucose tolerance or isolated IFG. The levels of 2-h OGTT C-peptide were higher among the individuals with isolated IGT. All groups had a relatively high prevalence of family history of diabetes, between 31% and 50%, but there was no difference in prevalence among the different groups of glucose tolerance.

5.2.1 Effect of puberty on glucose tolerance

In all the different groups of glucose tolerance the post-pubertal subjects had a tendency towards a lower Si than the prepubertal and the pubertal subjects (Fig 14 A). The post-pubertal individuals with normal glucose tolerance had a lower AIR (p=0.002) than the pubertal individuals with normal glucose tolerance (NGT) (Fig 14 B). DI was lower among the post- pubertal subjects, especially among those with combined IFG/IGT (p=0.0056) (Fig 14 C).

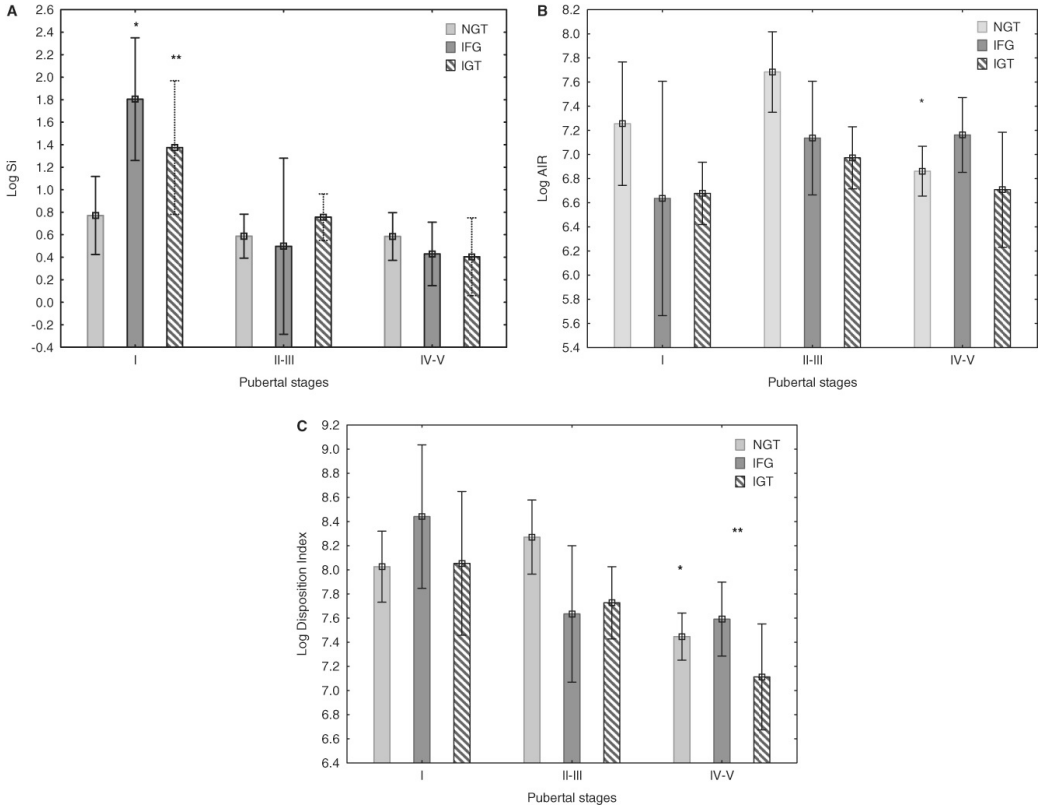


Figure 14 . *Insulin sensitivity A (log Si), insulin release B (log AIR) and disposition index C (log DI) in different categories of glucose tolerance in relation to puberty stages. Number of children in Tanner stage I/II-III/IV-V within the NGT group =11/10/35, IFG group=7/9/29, IGT group=6/10/8.*

5.2.2 Sex differences in glucose tolerance

The cohort of severely obese children in Study I encompassed 77 males and 57 females. The mean BMI SDS was 3.6 ± 0.6 in the whole cohort. The males and females were of approximately the same age and BMI SDS, but the females were more advanced in pubertal stages and had a higher total and abdominal fat mass ($p<0.05$). The females had a higher Si and level of CRF compared with the males ($p<0.05$). Differences are displayed in Table 4.

Table 4. *Anthropometric and clinical data among boys and girls in Study I*

	Females n=57	**Males n=77**	**p-value**
Age (years)	14.1±2.7	13.4±2.7	0.128
Tanner stage	5 (1-5)	3 (1-5)	0.002
BMI SDS	3.6±0.5	3.7±0.6	0.373
Total fat mass (%)	50.6±4.2	46.2±4.7	<0.001
Abdominal fat mass (%)	51.2±4.2	46.2±4.0	<0.001
Si (x10^{-5}/min/pM)	2.7±1.9	2.4±2.1	0.059
AIR (pM)	1093.7±634.5	1531.7±956.6	0.009
DI (10-5/min)	2382.1±1040.9	2927.8±2050.0	0.507
Relative VO_2 max (mL/kg FFM/min)	59.9±11.6	52.8±9.8	<0.001

5.2.3 Disposition Index

DI was one of the significant determinants of the variance (β=-0.49, p=0.0126), along with gender and Tanner stage, in 2-h OGTT glucose levels in multiple regression analyses with 2-h glucose as the dependent variable and sex, age, Si, AIR, DI, BMI, Tanner stage, and relative VO_2 max/kg FFM as independent variables. In a sub-analysis, in which we stratified the children into two groups with low (the 25th percentile) and high (the 75th percentile) DI, the children with low DI were older, had reached a more advanced pubertal status, a significantly higher 2-h glucose and C-peptide level and a tendency toward a lower relative VO_2 max, but there was no difference in BMI SDS or fat mass distribution. Separate analyses of females and males are shown in Figure 15.

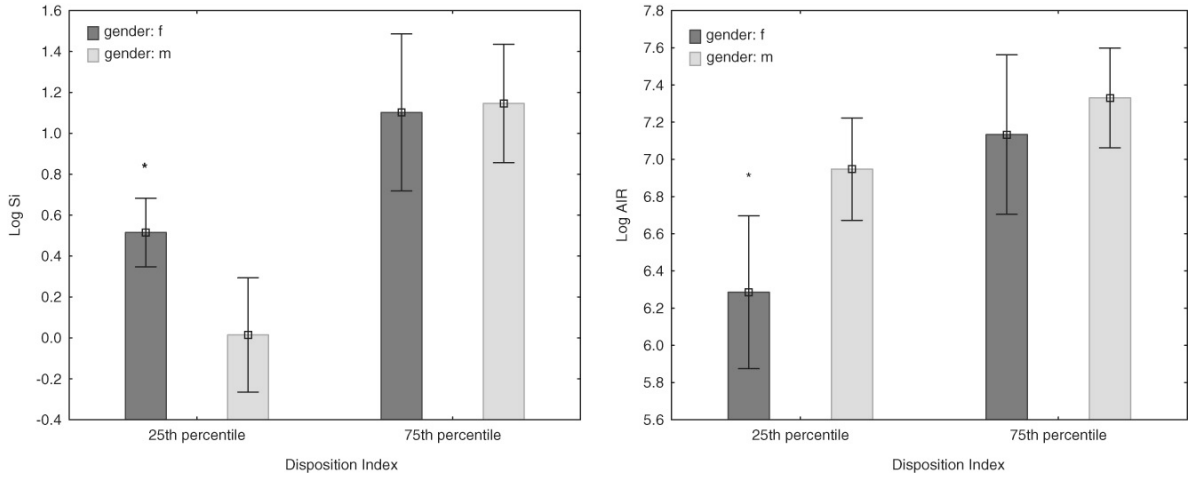


Figure 15. *Comparison between individuals with low and high DI in relation to gender. (A) Females with low DI had significantly higher Si than the males with low DI (*p = 0.0049). (B) Females with low DI had significantly lower AIR (*p = 0.0097).*

5.3 EARLY SIGNS OF DISTURBED GLUCOSE METABOLISM IN THE NON-DIABETIC RANGE

In Study II 333 children and adolescents were investigated with fs-IVGTT. The mean age in this cohort was 14.8 years, mean BMI SDS 3.09 ±0.39, and the majority were in Tanner stage 4/5. Normal glucose tolerance was present in 268 patients. The exclusive IFG interval provided by ADA (5.6-6 mmol/L) was found in 44 patients, and 21 patients had IFG according to WHO (6.1-6.9 mmol/L).

In models adjusted for age, sex, migrant background and degree of obesity, fasting glucose levels ranging from 6.1-6.9 mmol/L had 48 % lower AIR and 57 % lower DI than individuals with normal glucose tolerance (p<0.0001). In children and adolescents with obesity and fasting levels ranging from 5.6-6.0 mmol/L, the same models showed similar AIR, Si, and DI as individuals with normal glucose tolerance, as illustrated in Figure 16.

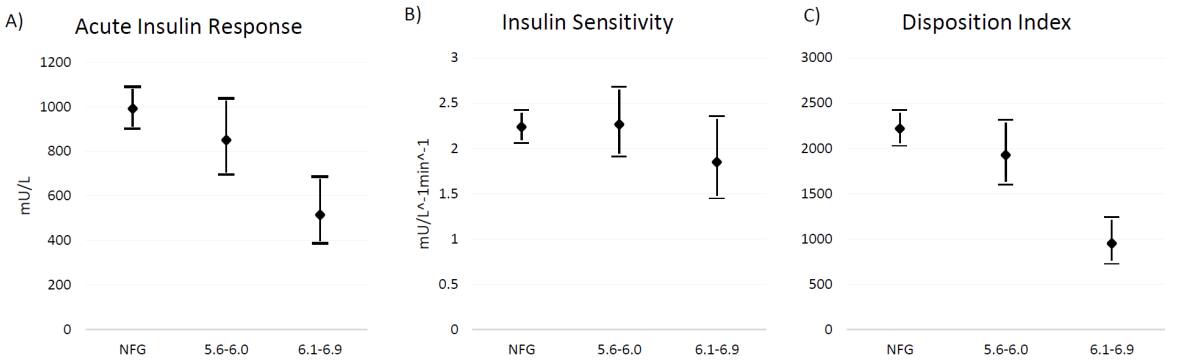


Figure 16. *Geometric means of A) AIR), B) Si and C) DI by fasting glucose category adjusted for sex, age, migrant background, and degree of obesity. Intervals represent 95% confidence limits.*

5.4 CLINICAL CHARACTERISTICS OF PATIENTS WITH DIABETES AT FOLLOW-UP

5.4.1 Adolescents with diabetes

In Study III, the adolescents with type 2 diabetes were more overweight and obese, had lower HbA1c and had fewer clinical visits than adolescents with type 1 diabetes (2.8 visits per year compared with 4.1 visits per year). At follow-up of the adolescents with type 2 diabetes had the following treatment: 37.7 % were on oral medication, 16.9% were on insulin and oral medication, and 17.9% were on insulin only, and 17.7% on diet only, while 6.9% (n=9) individuals were on GLP-1 and/or combined treatment. The prevalence of complications at approximately five years of follow-up is displayed in Table 5.

Table 5. *Five-year follow-up of adolescents with a diabetes diagnosis at 10-17.9 years of age (SWEDIABKIDS)*

	Type 1 diabetes n=499	**Type 2 diabetes n=130**	***p*-value**
Retinopathy n (%)	85/442 (19.2)	32/103 (24.6)	0.008
Microalbuminuria n (%)	16/416 (3.8)	10/113 (7.7)	0.029
Systolic hypertension >95 th percentile (%)	24/328 (7.3)	12/91 (13.2)	0.077
Diastolic hypertension >95 th percentile (%)	8/328 (2.4)	9/91 (9.9)	<0.001

5.4.2 Young adults with diabetes

At 10-year follow-up, the young adults with type 2 diabetes (n=297) were more overweight and obese and had higher blood pressure than the young adults with type 1 diabetes (n=883). Most of the patients with type 2 diabetes were on oral medication only (33.7 %), while 26.9 % were on a combination of insulin and oral medication, 24.2% on insulin only, and 12.8% were on diet only, and 0.8% were on GLP-1 and/or combined treatment. Frequency of diabetes-related complications is shown in Table 6.

Table 6. *10-year follow-up of young adults with a diabetes diagnosis at 18-25 years of age* (NDR)

	Type 1 diabetes n=883	**Type 2 diabetes n=297**	***p*-value**
Retinopathy n (%)	373/851 (43.8)	98/247 (39.7)	0.273
Microalbuminuria n (%)	42/883 (4.8)	45/297 (15.2)	<0.001
Systolic hypertension >95 th percentile (%)	39/799 (4.9)	39/253 (15.4)	<0.001
Diastolic hypertension >95 th percentile (%)	35/799 (4.4)	32/253 (12.6)	<0.001

5.4.3 Factors associated with outcomes

The association between the variables BMI, systolic and diastolic blood pressure, HbA1c and the two separate outcomes microalbuminuria and retinopathy is tested in a sensitivity analysis and displayed in the ROC curves in Figure 17. BMI had the strongest association with microalbuminuria in both types of diabetes, and HbA1c had the strongest association with retinopathy in both types of diabetes.

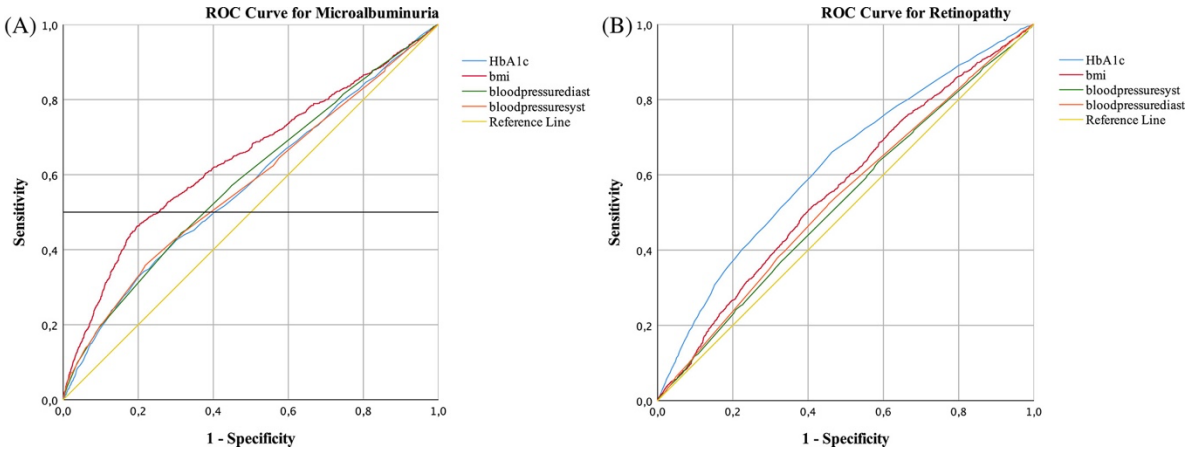


Figure 17. *ROC curves for the association between variables and outcomes microalbuminuria (A) and retinopathy (B) in both type 1 and 2 diabetes.*

5.4.4 Association model for type 2 diabetes and outcomes microalbuminuria and retinopathy

The risk factors which contribute to the higher risk in type 2 diabetes vs type 1 diabetes were tested in sequentially adjusted models.

The base model was adjusted for age, sex and diabetes duration and showed significantly higher rates of microalbuminuria and retinopathy in type 2 diabetes than in type 1 diabetes. The association was weaker with a further adjustment for BMI, Hba1c, and blood pressure but still significant. The results of the Cox proportional hazard model are displayed in Table 7.

Table 7. *Cox proportional hazards for the association between type 2 (vs. 1) diabetes adjusted for sex, BMI, HbA1c, and blood pressure*

Outcome	Base Model (Type 2 vs 1, age, sex, diabetes duration)	Model adjusted for base model, BMI, HbA1c, systolic and diastolic blood pressure
Microalbuminuria		
HR (95% CI)	3.99 (3.49-4.36)	3.32 (2.86-3.85)
p-value	<0.001	<0.001
Retinopathy		
HR (95% CI)	1.24 (1.14-1.35)	1.17 (1.06-1.30)
p-value	<0.001	0.04

5.4.5 Sex differences in risk pattern

The risk pattern was different for the two diabetes types and also between the sexes. A Cox proportional hazards analysis of the clinical and biochemical characteristics predicting development of risk in the different diabetes types was carried out to investigate the differences. The development of type 2 diabetes-related microalbuminuria is associated with male sex (HR 1.276, 95% CI 1.06-1.54, p-value 0.012), BMI, systolic blood pressure and a significant but small association with HbA1c levels. The development of retinopathy in type 2 diabetes was associated with male sex (HR 1.44, 95 % CI 1.21-1.71, p-value <0.001), HbA1c levels and diastolic blood pressure.

In type 1 diabetes patients the development of microalbuminuria was associated with female sex (HR) and HbA1c levels. The development of retinopathy in type 1 diabetes was associated with female sex, BMI, HbA1c and higher diastolic blood pressure.

In a Cox proportional hazards analysis of the association between type 2 (vs. 1) diabetes and the outcomes in males and females separately, a difference between the sexes is discernable. Males with type 2 diabetes are at high risk of developing both microalbuminuria and retinopathy, whereas it seems that females with type 2 diabetes have a risk of developing microalbuminuria but not retinopathy to the same extent. Table 8 reveals the different risk situations for each sex.

Table 8. *Cox proportional hazards for the association between type 2 (vs. 1) diabetes and outcomes in males and females separately*

Outcome	Base Model	Model adjusted for BMI, HbA1c, and systolic and diastolic blood pressure
Microalbuminuria	Males	
HR (95% CI)	5.49 (4.68-6.24)	4.92 (3.99-6.07)
***p*-value**	<0.001	<0.001
	Females	
	2.89 (2.48-3.37)	2.36 (1.91-2.91)
	<0.001	<0.001
Retinopathy	Males	
HR (95% CI)	1.59 (1.42-1.78)	1.64 (1.42-1.89)
***p*-value**	<0.001	<0.001
	Females	
	0.97 (0.83-1.1)	0.8 (0.68-0.95)
	0.676	0.008

5.4.6 Time to event

The probability of event-free cumulative survival, that is, developing signs of nephropathy or retinopathy, related to diabetes duration was calculated. The mean time to event, in the case of microalbuminuria, was 123 months in type 1 diabetes (with a 95% CI 122-124 months) and 108 months in type 2 diabetes (with a 95% CI 107-110 months). The mean time to event in the case of retinopathy was 113 months in type 1 diabetes (with a 95% CI of 112-113 months) and 108 months in type 2 diabetes (with a 95% CI 109-112 months).

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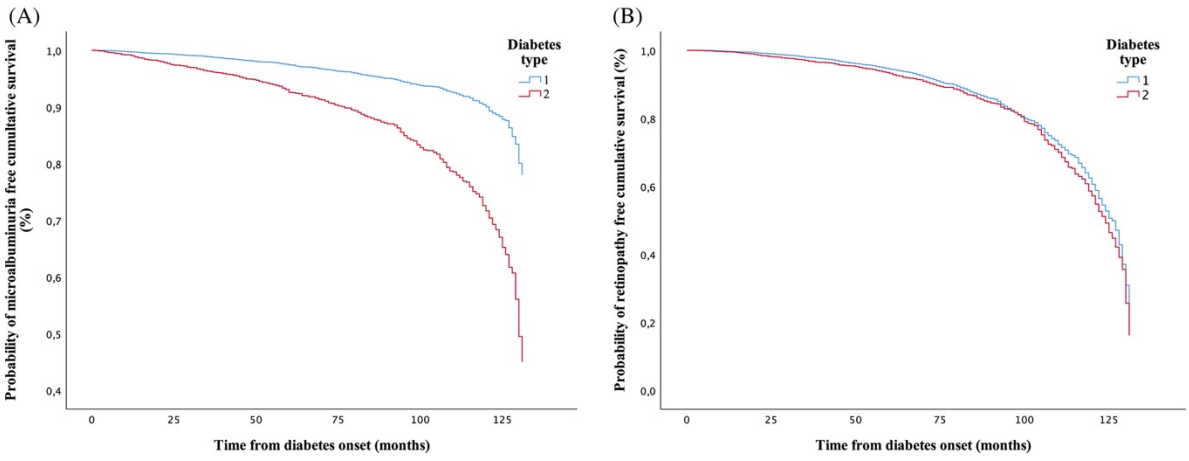


Figure 18. *A) Kaplan-Meier survival curve displaying the probability of microalbuminuria free survival in type 1 and 2 diabetes at means of the covariates age, HbA1c, BMI, and blood pressure. Log rank test p<0.001. B) Kaplan-Meier survival curve displaying the probability of retinopathy-free survival in type 1 and 2 diabetes at means of the covariates age, HbA1c, BMI, and blood pressure. Log rank test p=0.029.*

6 DISCUSSION

6.1 MAIN FINDINGS

This thesis presents three main findings. First, we found a high prevalence of prediabetes among the children and adolescents with severe obesity. Secondly, we found that IFG_{WHO} is associated with reduced β-cell dysfunction and a lower DI. The third main finding is the high prevalence of diabetes-related complications among the adolescents and young adults with diabetes, with the risk being more evident among those who had early-onset type 2 diabetes despite lower HbA1c levels.

6.2 PREVALENCE OF PREDIABETES IN OBESE CHILDREN

The prevalence of prediabetes varies considerably depending on population observed, and naturally also the cut-off level used. In Study I, the prevalence of combined prediabetes was 14.8 % and isolated IFG_{ADA} was 35.8%. This is almost as high as other studies have reported from similar cohorts of obese children in the USA and New Zealand [31, 277], but a much higher prevalence than other European studies of obese children and adolescents [278, 279].

We have also previously shown that the prevalence of IFG_{ADA} in a large nationwide cohort of obese children in Sweden is high (17.1%), three times higher than in a similar cohort from Germany [280]. In a recent population-based study in the USA the prevalence of prediabetes in healthy adolescents was 18% and was associated with obesity and male sex [204], as we also noted in the present study. The reason for the variations in prevalence of prediabetes among obese and healthy pediatric populations is unclear. However, differences in degree of adiposity, physical activity, pubertal status and diet can all contribute to the changes in glucose homeostasis in the growing child. In individuals with multiple risk factors the variation in insulin sensitivity during puberty can cause disturbed glucose homeostasis transiently, with recovery when puberty ends. This makes the diagnosis of prediabetes, and the understanding of the possible associated risk in children and adolescents more complicated than in adults.

The prevalence of prediabetes was not as high in Study II. This study was a larger cohort of children with obesity and with a lower mean BMI SDS than in Study I. Different BMI SDS references were used, Study I used BMI SDS according to Karlberg and while Study II used the extended international Cole BMI SDS, which makes comparison somewhat complicated since the two references are slightly different at different ages [3, 4]. The international reference is based on childhood growth data from Hong Kong, Britain, the Netherlands, Brazil, Singapore, and the USA whereas Karlberg is based on a growth patterns in Swedish children. In a comparison between the two references at the age of 14 in both genders, which was the approximate mean age in both studies, an average BMI SDS of 3.6 according to Karlberg corresponded approximately to an average BMI SDS according to Cole of 4 implying that the children in Study I had a higher degree of obesity.

The higher prevalence of prediabetes in Study I, compared with Study II, can partly be explained by the more severe obesity of the participants. This is in accordance with previous studies who have shown increasing prevalence of prediabetes, insulin resistance and other cardiovascular risk factors with increasing degree of obesity [28, 281, 282].

6.3 WHICH CUT-OFF GLUCOSE LEVEL IS IMPORTANT?

Earlier studies have shown different risk of complications depending on which glucose level used as cut-off [283], and the associations between different glucose levels and clinical complications are unclear in children. The levels of both fasting and postprandial glucose vary widely in individuals with prediabetes which makes classification difficult [284]. However, even slightly elevated fasting glucose levels in adults predicts an increased risk of type 2 diabetes [285] and fasting glucose levels in the upper range in childhood is a predictor of prediabetes and diabetes type 2 in younger adulthood [286]. In our Swedish cohort of individuals with childhood obesity fasting glucose levels over 6.1 to 6.9 mmol/L (IFG_{WHO}) predicted future use of type 2 diabetes medication, but the lower interval of fasting glucose over 5.6 to 6.0 mmol/L (exclusive interval for IFG_{ADA}) was not associated with increased use of type 2 diabetes medication [287]. Study II investigated which level of fasting of exclusive IFG_{ADA} range and IFG_{WHO} levels had an association with impaired glucose-insulin regulation, and only IFG_{WHO} cut-off glucose levels of 6.1 to 6.9 mmol/L were associated with lower first phase insulin secretion and lower DI. Although the patterns were not as clear as those seen in Study I, we could see that insulin secretion is affected in those with a combined IFG/IGT or isolated IGT and also that DI was one of the factors explaining 2-hour glucose levels after an OGTT. DI has been shown to predict conversion to diabetes [288-291], and in this high-risk population signs of disturbed glucose regulation are evident. A meta-analysis of 26 prospective cohort studies in adults investigating the significance of different cut-off levels of glucose for future disease showed increased risk of cardiovascular disease and all-cause mortality at IFG levels of 6.1 mmol/mol (WHO cut-off) and IGT or IFG/IGT combined [283].

We observed a high prevalence of IFG_{ADA} in Study I and also a relatively high number of patients with combined IFG_{ADA} and IGT. Although the risk associated with prediabetes in the lower range according to ADA criteria is still unclear in children, it seems that IFG in the lower ADA range does not exclude IGT in children with obesity. This finding - that a disturbed glucose homeostasis even in the lower range can be associated with IGT - suggests that an OGTT must be performed to accurately assess their future risk. Even though repeated OGTTs in the same individuals have not shown consistent results [292], there is clear evidence that IGT is associated with future risk of type 2 diabetes [285, 293]. Prediabetes is not only associated with a higher risk of developing type 2 diabetes but is also a risk factor for cardiovascular disease and nephropathy [294-296], which underscores the importance of repeated investigation of associated risk factors.

6.4 GENDER DIFFERENCES IN PREDIABETES

We can see from Study I that prediabetes seems to develop earlier and is slightly more prevalent in males than in females. This is in accordance with a few earlier studies which have found that obese males have more risk factors such as prediabetes, higher blood pressure, higher prevalence of dyslipidemia, and non-alcoholic fatty liver disease than females [277, 297-299]. The reasons for the increased risk in males are unclear. In Study I we observed that the males had a lower cardiorespiratory fitness (CRF) than the females. They were equally obese, according to BMI and BMI SDS, but the males had a lower total and abdominal fat mass so increased fat mass or abdominal distribution of fat can probably not explain the differences. In spite of a lower percentage of body fat, the males had a lower CRF and lower degree of insulin sensitivity. Boys, in general, have a higher level of CRF than girls [300] and tend to be more physically active [301, 302]. Although we do not have information on their level of physical activity in this cohort, it could be interpreted that the obese boys in

this cohort are less physically active than the girls. Previously published data has indicated that overweight boys have more sedentary behaviors than overweight girls [303], which is in accordance with our observation. In an attempt to see whether gender differences were present among those with a low DI, which is predisposing for type 2 diabetes [190], we noted that the males with low DI had a more pronounced insulin resistance and a higher compensatory AIR compared with females with low DI. The individuals with a low DI in Study I were older, and had signs of β-cell stress, and a tendency towards a lower relative VO_2 max. A longitudinal study is required to confirm and interpret these findings. There were differences in physical maturity between the sexes so we can speculate that also puberty with the associated changes in growth hormones, may also affect the differences.

6.5 PREDIABETES, OBESITY AND PHYSICAL FITNESS

In Study I we can see that CRF correlates with insulin sensitivity and pubertal development, but the level of CRF does not seem to affect the risk of developing prediabetes in this cohort of severely obese children and adolescents. All of the obese children in this cohort had a lower CRF than normal weight peers [304, 305], as other studies on CRF in obese populations have also reported. During the last thirty years the level of CRF has declined among the general population of children [306-308], and in obese children the level of CRF is even lower and decreases with age in both girls and boys [309].

Previously published data from this cohort of obese Swedish children have reported that only 20-26 % of the variance in insulin sensitivity is explained by CRF, sex, body composition, age, or pubertal status [310]. However, the earlier study found that the relative VO_2 max had a stronger association with insulin sensitivity than percentage body fat, implying that physical activity is of importance in the development of insulin resistance [310]. Although we could not see any difference in CRF in relation to the different groups of glucose tolerance, obese children with low DI had a lower CRF than those in the upper range of DI, indicating that CRF is of importance in this group of severely obese children and adolescents.

6.6 EFFECT OF PUBERTY ON THE PRESENCE OF PREDIABETES

Growth development during puberty is dependent on the GH/IGF-1 axis, and this is associated with pubertal insulin resistance and can be of importance in affecting the risk of diabetes [84, 150, 166]. In Study I, the results show that puberty is a contributing factor to the variance in 2 h-glucose in this cohort of obese children. Surprisingly both IFG and IGT were present even in the prepubertal children. The number of prepubertal children was small, which made sub-analysis unsuitable, but we observed a surprisingly high insulin sensitivity in the prepubertal boys with prediabetes in comparison with the other pubertal stages, which we cannot fully explain. In this cohort of obese children insulin sensitivity was low even in the post-pubertal adolescents irrespective of their glucose tolerance status; it was apparent that they did not regain insulin sensitivity after puberty, as has been shown in other studies in healthy, normal weight children [150, 165]. A compensatory increase in insulin secretion was noted irrespective of glucose tolerance status, in all pubertal groups, suggesting that a compensatory insulin secretion to meet the insulin resistance to achieve normal glucose tolerance. The effect of puberty on metabolic health was investigated in a 1-year longitudinal German survey of 2017 obese children and, in agreement with our results, the authors found a strong association with pubertal development in the progression to a more metabolically unhealthy obese condition [311].

6.7 DIFFERENT PREDIABETES PHENOTYPES?

The pattern of β-cell dysfunction is proposed to be different in IFG and IGT. Impaired early-phase insulin secretion is seen in both IFG and IGT, but late-phase insulin secretion is impaired in IGT, resulting in abnormal glucose levels after a meal [312]. Approximately 80 % of glucose disposal after a meal occurs in skeletal muscle, and a previous study investigating glucose tolerance suggests that skeletal muscle insulin resistance is more prominent in IGT [313]. IFG appears to be more closely associated with hepatic insulin resistance than IGT [314]. Quite a few of the severely obese children (14.8%) in Study I had combined IFG and IGT, and one can assume that they have a combined peripheral and hepatic insulin resistance because of their combined prediabetic condition.

Study I could not reveal explanations to the causes or risk factors of IFG_{ADA}. However, we did not measure hepatic insulin resistance, which has been suggested as one key cause of IFG [315, 316]. The evaluation of insulin resistance was based on fs-IVGTT, which has been validated by numerous studies [179, 316], but is known to reflect both peripheral and hepatic insulin resistance [180]. In Study I the obese children with combined prediabetes had significantly lower AIR, and also higher 2 h OGTT insulin and c-peptide levels as signs of β-cell stress than those who had normal glucose tolerance or IFG. This is in accordance with previous studies in different ethnic groups, which implies that β-cell dysfunction exists early in the development of prediabetes [31, 150, 291, 317]. The RISE consortium has investigated differences in glucose and insulin homeostasis in youth and adults with a similar degree of obesity with clamp and extended OGTT models. Adolescents had much higher levels of C-peptide and insulin levels to compensate for lower insulin sensitivity indicating that the process of type 2 development differs between adolescents and adults [318, 319]. Our results in Study I are in agreement with this as we also observed high C-peptide and insulin levels among the severely obese children and adolescents.

6.8 INSULIN RESISTANCE – A SIMPLE INSULIN-GLUCOSE CROSS-TALK OR A COMPLEX WHOLE-BODY AFFAIR?

We discerned a combination of different prediabetes stages in Study I, suggesting that some of the children had a more advanced prediabetic stage possibly due to a more severe whole-body insulin resistance and signs of β-cell stress. We could not discern any differences in fat distribution explaining glucose tolerance differences, but all children in the cohort were severely obese and similarly insulin-resistant.

Study I showed that oral glucose stimulated a high β-cell response in those with IGT or combined prediabetes, but a lower AIR was elicited during the intravenous test. Earlier studies have shown that both prediabetes stages IFG and IGT have a reduced AIR, while IGT also has a reduced second-phase insulin secretion leading to elevated glucose levels after a meal [313]. It is possible that the increased insulin response to the oral glucose load is due to the incretin effect, but this effect is still not enough to maintain normal glucose levels after the OGTT. In a study performed to investigate the differences in incretin effect in obese youth with type 2 diabetes and normal glucose tolerance, they revealed a reduced incretin effect in the youth with type 2 diabetes, but also an increased insulin secretion in the obese NGT individuals during the intravenous glucose test [134]. Defects in the incretin effect have been reported both in obesity, prediabetes and type 2 diabetes, and are proposed to be early markers of β-cell dysfunction [131, 133]. We did not measure the incretin effect in our study, but it is possible that this had an effect on the results.

In Study I we focused on some of the factors involved in the progression from normal glucose tolerance to diabetes, with a main focus on puberty and CRF. The pathophysiology of the development of insulin resistance associated with obesity, disturbed glucose homeostasis and type 2 diabetes is much more complex and involves many other factors such as ethnicity, genetic background, gene-environment interactions, complex insulin signaling pathways, inflammation, nutritional factors, and gut microbiota.

6.9 PROGRESSION FROM PREDIABETES TO DIABETES

Several studies have estimated the risk of developing type 2 diabetes in adults with prediabetes. The annual incidence of type 2 diabetes varies between 5% and 10% in different prediabetic conditions and depends on the age and ethnicity of the studied population [320-322]. The risk seems to differ between the different prediabetes stages: isolated IFG or IGT is associated with 4-9 % annual incidence of type 2 diabetes whereas a combined prediabetic stage has a higher risk to developing type 2, with a progression rate of 15–19% [320]. In children the progression from prediabetes to diabetes type 2 is unclear, depending on population observed. A study from the USA followed severely obese children with normal glucose tolerance at baseline, 9.5% of whom progressed to IGT, and among those with IGT at baseline 24.2% developed type 2 diabetes within two years [323]. A European study in a high- risk adolescent population observed a 2% progression from IGT to type 2 diabetes in three to five years, and a high reversion rate from IGT to normal glucose tolerance [324, 325]. Although there are differences in the progression of glucose disturbances due to differences in ethnicity and probably also other factors, a study of healthy children has shown that both fasting and 2 h glucose after OGTT were strong predictors of future T2D [326].

As our Studies I and II are cross-sectional in design, we cannot draw conclusions on the progression rate from prediabetes to diabetes in this cohort although we have observed signs of β-cell stress which may be an early sign of type 2 diabetes development as observed in earlier studies [291, 327].

6.10 TYPE 2 DIABETES PREVALENCE

We were surprised that no cases of silent type 2 diabetes were found in the high-risk cohort of severely obese children in Study I; it may be that the progression of type 2 diabetes takes longer time to develop. The progression of type 2 diabetes in adults is highly variable, possibly depending on age of diagnosis. It is proposed that the pathophysiological process is slow at the beginning and that type 2 diabetes presents when a tipping point is reached. The prospective Whitehall study in adults, which followed a cohort of 6538 British adults for 10 years, showed that in those who developed type 2 diabetes a linear increase in fasting glucose preceded a rapid, steep increase in fasting glucose level approximately three years before diagnosis of diabetes [327].

In Study III, a population-based study with information from two national databases in Sweden, we found a successive increase in early-onset type 2 diabetes among adolescents and young adults. The number of type 2 diabetes patients below 18 years of age is still small, but we found a surprisingly high number of young adults with type 2 diabetes. Earlier epidemiological studies in Sweden have not discerned an increasing prevalence of childhood diabetes type 2 [328, 329]. However, one relatively recent Swedish study from our group reported increased use of type 2 diabetes medication in individuals over 18 years of age who had previously been treated for childhood obesity [287]. There is a possibility that type 2

diabetes does not present in childhood, but instead in early adulthood. Another concern is that there might be differences in the reporting of new diabetes patients in the pediatric register depending on diabetes type. Hence, there is a risk that misclassification occurs, and also that some cases of type 2 diabetes may not be reported.

6.11 RATE OF COMPLICATIONS DIFFER AMONG THE DIABETES TYPES

The individuals with diabetes were followed from diagnosis with repeated measures of risk factors and complications over five to ten years; 15% of individuals with type 2 diabetes had microalbuminuria and almost 40% developed retinopathy. The development of microalbuminuria was more frequent in youth with type 2 diabetes, which is seen in other parts of the world, such as Canada, the USA, and Australia, all of which have reported earlier and higher prevalence of complications in youth with type 2 diabetes compared with type 1 diabetes [225, 228, 229, 330]. The youngest patients with type 2 diabetes in Study III had, despite a lower HbA1c, a higher prevalence of microalbuminuria than those with type 1 diabetes. This is in accordance with a report on type 2 diabetes patients from Australia [225], which indicates that factors other than HbA1c levels are important for the risk of developing complications. Those factors, such as hypertension and hyperlipidemia, are probably related to the obese state, which also can affect individuals with type 1 diabetes. Treatment in both diabetes types should focus not only on HbA1c levels, but also attempt to find and treat other risk factors. We also found a small but significant difference in prevalence of retinopathy between type 1 and 2 diabetes, which is in accordance with some [228], but not all, previous studies [248].

6.12 SEX DIFFERENCES IN FREQUENCY OF DIABETES-RELATED COMPLICATIONS

There was a markedly higher prevalence of microalbuminuria and retinopathy among males than among females with type 2 diabetes, despite the same degree of obesity and diabetes duration, suggesting that men are more at risk of microvascular complications than women. Our results are in agreement with a few earlier studies which reported that men seem to be more at risk, both in regard to the development of retinopathy and an increased risk to develop kidney failure [241, 331, 332]. Type 2 diabetes manifests at a comparatively lower BMI in males than females and is also associated with a higher degree of comorbidities such as hypertension, microalbuminuria and dyslipidemia [333]. The reason for the differences is unclear. Whether they are true sex differences, as mentioned above, or gender differences - due to, for example, behavioral patterns or level of physical activity - remains to be investigated.

6.13 THE MULTIFACTORIAL CAUSE OF COMPLICATIONS

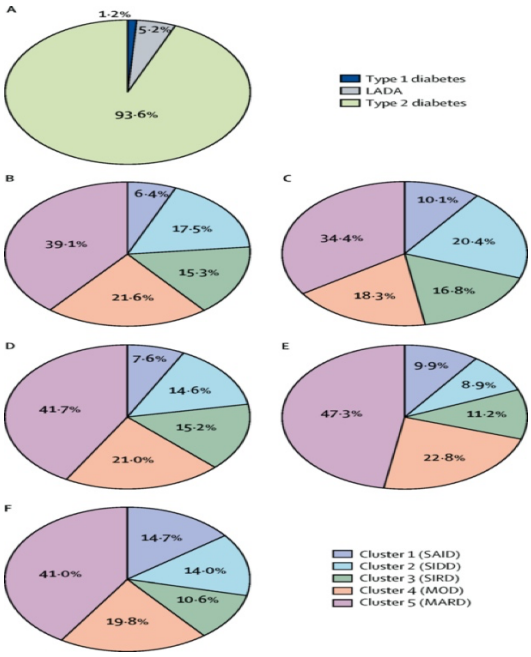
The progression of the main complications assessed in this thesis seems to be caused by different mechanisms. HbA1c levels are of importance but do not represent the whole truth behind the development of complications. The results suggest that HbA1c levels are of importance for the development of retinopathy, and that BMI is the most important predictor for the development of microalbuminuria in both types of diabetes. Individuals with type 2 diabetes had a high degree of comorbidities, and the insulin resistance associated with obesity can be one explanatory factor. The development of insulin resistance is different in different tissues, and it probably leads to different complications in, for example, the kidneys and the eyes. Hyperglycemia per se is associated with changes in the microvasculature in both the eye

and kidney. The causes of retinopathy are not fully understood, although the most important factor is hyperglycemia in both type 1 and 2 diabetes [238, 239]. Insulin resistance has been associated with renal complications [334], and insulin signaling in the kidney podocytes seems to be of importance for normal kidney function. Knock-out INSR mice with subsequent insulin resistance developed diabetic kidney disease even in the presence of normoglycemia, which implies that not only glucose levels but also normal insulin signaling is of importance to sustain normal kidney function [335]. Human studies and animal models have shown that when the podocytes in the kidney are exposed to SAA, this increases NF-κB activity activating numerous inflammatory responses [234], which might be a factor in the development of kidney failure. In skeletal muscle insulin signaling is inhibited by the inflammatory pathways, in particular by the NF-κB cascade [95, 96], leading to insulin resistance and subsequent prediabetes and diabetes.

Our results in Study III show a high degree of comorbidities and complications in youth-onset type 2 diabetes, and men seem to be affected to a higher degree. This is in accordance with other studies. A study from the USA of 699 adolescents with type 2 diabetes found that the prevalence of hypertension and microalbuminuria increased over time. A higher BMI and male sex was associated with hypertension, whereas the risk of microalbuminuria was more related to glycemic control in contrast to our study [336]. The difference in complications between the different diabetes types in Study III might be due to differences in intensity of treatment: The majority of the patients with type 2 diabetes had pharmacologic treatment, possibly due to the challenge to encourage and maintain lifestyle changes, but there are also indications that early onset type 2 diabetes is more aggressive [34].

Diabetes classification is presently made using two main types, type 1 and 2 diabetes, but both types seem to share features from each other. Type 2 diabetes is a multifaceted and heterogenous disease, with some clear features such as the overweight condition, is often associated with heritability but varies in many aspects. Type 1 diabetes results from an immunological destruction of β-cells and all have a subsequent insulin deficiency, however individuals with type 1 diabetes have different HLA types and a variety of autoantibodies indicating a heterogenous pathophysiology also in type 1 diabetes. LADA is another diabetes type in adults, similar to both type 1 and 2 diabetes, with autoimmunity and a slow progression towards insulin dependency.

In adults this has led to different proposals to make stratifications of the main two diabetes types into multiple diabetes subtypes based on clinical picture with the existence of autoantibodies, BMI, age at onset, HbA1c and estimates of β-cell function and insulin resistance. A large Scandinavian study made clustering of type 1, 2 diabetes and LADA into five different clinical subtypes with shared genetic associations revealed differing risk of complications among the groups, the group with relative insulin deficiency had a greater risk of diabetic retinopathy and the cluster with insulin resistance had a higher risk of diabetic nephropathy [337, 338]. The different subtypes are displayed in Figure 19.



(A) Distribution of ANDIS patients (n=8980) according to traditional classification.
(B) Distribution of ANDIS patients (n=8980) according to k-means clustering.
(C) Distribution of patients in the Scania Diabetes Registry (n=1466) according to k-means clustering.
(D) Distribution of patients in the All New Diabetics in Uppsala cohort (n=844) according to k-means clustering.
(E) Distribution of DIREVA patients with newly diagnosed diabetes (n=878) according to k-means clustering.
(F) Distribution of DIREVA patients with longer-term diabetes (n=2607) according to k-means clustering.

LADA=latent autoimmune diabetes in adults. SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=mild obesity-related diabetes. MARD=mild age-related diabetes. ANDIS=All New Diabetics in Scania. DIREVA=Diabetes Registry Vaasa.

Figure 19. *Novel subgroups of adult-onset diabetes and their association with outcomes: A data-driven cluster analysis of six variables. Ahlqvist, E et al. Lancet Diabetes Endocrinol. 2018.*

Although these results might not be fully applicable to young adolescents with diabetes, our results in Study III are in agreement with these results since youth-onset type 2 diabetes seem to develop kidney related complications more frequently, not only depending on glycemic levels. If we were to use these novel subgroups in children, the main diabetes type in children would still be severe autoimmune diabetes or severe insulin deficient diabetes. Today 95% of all childhood diabetes in Sweden are categorized as type 1 diabetes, but approximately 12% of all who develop diabetes are autoantibody negative at diabetes diagnosis, of whom 1.5 % have a MODY variant [209]. There are some indications in childhood diabetes that type 1 diabetes is more heterogenous than previously believed. With the increasing BMI among children, BMI has been proposed to be of importance also in the development of type 1 diabetes. Children with type 1 diabetes and a low-risk HLA types has been associated with a higher BMI [339, 340], and there are a few recent studies presenting an association between an increased BMI and risk of developing type 1 diabetes [341-343]. There are also reports that children and adolescents with type 1 diabetes are more overweight and obese comparing than the general population, with an association to cardiovascular risk factors [344, 345]. Children with type 1 diabetes are at risk in several ways: Besides having a risk of developing obesity, poor metabolic control in childhood can be associated with a higher risk of premature diabetes-related death [346].

6.14 THE PEDIATRIC SETTING

During the last decades the pathophysiology in prediabetes and type 2 diabetes has been explored extensively though mechanistic and molecular studies, biomarkers, genetic and epigenetic factors and large clinical trials. These studies have led to many insights in the pathophysiology, as well as evidence of the heterogeneity of the prediabetic condition and

type 2 diabetes. Nonetheless, the classification and characterization of prediabetes and diabetes is still challenging for healthcare professionals.

It is not easy to establish and understand the risk for the children with obesity, and we need to have simple and clear models for clinical practice. Studies I and II show that children with obesity have many comorbidities, such as prediabetes, and that the condition can present early, especially in males. They often have a family history of diabetes and puberty needs to be considered as it can cause a transient dysregulation of glucose levels. IFG_{ADA} can be associated with IGT, so further investigation with OGTT must be kept in mind. Besides determination of glucose tolerance and further investigation of type 2 diabetes, type 1 diabetes must also be kept under consideration regardless of the child's BMI, since it is the most common type of diabetes in childhood.

Children with severe obesity can all benefit from physical activity, and it is more difficult to make life style changes when obesity continues into adolescence and adulthood [347]. Therefore, it is vital to encourage and increase physical activity at an early age, for all children but especially for children with obesity, independent of other diagnoses. In obese youth with prediabetes or type 2 diabetes the main modifiable factors are the associated obesity and sedentary lifestyle. Efforts to encourage youth to become more physically active and change their lifestyle requires intensive support and often needs to involve the whole family [347, 348], schools, and the surrounding environment to be effective. Studies attempting to increase physical activity have proved that doing so has positive effects on insulin resistance and cardiovascular risk factors even without changes in weight [349, 350], so the level of CRF is a modifiable factor to improve insulin resistance and reverse comorbidities. Insulin resistance and prediabetes in obese adolescents improved after an intensive lifestyle modification program in a randomized control trial, the Bright Bodies Healthy Lifestyle Program [351], changing the trajectory from prediabetes to diabetes. For obese adolescents who develop type 2 diabetes a multidisciplinary team is needed to promote intensive lifestyle weight-loss interventions, by education in diabetes, self-glucose measurements, assessment of comorbidities, and pharmacologic intervention.

6.15 STATISTICAL CONSIDERATIONS

Study I and II

Internal validity
All registry-based studies rely on the quality of the register. Internal validity refers to the extent that the results of a study are reflect reality. The data in the BORIS register are validated every third year through random and selective controls on specific data; validation is also carried out in connection with the annual report and when linkage with other registers is made. This guarantees a high level of internal validity.

External validity
External validity refers to how applicable the results are to the general population. Since all children in Studies I and II had obesity and severe obesity, and were seeking medical help for their condition, the results are applicable for children with severe obesity but might not be true for all children with overweight and obesity.

Confounding factors
Even though many potential confounding factors were considered in Study I, residual confounding is likely to be present. We did not assess ethnicity, genetic background, incretin effect, or grade of inflammation, all of which, can affect the risk of insulin resistance, prediabetes and diabetes.

Study III

Internal validity
The NDR and SWEDIABKIDS are established national quality registers with a high degree of coverage. Data are validated continuously, which guarantees a high level of internal validity.

External validity
The study was a retrospective cohort study, where we could follow individuals with diabetes over time. The data were prospectively collected and population-based, which makes the results applicable for children, adolescents and adults with diabetes in Sweden during the years the data were collected. The cohort can be classified as an open, dynamic cohort, since new cases are registered during the follow-up period.

Lost to follow-up
There is always a potential risk of bias in a cohort study, and some of the risks can be addressed. There is a possibility that some cases are lost to follow-up, especially among those with type 2 diabetes. Type 2 diabetes is a disease where symptoms might not be present, and this can lead to missed appointments and less adherence to medical advice. We do not have information on the patients who are lost to follow-up, but one can assume both that they are healthier and that they could have a more advanced disease. The potential effect on the results could be that the effect we see is smaller than the actual differences. To mitigate this problem to some extent, we carried out statistical analyses on the whole combined cohort and also in the two separate groups of adolescents and young adults.

Selection bias
The study cohort included both type 1 and 2 diabetes patients; hence, it was a nested case-control study within a cohort study. To minimize selection bias the adolescents and young adults with diabetes were withdrawn from the same study base, the National Diabetes Registry, and the controls were randomly chosen.

Misclassification
There is a possibility that some patients were misclassified. In the adult register MODY diagnosis is not registered and could be interpreted as having type 2 diagnosis. These patients exist but there are not many of them, around 1.5 % of all diabetes patients in children and adults. The study relies on the diagnosis from the physician responsible, both in regard to type 1 and 2 diabetes. Type 1 diabetes is less likely to be misclassified, since we can see that all type 1 patients are treated with insulin both in the first registration and at follow-up. A small number of patients changed diagnosis when changing from the pediatric to the adult register, and they were excluded from the study. However, if non-differential misclassification exists with randomly misclassified patients, especially among those who are classified as type 2 diabetes, this makes the difference smaller between the diabetes types than it actually is. The information on exposure (BMI, HbA1c) was prospectively collected to minimize information bias and the collection was made in the same manner for type 1 and 2 diabetes.

Missing data
Missing data is another obvious problem, but we could not discern differences in missing data between type 1 and 2 diabetes. Type 2 diabetes patients did not have as many visits as type 1 diabetes patients, which might affect the collection of urine samples; one can therefore suspect that the differences in complications are even greater. To address the differences in sex, we carried out regression analyses and stratification of analyses, and it seems that male sex is associated with a higher risk of developing complications.

6.16 LIMITATIONS

Several limitations must be acknowledged. As Studies I and II are cross-sectional studies on a high-risk population, the results cannot be interpreted and generalized in a broader sense to all children, but the results would be applicable to children and adolescents with severe obesity. The patients were consecutively investigated concerning risk factors associated with obesity and represent a population of children with obesity seeking medical help for obesity. We did not assess ethnicity, which is a factor affecting insulin resistance and the risk of type 2 diabetes, but the cohort originated from several different countries representing a mixed Swedish population mainly from an urban area. Another limitation is that we did not perform repeated testing with fs-IVGTT or OGTT in Study I, so we do not have a measure of the intrapatient variability. Several studies have shown relatively poor correlation when performing repeated OGTTs in the same individual [292]. However, a strength of this study is that the obese children performed two different glucose regulation tests. A major advantage of the OGTT is that it encompasses many of the physiologic processes in the measure of β-cell response [133], and fs-IVGTT is an elaborate test for both AIR and Si. Also, all the investigations were performed by well-trained nurses in the same clinical setting and conditions, providing a familiar setting for the children.

Several methods are available to assess insulin resistance, and every model is an approximation of reality. In this thesis the fs-IVGTT with minimal modeling was used to measure the AIR and Si. This method has been validated against clamp and is proven to be equivalent if different cut-off limits are used in diagnosing the insulin-resistant state [185]. The model assumes a linear increase of insulin from beta-cells, and the two-compartment model can be criticized since it does not regard the insulin-glucose interactions as an integrated system. It may be a too simplified assumption that linearity exists in glucose and insulin interaction. This may have an effect on the insulin sensitivity estimates, which, therefore might not describe the reality accurately. The model cannot distinguish between the separate effects of insulin, glucose utilization and suppression of hepatic glucose production, which might be affected differently in severe insulin resistance. Since all the individuals in Study I have a similar degree of obesity and insulin resistance, the test might not be able to discern differences in insulin sensitivity nor properly reflect the actual insulin resistance.

In Study II only one glucose measure was used to define the groups of glucose tolerance, and since fasting glucose levels vary [352], this may have resulted in overlapping between the groups.

Matching in Study III was not possible according to the study plan since there was an unexpectedly high number of cases of type 2 diabetes in relation to the matched individuals with type 1 diabetes. The registers have developed over time, with an increasing coverage. It is possible that what we see is an increasing completeness of the registers rather than an increasing number of early-onset diabetes diagnoses.

7 CONCLUSION

Overall conclusion
The findings of this thesis confirm that obesity in childhood is associated with prediabetes and also β-cell failure. The prevalence of prediabetes among severely obese children and adolescents in Sweden is very high and associated with β-cell dysfunction and severe insulin resistance. Obese children with IFG in the WHO range (6.1-6.9 mmol/L) have significantly impaired β-cell function and DI. Factors with a clear association to IGT in the cohort of severely obese children were DI and puberty. Early-onset type 2 diabetes is associated with a higher degree of complications such as microalbuminuria and retinopathy than in type 1 diabetes despite lower HbA1c levels. The risk seems to differ between the sexes, it seems that obese males develop prediabetes and also type 2 diabetes-related comorbidities and complications more frequently than females.

Specific conclusions
In this cohort of obese children and adolescents IFG_{WHO} had a clear association with a disturbed glucose homeostasis, with a lower AIR than seen among those with normal glucose tolerance. With the methods used in this thesis we could not find any explanations of the causes of IFG_{ADA}. A substantial number of the obese children had a combination of IFG_{ADA} and IGT, and a small number of children with normal fasting glucose had IGT. Thus, investigation with an OGTT is often needed, especially in a high-risk population, to define glucose tolerance.

Puberty and DI were some of the explanatory factors in IGT. The obese children with a low DI, which is associated with a risk of type 2 diabetes development, were older and had a lower CRF. We cannot draw conclusions about future risk, but an elevated glucose level in both the ADA range, and especially WHO range can pose a risk to the child.

There is most likely not only one factor causing complications in diabetes, but it seems that early-onset type 1 and 2 diabetes are associated with different complications. The more common comorbidities observed in early-onset type 2 diabetes, such as hypertension, most likely affect the risk of developing microalbuminuria but it may also be an effect of insulin resistance. Long-term moderately-elevated levels of glucose beginning in early childhood may, although not in the diabetes range, be a cause of insulin resistance due to obesity and, possibly, also lead to an increased risk of complications. A high BMI, HbA1c and hypertension is not favorable in either of the diabetes types, but we have some evidence that type 2 diabetes is earlier and more prevalently affected by complications than in type 1 diabetes. Youth with established type 2 diabetes have a high risk of having complications early, which highlights the importance of screening for comorbidities and complications at diagnosis and repeatedly over time.

8 FUTURE PERSPECTIVES

In writing this thesis, the existing literature as well as our own results further emphasize the evidence of the risk of childhood obesity, which is almost overwhelming. Society all over the world is changing rapidly: with a rising BMI, increasing screen time for both children and adults, lower physical activity and greater social inequalities afflicting many children. We are all aware of the risk associated with obesity and know that changes in lifestyle are difficult to achieve. However, we need to further improve what we can do to increase the possibilities for movement and to stimulate physical activity in both children and adults, since the cost and consequences of obesity are so high. This has to be achieved by the authorities and healthcare services; but all of us who are in contact with children have a role to play.

Besides the prevention of sedentary behavior and obesity, we need to explore the trajectories of prediabetes through longitudinal studies to increase our knowledge on how puberty affects IFG, IGT and type 2 diabetes progression. We need to further investigate to the extent to which elevated glucose levels are transient effects or whether disturbed glucose regulation is a sign of type 2 diabetes development.

Since treatment options for type 2 diabetes are somewhat scarce, we need to carry out studies comparing conventional treatment with more modern type 2 diabetes medications to prevent the development of complications.

Microalbuminuria is an early sign of diabetes-related kidney disease, but it can also be a result of hypertension and obesity in itself. Further explorations are warranted of the effect of insulin resistance in obesity, early type 2 diabetes, and the possible development of kidney disease.

We also need to find treatment options for children with type 1 diabetes and obesity, since treatment differs from regular obesity treatment. The same basic lifestyle interventions are needed, but the necessary insulin treatment poses other demands on both diet, physical activity, and pharmacologic treatment.

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