From DEPARTMENT OF CLINICAL SCIENCE, INTERVENTION AND TECHNOLOGY
Division of Pediatrics
Karolinska Institutet, Stockholm, Sweden

ELEVATED FASTING GLUCOSE LEVELS IN OBESE CHILDREN AND ADOLESCENTS: PREVALENCE AND LONG-TERM CONSEQUENCES

Emilia Hagman

Stockholm 2016

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Published by Karolinska Institutet.
Cover illustration: Rebecka Högdahl Hornburg
Printed by E-Print AB 2016

ISBN 978-91-7676-199-1

Research is formalized curiosity. It is poking and prying with purpose.

Zora Neale Hurston (1891-1960)

ELEVATED FASTING GLUCOSE LEVELS IN OBESE CHILDREN AND ADOLESCENTS: PREVALENCE AND LONG-TERM CONSEQUENCES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

For the degree of Ph.D. at Karolinska Institutet. The thesis is defended in hall C1.87, Karolinska University Hospital Huddinge

Friday February 19, 2016, 10:00

By

Emilia Hagman
M. Sci

Principal Supervisor:
Professor Claude Marcus
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Pediatrics

Co-supervisors:
Professor Anders Ekbom
Karolinska Institutet
Department of Medicine, Solna

PhD Pernilla Danielsson
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Pediatrics

PhD Ricard Nergårdh
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Pediatrics

Opponent:
Professor Peter Bergsten
Uppsala University
Department of Medical Cell Biology

Examination Board:
Associate Professor Sofia Carlsson
Karolinska Institutet
The Institute of Environmental Medicine
Division of Epidemiology

Associate Professor Rønnaug Astri Ødegård
Norwegian University of Science and Technology
Department of Laboratory Medicine, Children's and Women's Health

Professor Anders Hjern
Karolinska Institutet
Department of Medicine, Solna

ABSTRACT

Background: Obesity in childhood and adolescents is a major concern in Sweden, as in many parts of the world. Already during the pediatric years is obesity associated with several metabolic complications, which often persists into adulthood. An important metabolic concern is the disturbance of the glucose-insulin homeostasis. One early sign is impaired fasting glycaemia (IFG) which is considered a prediabetic stage as IFG is associated with markedly increased risk for development of type 2 diabetes mellitus (T2DM) in adults. IFG refers to elevated, but not yet diabetic, glucose levels in the fasting state. At present, two different cut-off values for IFG are used in parallel; the American Diabetes Association (ADA) suggest 5.6 mmol/L and the World Health Organization (WHO) promotes 6.1 mmol/L as the cut-off for IFG. In adults, IFG has been associated with increased risk for cardiovascular disease, cancer, and premature also in the absence of the development of T2DM.

The prevalence of IFG in the obese pediatric population has been reported with vast differences across different countries and populations. Further, the long-term consequences of IFG in the obese pediatric population are not clear. However, obesity as well as obesity-induced dysglycemia, may affect several brain functions important for schooling. The aim of this thesis is to investigate the prevalence, risk groups, and consequences of IFG in obese children and adolescents.

Method: All studies included in this thesis contains data from the Swedish Childhood Obesity Register – BORIS (Barn Obesitas Register I Sverige). BORIS is a national quality register for obesity treatment in childhood and adolescence and was initiated in 2005. In addition, data from Germany and Poland regarding children and adolescents who have been undergoing obesity treatment are included. Study I and II are cross-sectional observational studies and Study III and IV are prospective cohort studies, which in addition to BORIS data using data from several national registries.

Results: The total prevalence of IFG among obese children in the German cohort according to the ADA was 5.7% and according to the WHO it was 1.1%. In Sweden, the corresponding prevalence was 17.1% and 3.9%, respectively. IFG risk was associated with increasing age, male sex and degree of obesity. Further, Swedish obese young children had higher glucose levels than Polish obese young children.

The use of T2DM medication retrieved from the national prescribed drug registry was used as a proxy for the diagnosis of T2DM. The pediatric obese population in Sweden, based on the BORIS cohort, had a 24 times increased use of T2DM medications in early adulthood in relation to a population-based comparison group, regardless of gender and ethnicity. While the WHO-defined IFG predicted future use of T2DM medication in early adulthood with an adjusted hazard ratio of 3.84 compared with those who had fasting glucose levels <5.6mmol/L. A fasting glucose level of 5.6-6.0 mmol/L, i.e. the IFG glucose interval added by ADA, did not increase the use of T2DM medication in young adults more than pediatric obesity itself. Female gender and more severe degree of obesity increased the risk for future T2DM medication.

In the obese cohort, 55.4% completed ≥12 years in school, compared with 76.2% in the comparison group. IFG did not correlate significantly with school completion; 50.8% for those with IFG according to ADA and 48.4% for those with IFG according to WHO completed school compared with 55.8% for non-IFG. When analyzing glucose as a continuous variable, a non-significant tendency on school completion could be seen (adjusted p=0.06).

Conclusion: IFG is highly prevalent among obese children in Sweden compared with Poland and Germany. There are no known explanations for the large regional differences in both IFG prevalence and fasting glucose levels. IFG according to WHO, but not the additional interval added by ADA (5.6-6.0 mmol/L) can be considered as a prediabetic stage in the obese pediatric population. Thus, these studies indicate that risk markers identified in adults can not directly be transferred to children. Obesity in childhood and adolescence was associated with low educational level in early adulthood but IFG did not statistically significantly increase the risk for low education in obese individuals.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING
(SWEDISH LAY SUMMARY)

Fetma hos barn och ungdomar hör till våra största hot mot folkhälsan. Hos vuxna ökar fetma risken för en rad sjukdomar, bland annat hjärtkärlsjukdom och typ 2 diabetes (T2D). Hos barn med fetma är dessa följdsjukdomar sällsynta, men förstadier till dem finns. Ett sådant tidigt tecken är förhöjt fasteblodsocker, eller engelskans impaired fasting glycemia (IFG), som hos vuxna är ett förstadium till T2D. I dagsläget finns det två olika gränser för IFG: en lägre gräns satt av American Diabetes Association (ADA) med blodsocker tagna efter en natts fasta på ≥5,6 mmol/L och en högre gräns som är satt av Världshälsoorganisationen (WHO), ≥6,1 mmol/L. Vuxna individer med IFG har ökad risk för hjärtkärlsjukdom, cancer och förtidig död, även om diabetes inte utvecklas. Både fetma i sig, T2D och förhöjda fasteblodsocker kan påverka hjärnans mentala processer, såsom inlärning och minne. Huruvida IFG hos barn och ungdomar som lider av fetma medför samma risker som IFG gör bland vuxna är inte känt.

Den här avhandlingens syften är att utvärdera

1. förekomsten av IFG hos barn och ungdomar som lider av fetma
2. om det går att identifiera vilka barn som har högst risk att få IFG
3. huruvida de barn och ungdomar som har IFG utvecklar diabetes i vuxen ålder
4. om IFG minskar chansen att avsluta gymnasieutbildning

Metod

Alla studier i den här avhandlingen innehåller material från BarnObesitasRegistret I Sverige (BORIS). BORIS är ett kvalitetsregister som inkluderar barn och ungdomar som får fetmabehandling på barnläkarmottagning eller barnklinik. Utöver det registret kommer data även från ett motsvarande nationellt barnfetmabehandlingsregister i Tyskland och ett lokalt behandlingsregister i Polen. För patienterna i BORIS har vi också samlat in data från svenska nationella register avseende utbildning, läkemedelsanvändning och sjukdomsdiagnoser. För de longitudinella studierna av patienterna i BORIS finns en kontrollgrupp som Statistiska centralbyrån matchat på kön, födelseår och bostadsområde. All data i avhandlingen är därmed registerbaserad.

Resultat

I den tyska gruppen var den totala förekomsten av IFG enligt ADA 5,7% och enligt WHO 1,1%. I Sverige var den respektive förekomsten påfallande mycket högre, 17,1% respektive 3,9%, trots att vi justerat för ålder och grad av fetma, vilket annars skulle kunna påverka resultaten. Vidare hade de svenska barnen med fetma högre blodsockernivåer än polska barn. Risken för IFG ökade med stigande ålder, grad av fetma och om man var pojke.

Vi använde diabetesläkemedel som markör för T2D eftersom diabetesdiagnoser är mer osäkert registrerade än läkemedelsanvändning. Detta beror på att T2D ofta sköts i primärvården, vars diagnoser inte rapporteras till nationella register. Barn med fetma i Sverige hade en markant ökad risk att få diabetesläkemedel i tidig vuxen ålder i jämförelse med en populationsbaserad kontrollgrupp, oberoende av kön och etnicitet. IFG definierat av WHO förutspådde framtida användning av T2D-medicinering hos vuxna. Däremot, det utökade sockerintervallet som ADA definierat, ökade inte användningen av T2D-medicinering hos unga vuxna mer än barnfetma i sig. Dessutom hade kvinnor högre risk för framtida T2D-medicinering trots att IFG var vanligare bland pojkar. Allvarlig fetma i barndomen ökade också risken för T2D-medicinering hos unga vuxna.

Av de barn som led av fetma avslutade 55,4% gymnasiet, jämfört med 76,2% i kontrollgruppen. Av dem med lätt förhöjda blodsockervärden (IFG ADA) avslutade 50,8% gymnasiet, och 48,4% av de med mycket förhöjda blodsockervärden (IFG WHO), jämfört med 55,8% för de med normalt fasteblodsocker, men skillnaderna var inte statistiskt säkerställda.

Slutsats

Den här avhandlingen visar att barn och ungdomar med fetma i Sverige har en hög risk att ha förhöjda fasteblodsocker, både jämfört med normalviktiga men även jämfört med barn och ungdomar med fetma i såväl Tyskland som Polen. Vidare visar den att lätt förhöjda fasteblodsocker (IFG ADA), som hos vuxna är ett förstadium till diabetes, inte förutspår diabetes mer än fetma i sig hos barn och ungdomar. Angående samband mellan höga fasteblodsocker och sannolikheten att avsluta gymnasiet fanns det en trend, men den var inte statistiskt säkerställt. Fetma i sig påverkade däremot allvarligt sannolikheten att ta studenten.

LIST OF SCIENTIFIC PAPERS

I. **Major differences of impaired fasting glucose prevalence in two nationwide cohorts of obese children.**
Hagman E*, Rinehr T*, Kowalski J, Ekbom A, Marcus C‡, Holl RW‡.
International Journal of Obesity (Lond) 2014; 38(1): 40-5

II. **Blood sugar levels are higher in young obese children in Sweden than in Poland**
Hagman E, Ighani Arani P, Fischer M, Danielsson P, Marcinkiewicz K, Petriczko E, Marcus C.
Acta Paediatrica 2014; 103(11): 1174-8

III. **Association between impaired fasting glycemia in pediatric obesity and type 2 diabetes in young adulthood**
Hagman E, Danielsson P, Brandt, L, Ekbom A, Marcus C.
Manuscript

IV. **The association between childhood obesity and educational level is counteracted by successful obesity treatment: A prospective cohort study**
Hagman E*, Danielsson P*, Brandt L, Svensson V, Ekbom A, Marcus C.
Submitted

* Shared first authurship
‡ Shared last authorship

CONTENTS

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ADHD	Attention Deficiency Hyperactivity Disorder
ATC	Anatomic Therapeutic Chemical
BMI	Body Mass Index
BMI SDS	Body Mass Index Standard Deviation Score
BORIS	The Swedish Childhood Obesity Register
CVD	Cardiovascular Disease
DNA	Deoxyribonucleic Acid
EGP	Endogenous Glucose Production
FFA	Free Fatty Acid
fMRI	Functional Magnetic Resonance Imaging
GH	Growth Hormone
GLUT	Glucose Transporter
HDL	High Density Lipoprotein
HR	Hazard Ratio
IFG	Impaired Fasting Glycemia
IGT	Impaired Glucose Tolerance
IOTF	International Obesity Task Force
LADA	Latent Autoimmune Diabetes of Adulthood
LDL	Low Density Lipoprotein
LMBB	Laurence Moon Bardet Biedel (Obesity syndrome)
MODY	Maturity Onset of Diabetes in the Youth
NAFLD	Non-Alcoholic Fatty Liver Disease
NFG	Normal Fasting Glycemia
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PCOS	Polycystic Ovary Syndrome
PWS	Prader Willi Syndrome (Obesity syndrome)
QOL	Quality of Life
RR	Risk Ratio
SAM	S-adenosylmethionine
SD	Standard Deviation
SES	Socio Economic Status
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

1 PERSONAL NOTES

Ever since I was a child I have been eager to understand how things work within the human body. Consequently, the nutritionist education was a natural choice for me and created a path into the field of science. I am grateful that I have been given the opportunity to conduct my PhD-studies at Karolinska Institutet.

This thesis provides epidemiological research within the field of disturbed glucose metabolism as a consequence of obesity in childhood and adolescence. Even though we are getting closer to be able to identify individuals in the obese pediatric population who display a higher risk for obesity related co-morbidities, there are still more research needed to be able to make a fair risk prediction. Unfortunately, it is evident that children and adolescents suffering from obesity not only fight the battle against health risks, but also a battle in their daily living life. The stigmatization and prejudice that obesity is associated with, should not primarily be a battle for obese, but for everybody in society and especially for staff who meet and work with obese individuals.

The years of my PhD studies have been the most eventful time in my life. Thanks to participation in international congresses, I have got the opportunity to experience different cultures and seen many places I would not have done otherwise. I am also thankful for international collaborations which have resulted in two articles, which is included in this thesis. Even though this thesis is based on epidemiological research, I have a great interest in the cellular and molecular world, a topic I hope one day will have the chance to explore further.

As for now, I am wrapping up my PhD studies, but I feel that I am far from done with research. I am confident in that life will offer more academic adventures and that I will keep on developing as a researcher. This prospect makes me really excited.

2 BACKGROUND

2.1 OBESITY IN CHILDHOOD AND ADOLESCENCE

2.1.1 Prevalence

The prevalence of overweight and obesity in the pediatric population increased drastically from the 1980s in many European countries. The rise was leveling off in Sweden and some years ago, the prevalence of overweight in 10-year-olds was about 20%, including approximately 3-5% obese subjects.[1] Overweight and obesity are more prevalent in southern Europe. The prevalence of overweight has been reported to be more than 30% in Spain, Italy, and Greece, whereas a prevalence of 15-20% has been reported in Germany, Poland, and Sweden.[2] Obesity prevalence is higher in adolescents than in younger children, but it is also prevalent in children under 10 years of age. It has been reported to be 2.2% in Sweden and 4.7% in Germany.[3] Data from studies of four-year-olds[4] and from studies of cohorts with limited representativity[5] may indicate that the prevalence of obesity in young Swedish children reported in international reviews is underestimated. Recent and systematically collected data for children and adolescents over 10 years of age are scarce and therefore, the development of obesity prevalence remains uncertain at the moment. The prevalence of obesity is lower in Europe than in many other areas of the world, i.e., the United States and Near/Middle East.[6,7]

Rapid changes in the prevalence of childhood obesity in recent decades within a relatively stable population indicate that genetic factors are not the primary cause. Therefore, changes in the nature of the environment towards a more obesogenic society are the most likely cause for the rise in prevalence.[8] Genetic or epigenetic, i.e. DNA-methylation and histone modification, differences predispose some individuals to be more susceptible for the obesogenic environment.[9] Important factors for childhood obesity prevalence include: 1) societal factors, such as the marketing of energy-dense foods on television around the clock and an increase in traffic hazards for walkers and cyclists; 2) socioeconomic factors, such as income inequality; 3) physical inactivity, such as screen time, less participation in sports and increased motorized transportation, and 4) dietary habits, such as more widespread food purchasing opportunities, larger portion size, junk food consumption and sugar-sweetened beverages.[6,10,11] However, other factors, such as viral infections may also contribute to the development.[12]

2.1.2 Some Comorbidities Linked to Early Obesity

Obesity related comorbidities or abnormalities are already evident in the pediatric population. Even if not all subjects in the pediatric obese population suffer from obesity-related sequelae, early disturbances should be taken seriously. Childhood obesity can adversely affect almost every organ system and serious consequences often become present, including hypertension, dyslipidemia, insulin resistance, non-alcoholic fatty liver disease (NAFLD), and psychological complications.

2.1.2.1 Metabolic Consequences

Pediatric obesity is associated with several metabolic complications during the pediatric years, as well as complications that persist into adulthood. Insulin resistance and increased levels of

circulating insulin are early signs of obesity that can be observed already in youth.[13-16] Dyslipidemia may occur as a result of pediatric obesity. Convincing evidence suggests that obesity-related dyslipidemia tracks from early life into adulthood.[17,18]

2.1.2.2 Cardiovascular Consequences

High blood pressure has been shown to track from adolescence into adulthood.[16] Hypertensive adults with concomitant prediabetes have been shown to have a more rapid progression to Type 2 diabetes (T2DM) than prediabetic subjects without hypertension.[19] Non-occurring nocturnal blood pressure dipping (non-dipping) is common among severely obese adolescents, and has been shown to be negatively associated with measures of insulin metabolism regardless of the degree of obesity or daytime blood pressure in obese, non-diabetic adolescents without diagnosed hypertension.[20]

2.1.2.3 The Metabolic Syndrome

The metabolic syndrome includes a cluster of metabolic, cardiovascular and auxological abnormalities involving high BMI or waist circumference, dysglycemia, elevated blood pressure, and dyslipidemia. There are no universal definitions of the metabolic syndrome in childhood, hence different suggestions are present.[21-23] Children and adolescents with the metabolic syndrome have an increased risk of developing metabolic abnormalities[24] and T2DM[25,26] as adults. However, the metabolic syndrome is not a better predictor than high childhood BMI itself.[26]

2.1.2.4 Liver Consequences

NAFLD of different degrees of severity has become more prevalent in the pediatric population due to obesity[27] and is now the most common liver abnormality in children.[28] Up to 38% of obese children have been shown to have NAFLD[28], but it is often asymptomatic in children. However, almost all children with NAFLD are insulin resistant to some degree.[29]

2.1.2.5 Systemic Subclinical Inflammation

Obesity, and especially visceral obesity, leads to increased production of pro-inflammatory cytokines, i.e. C-reactive protein, interleukin 6, and tumor necrosis factor alpha. This is also observed in the obese pediatric population.[30,31] Systemic subclinical inflammation is believed to be a crucial contributing factor to most other obesity-related physical comorbidities, i.e. macro and micro-vascular dysfunction, and the development of diabetes.[32,33]

2.1.2.6 Psychological and Social Aspects

Obesity in the pediatric population has pronounced consequences on the psychological and social domains. Stigmatization from peers, parents, and teachers is well-documented.[34,35] Studies of self-esteem are inconclusive. Older children seem to be more prone to lower self-esteem compared with peers of normal weight, but associations are often weak.[6]

The association between obesity and depression is complex. Obese adolescents who seek treatment for their obesity display more symptoms of depression than subjects without obesity.[36] Some have found adolescent obesity to be associated with symptoms of depression[37,38]; some have

found modest associations[39], while still others have not detected such associations in children and adolescents.[40] It is also important to note that depression during childhood and adolescence is positively associated with BMI in adulthood; hence, reverse causality may be an explanation.[40,41]

Severely obese children and adolescents have been shown to have lower health-related quality of life (QOL) than their healthy peers, and similar QOL as those diagnosed with cancer.[42] Adults who suffered from childhood obesity have lower marital rates and lower income.[43,44]

The long-term social consequences of childhood obesity, such as lower income and lower marriage frequency, have been established for more than 20 years.[43,45] The potential relationship between obesity and school performance has also been addressed frequently, but the association is still unclear. Being obese in childhood and adolescence has been associated with poorer school performance and academic achievement in both school and population-based studies.[43,45-48] However, the conclusions are uncertain due to the fact that many studies have been cross-sectional, have used self-reported data for education, weight and height, or are based on a limited number of obese subjects. In addition, other studies have not been able to confirm any association between academic achievement and obesity[49,50] or, as in one study, in females, but not in males.[43]

In contrast to the physiological complications of obesity, the psychological and social aspects of obesity are highly linked to culture, where some cultures do not view excess weight as a negative attribute.[6]

2.1.2.7 Reversibility of Comorbidities

Weight reduction improves several of the abovementioned risks in young subjects with obesity. Reducing the degree of obesity in childhood and adolescence improves the lipid profile and insulin sensitivity.[51-53] Individuals who are obese in childhood and become non-obese in adulthood have the same risk for T2DM, hypertension, adverse lipid profile, and intima media thickness as subjects who were never obese.[54]

2.2 THE PHYSIOLOGY OF GLUCOSE HOMEOSTASIS

In healthy subjects, blood glucose is firmly regulated by a complex interplay between neurological and endocrine factors. Most of these factors increase blood sugar and only one hormone, insulin, lowers blood sugar. This proves how important it is from an evolutional perspective to avoid hypoglycemia (see below), whereas hyperglycemia has only recently become a threat to human health. The major hormones directly involved in the homeostasis of blood glucose are insulin, glucagon, cortisol, growth hormone (GH), and the catecholamines adrenaline and noradrenaline.[55,56] A schematic illustration of some of the major mechanisms of importance for blood glucose homeostasis is presented in Figure 1.

The effects of these hormones depend primarily on two factors: the amount released from the producing organ and the sensitivity at the target organ. This is of primary importance for insulin, as this is the only blood glucose lowering hormone. Insulin resistance is a reduced capacity by the body to handle a glucose load despite normal insulin release.[56]

2.2.1 The Pancreas

The Islets of Langerhans in the pancreas contain α-cells which produce glucagon, and β-cells which produce insulin. A basal plasma level of inulin is sustained by the pancreas and additionally secreted after ingesting carbohydrates. The postprandial release of insulin promotes glucose, amino acids, and fat uptake by the liver, muscles, and adipose tissue. Blood glucose levels are low when fasting, and under stress glucagon is released from the a-cells. Hence, insulin lowers the circulating glucose level, and glucagon maintains normal glucose levels or raises circulating glucose levels. Amylin is secreted from the β-cells in parallel with insulin and acts to decrease food intake, suppress glucagon release, and delay gastric emptying.[55,56]

2.2.2 The Liver

Insulin is not directly required for glucose uptake in the liver, as glucose is released or taken up in the liver depending on the concentration gradient via glucose transporter number two (GLUT 2). However, insulin stimulates phosphorylation of glucose and thereby increases the net uptake of glucose.[57] Insulin decreases utilization of glycogen and promotes formation of glycogen. Furthermore, insulin inhibits gluconeogenesis, promotes the storage of fat, inhibits β-oxidation of fats, and stimulates protein synthesis.[55]

Glucagon generally antagonizes the effects of insulin in the liver, where it promotes glycogen breakdown, gluconeogenesis and inhibits glycolysis. Additionally, glucagon promotes esterification of free fatty acids (FFA), which can either be stored or exported as VLDL particles. Alternatively, the liver can partly oxidize fatty acids and form ketone bodies, which can in turn be used as energy substrate in some tissues.[55]

2.2.3 Muscle Tissue

In the muscles, insulin increases GLUT 4 translocation to the cell membrane and thereby glucose uptake. Insulin promotes glycogen synthesis, glycolysis, and protein synthesis in muscles.[55] Low basal glucose uptake is mediated via GLUT 1.[58] Physical activity, independently of insulin, also activates glucose uptake via GLUT 4 translocation in the muscles.[59]

2.2.4 Adipose Tissue

In adipose tissue, insulin increases GLUT 4 translocation to the cell surface and thereby glucose uptake, promotes glycolysis to form glycerol-phosphate, and promotes conversion of pyruvate to FFAs and lactate. Further, insulin promotes formation of triglycerides.[55] In adipose tissue, GLUT 1 transporters are responsible for basal glucose uptake. Basal uptake is low in adults but probably higher in infants and young children.[60]

2.2.5 The Gastrointestinal Tract

Incretins are a group of hormones that are released from the gastrointestinal tract in response to food intake. At present, the two major incretins are glucose-dependent insulinotropic polypeptide (GIP) and glucagone-like peptide-1 (GLP-1). Both of these stimulate decreased blood glucose levels by enhancing insulin release and decreasing glucagon release from the pancreas. Extrahepatic effects involve reduced systemically inflammation and increased thermogenesis.[61] Targeting incretins is an emerging field within treatment for T2DM.[62,63]

2.2.6 Fasting and Hypoglycemia

Low blood glucose is acutely dangerous for any mammal. Therefore, the body has more mechanisms than the release of glucagon for raising blood glucose and switching energy sources. The body will respond with stress, and catecholamines, GH and glucocorticoids are released.[56,64-66] Catecholamines acutely increase lipolysis and FFA oxidation, which reduces glucose utilization and releases glycerol for gluconeogenesis.[67] GH slowly stimulates the release and oxidation of FFAs, which leads to decreased glucose utilization and protein oxidation, and preservation of glycogen stores.[66] Cortisol increases gluconeogenesis and protein breakdown, which provides substrate for gluconeogenesis.[67]

While fasting, similar mechanisms are involved to maintain normal blood sugar levels. The initial response involves a reduction in insulin levels and increased glucagon levels. At last, the stress response involving the release of catecholamines and glucocorticoids occurs.[67] GH in the fasting state also exerts mechanisms to maintain glucose concentrations.[66] As a result, normal blood sugar can be maintained over days of fasting, contrary to popular belief.[68]

It is noteworthy that the body has several metabolic responses to hypoglycemic levels, but only one metabolic response to hyperglycemia (Figure 1).[64-66]

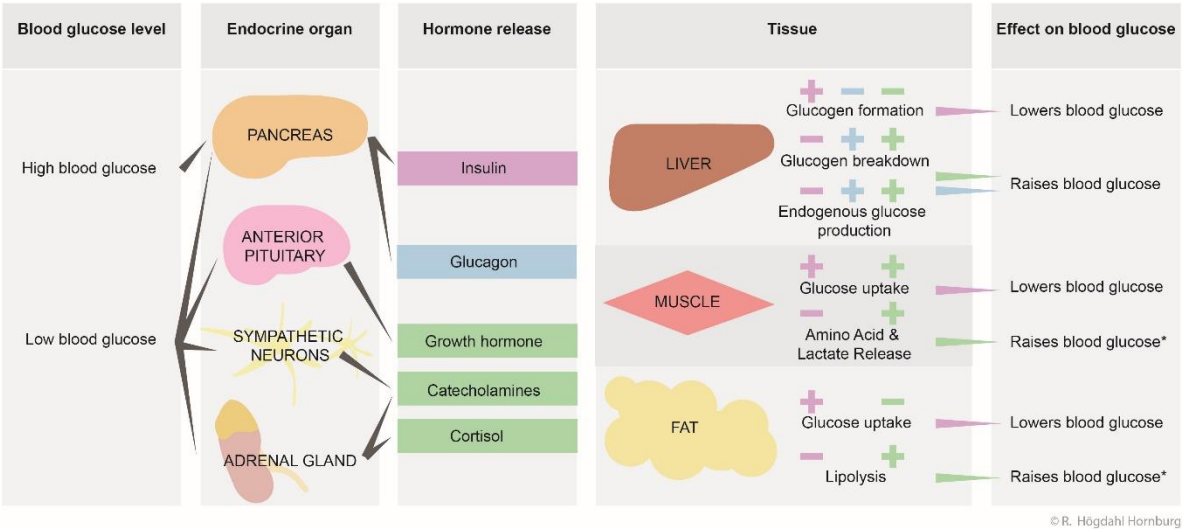


Figure 1. Schematic illustration of some of the major acute mechanisms of importance for blood glucose homeostasis. *Muscle and fat tissue can indirectly raise blood glucose by providing the liver substrate for glucose formation, and energy to reduce the glucose utilization.

2.2.7 Puberty and Pregnancy

In metabolically healthy young people, insulin sensitivity decreases before and during puberty, and is lowest in Tanner stage 3, returning to almost prepubertal levels in Tanner stage 5 (end of puberty). Pubertal insulin insensitivity is believed to be primarily mediated by GH.[69-72]

Pregnancy is normally attended by progressive insulin resistance that begins near mid-pregnancy and progresses until delivery. Pregnancy-induced insulin resistance results from the insulin-desensitizing effects of hormones produced by the placenta, combined with increased maternal adiposity. The degree of insulin resistance might be as high as in individuals with T2DM.[73]

Normally, insulin secretion increases to compensate for pregnancy-induced insulin resistance. However, if the insulin-producing β-cells are not able to compensate for the insulin resistance, gestational diabetes (GDM) with elevated glucose concentrations may occur.[73] The risk of developing GDM is higher if the mother is already obese and insulin-resistant before pregnancy. Most cases of GDM resolve after delivery. However, these women have a documented reduced insulin secretory capacity and thus a substantially higher risk of developing T2DM later in life.[73,74]

2.3 ALTERED GLUCOSE HOMEOSTASIS

Metabolic perturbation may occur in obese subjects, leading to dysregulation of the glucose-insulin homeostasis. Many factors mentioned above may contribute to disturbances in glucose metabolism and insulin resistance, such as disturbed liver function, inflammation and physical inactivity. The final endpoint – if the genetic predisposition negatively affects the capacity to increase insulin secretion – is T2DM, but before that, changes in the homeostasis can be observed. Early signs, such as elevated glucose levels and impaired insulin sensitivity and beta-cell function, can be observed over a long and fairly stable period. But during the 2-4 years preceding a diabetes diagnosis, a steep deterioration of insulin sensitivity and fasting glucose levels can be observed.[75] In children with obesity, altered glucose metabolism is an early comorbidity marker.[13]

2.4 PREDIABETES

Prior to the onset of T2DM, a period of moderate hyperglycemia is often present, which is referred to as prediabetes. This can be observed in the fasting and/or in the postprandial stage. Even though prediabetes is a high-risk state for developing diabetes, many people with prediabetes do not progress to diabetes, which is why some prefer to use a different term, such as intermediate hyperglycemia.[76,77] However, prediabetes is the most frequently used term.

2.4.1 Definitions

2.4.1.1 IFG

Impaired fasting glucose or glycaemia (IFG) refers to elevated but not yet diabetic glucose levels in the fasting state. Two different cut-off values for IFG are currently used in parallel, which perhaps causes some confusion: the American Diabetes Association suggests 5.6 mmol/L[76] and the World Health Organization promotes 6.1 mmol/L[77] as the cut-off for IFG. The origin of these two definitions is discussed below. Testing whether IFG is present is fairly uncomplicated. All that is required to determine the fasting glucose level is a drop of blood in the morning before breakfast and an automatic glucometer, which is easy to use by everybody.

2.4.1.2 IGT

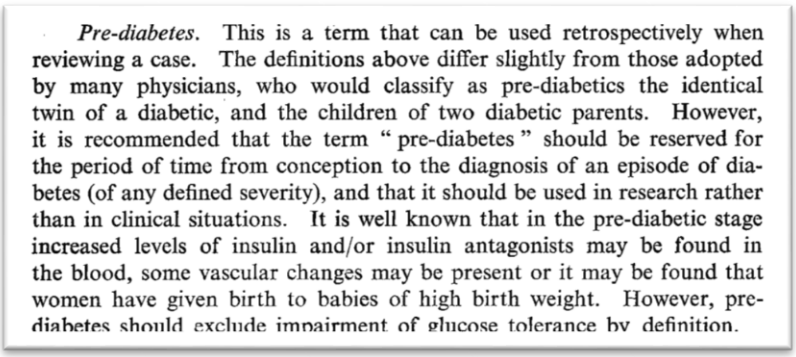
Impaired glucose tolerance (IGT) is defined by elevated postprandial glucose levels, which indicate an impairment in the body's capacity to handle carbohydrates in a meal. IGT is evaluated via an oral glucose tolerance test (OGTT). The range for IGT is a 2h glucose level after an OGTT of 7.8-11.0 mmol/L.[76,77]

2.4.1.3 HbA1c

HbA1c refers to glycated hemoglobin, which mirrors the overall blood glucose levels two-to-three months prior to testing. In recent years, HbA1c has emerged as a marker of prediabetes. Prospective studies show that subjects with HbA1c in the high range have a subsequent 5-year incidence of diabetes ranging from 12 to 25%.[78-80] However, HbA1c has not been a successful identifier of dysglycemia, i.e. IFG and IGT, in children suffering from obesity.[81-83]

2.4.2 The History of Prediabetes

One of the earliest references to prediabetes was in 1965 by the WHO, Figure 2. At that time, prediabetes was not a state itself that could be identified, but a "term that can be used retrospectively when reviewing a case". Hence, prediabetes could not be identified until the diagnosis of diabetes was made. WHO also stated that "it [prediabetes] should be used in research rather than in clinical settings" leaving it undiagnosed for the benefit of the patient, even though already in 1965, WHO concluded that "it is well-known that in the pre-diabetic stage, increased levels of insulin //… // may be found in the blood" and "some vascular changes may be present".[84]



Pre-diabetes. This is a term that can be used retrospectively when reviewing a case. The definitions above differ slightly from those adopted by many physicians, who would classify as pre-diabetics the identical twin of a diabetic, and the children of two diabetic parents. However, it is recommended that the term " pre-diabetes " should be reserved for the period of time from conception to the diagnosis of an episode of diabetes (of any defined severity), and that it should be used in research rather than in clinical situations. It is well known that in the pre-diabetic stage increased levels of insulin and/or insulin antagonists may be found in the blood, some vascular changes may be present or it may be found that women have given birth to babies of high birth weight. However, pre-diabetes should exclude impairment of glucose tolerance by definition.

Figure 2. The 1965 definition of prediabetes from the World Health Organization.

In 1997, the ADA Expert Committee introduced the category of Impaired Fasting Glucose (IFG) to describe the zone between the upper limit of normal fasting plasma glucose and the lower limit of diabetic fasting plasma glucose. At that time, IFG was defined as 6.1-7.0 mmol/L. This was believed to correspond with the zone between the upper limit of a normal 2h plasma glucose and the lower limit of the diabetic 2h plasma glucose described as IGT. The cut-off of 6.1 mmol/L was adopted by WHO in 1999.[77] In 2003, the ADA Expert Committee decided to reduce the cut-off point for defining IFG from 6.1 mmol/L to 5.6 mmol/L. This was motivated by the fact that the prevalence of IFG should be similar to the prevalence of IGT. However, the reduced IFG cut-off point has been questioned.[85]

2.4.3 Prevalence of IFG in Childhood and Adolescence

The prevalence of IFG in the obese pediatric population has been reported with vast differences across different countries and populations.[86-91] However, many studies have been based on samples that are too small to be representative of entire populations. It is therefore unclear whether these variations in prevalence represent a true variation in the prevalence across different geographic areas.

European studies have reported prevalence from 0-2% in Italy[87,92] to 16% in the Netherlands (both using the ADA criteria).[88,90,93] American studies have reported higher prevalence, ranging from 2-9% (WHO criteria) and 15-47% (ADA criteria).[86,91,94] Other countries, including India, China and Mexico, have reported a few percent with IFG in the obese pediatric population,[89,95,96] whereas in Taiwan, 28% of obese adolescents have IFG_{ADA}.[97] Several challenges arise when attempting to compare these prevalence numbers, because different definitions of obesity have been used and different methods were used to recruit the sample populations, including both population-based samples and obesity clinic samples. It is thus unclear whether the variations in prevalence across different geographic areas are accurate.

2.4.4 Who Will Develop Prediabetes as an Adult?

Pediatric risk factors for developing IFG and T2DM have been reported to be BMI, TG, HDL cholesterol, IFG, and family history of T2DM.[98,99]

Regarding parental factors, the longitudinal Cardiovascular Risk in Young Finns Study could identify maternal BMI as an important predictor of T2DM in the offspring.[100] Furthermore, elevated fasting insulin concentrations in children aged 3-6 years are independently associated with an elevated risk of T2DM in adulthood. This relationship was not present in adolescents.[101]

Other factors that have been shown to affect the risk of developing prediabetes and T2DM include exposure to certain antibiotic groups[102] and persistent organic pollutants.[103] In epidemiological studies, consumption of dairy products has been shown to be protective against T2DM.[104]

2.4.5 Pathophysiology of Prediabetes

Plasma glucose in the fasting state is affected by several factors (Figure 3). The pathophysiology of IFG and IGT differ. Both stages represent insulin resistance, but at different locations in the body, and intermediate hyperglycemia, but on different occasions.

IFG is associated with reduced hepatorenal insulin sensitivity, causing higher hepatic endogenous glucose production (EGP), combined with insufficient basal insulin secretion, resulting in elevated fasting glucose levels.[105-108] Contrary, IGT is associated with peripheral insulin resistance, primary in the skeletal muscles.[109] Furthermore, first phase insulin secretion has been shown to be impaired in subjects with both IFG and IGT. Second phase insulin secretion and peripheral insulin resistance in skeletal muscles seems to be normal in subjects with IFG but impaired in subjects with IGT.[105,106,108,110] However, some physiological differences have been observed across different populations, indicating that the pathogenesis of IFG may differ in different ethnic groups.[106] Because IFG and IGT are distinct metabolic abnormalities, subjects with combined IFG and IGT have a more severe progression towards T2DM.[19,111,112]

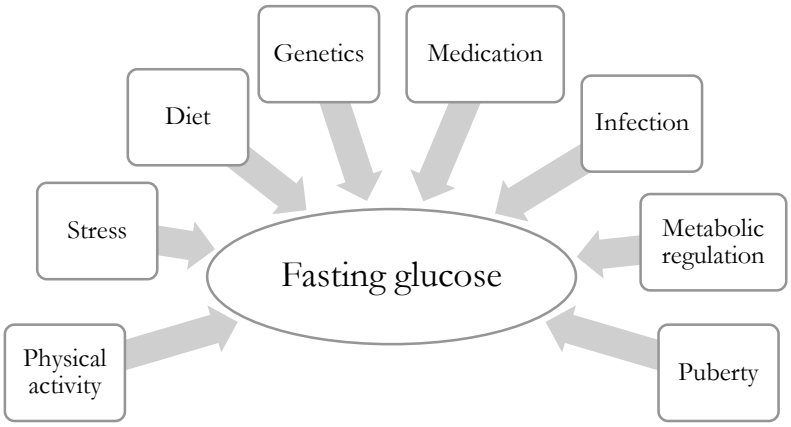


Figure 3. Fasting glucose levels are affected by several factors, including those that can and cannot be influenced by lifestyle changes.

2.4.6 Prediabetes and Related Comorbidities in Obese Adolescents

Prediabetes in obese children and adolescents has been associated with several cardiovascular changes. Young individuals with prediabetes have been shown to have increased arterial thickness and stiffness[113], increased intima media thickness[114] and elevated systolic blood pressure.[113] Furthermore, adolescents with prediabetes have been shown to have an atherogenic lipid profile.[115] Taken together, early in life, prediabetic obese youth display an adverse physiological profile that is most likely mediated by their dysglycemia.[116]

2.4.7 Prediabetes, Comorbidities, and Risks in Adults

Prediabetes is not only a risk factor for future diabetes, but also for other non-communicable diseases and for all-cause mortality. Even though IFG and IGT are often used together, they have separate pathophysiology and etiology and may have different associations with clinical events and mortality.[108]

2.4.7.1 Micro and Macrovascular Disease

Prediabetes might be associated with an increased risk of retinopathy, neuropathy, early forms of nephropathy and chronic kidney disease.[117] Prediabetes is also linked with increased risk of manifestations of vascular disease.[118] However, the epidemiological relationship between prediabetes and macrovascular disease can be confounded by the clustering of vascular risk factors in individuals, since glucose levels in the prediabetic range are correlated with many risk factors, including general and central obesity, blood pressure, and dyslipidemia.[118]

2.4.7.2 Cancer

A meta-analysis published in 2014 using data from 16 prospective studies including more than 890,000 subjects investigated the association between prediabetes and cancer development. They found that prediabetes was associated with an increased risk of cancer overall, Risk ratio (RR) 1.15 95% [CI 1.06-1.23].[119] Highly represented types of cancer included stomach/colorectal, liver, and endometrial cancer. Oddly, the authors found a higher RR for IFG_{ADA} than IFG_{WHO}[119], suggesting that there are no dose-responses to the observed relationship.

2.4.7.3 Cardiovascular Disease

Higher glucose levels in the non-diabetic range increase the risk for (Cardiovascular disease) CVD. For post challenge glucose levels, a linear relationship with CVD is observed. However, for fasting plasma glucose, a light threshold appears around 5.6 mmol/L. The relationship is more pronounced in women than in men.[120]

2.4.7.4 Mortality

Already in the year 2000, data from the Hoorn Study showed that subjects with IFG_{WHO} have increased risk for premature all-cause mortality.[121] A later study of patients with coronary heart disease showed that prediabetes significantly increases the risk for premature mortality.[122] A recent meta-analysis using data from 26 prospective studies investigated the association between prediabetes and the risk for premature all-cause mortality.[123] They found that IFG according to WHO and IGT increase the risk for premature all-cause mortality and cardiovascular mortality, with a relative risk of about 1.15. However, IFG according to the ADA criteria was not associated with increased risk for premature mortality.[123]

2.4.8 Prediabetes and Brain Function and Cognition

In large longitudinal studies, fasting glucose has been associated with reduced grey matter in the brain in adults.[124,125] T2DM in adults has been associated with impaired memory and attention performance.[124] Adolescents suffering from obesity and T2DM show signs of brain alteration compared with adolescents with obesity, but without T2DM.[126] Yau and associates compared 8 obese subjects with T2DM with 18 obese subjects without T2DM using tests for cognitive function and fMRI. Adolescents with T2DM consistently performed worse in cognitive domains, and brain structural analyses revealed reduced white matter volume, but no obvious grey matter volume reduction.[126] Taken together, obesity-induced dysglycemia may affect several brain functions that are important for schooling.

2.4.9 Progression from Impaired Fasting Glycemia to Diabetes

In the adult population, IFG results in a yearly incidence of T2DM of 5-17% based on various IFG definitions and the populations used.[112,127-129] The cumulative incidence over 6-9 years has been reported with a range of 29-39%.[130-132] On the contrary, IFG has also been shown to have a reversal rate to normal fasting glycemia (NFG) of 19-80% over 10 years.[127,133]

The progression of IFG to T2DM in the pediatric obese population has not previously been systematically investigated. However, the transition from the prediabetic stage of IGT to T2DM has been shown to be more rapid in children and adolescents compared with adults.[134]

2.5 TYPE 2 DIABETES MELLITUS

2.5.1 Definition

Type 2 Diabetes Mellitus (T2DM) is caused by the combination of insulin resistance and the failure of insulin-producing cells to compensate for the increased needs of insulin release, resulting in a diabetic state.[55,67] In general, T2DM is not insulin-dependent and is not caused by autoimmune destruction of the insulin producing cells, as in type 1 diabetes (T1DM).[135] Other types of diabetes resemble T2DM, such as dominant genetic forms of non-insulin dependent diabetes, including Maturity Onset Diabetes of the Young (MODY) diabetes.[136] Latent Autoimmune Diabetes in Adults (LADA), a late form of slow-onset autoimmune diabetes, can clinically be interpreted as T2DM, but the pathophysiology differs.[137] Differential diagnosis are describes in chapter 2.6.

T2DM is defined equally in adults and children by a fasting plasma glucose ≥7.0mmol/L or a plasma glucose of ≥11.1mmol/L two hours after an OGTT.[76,77] In contrast to WHO, ADA also allows an HbA1c of 48 mmol/L to define T2DM.[76] The test should always be confirmed by repeated testing before a final diagnosis is made.

2.5.2 Epidemiology and Risk Factors

2.5.2.1 Prevalence

T2DM is a non-communicable disease and a growing public health issue. The International Diabetes Federation (IDF) estimated in 2013 that 382 million adults worldwide have some form of diabetes, and that the number will increase by 55% to more than 592 million in 2035. The situation in Europe is somewhat different, where the increase is estimated to be about 22%, from 56 million to 69 million. Europe has the highest prevalence of children with T1DM, corresponding to about 130 thousand individuals in 2013.[138] In Sweden, approximately 323 thousand individuals had T2DM in 2014, with 2,460 individuals from the age group ranging from 18-35.[139]

2.5.2.2 Risk Factors

Predicting adult T2DM in young years has been investigated by several prospective cohort studies. Demographic, hereditary, and physiological factors have been investigated. On a population basis, it is well established that high childhood BMI is associated with adult T2DM.[25,101,140-142] The degree of obesity in adolescence has also been shown to be higher among those who develop T2DM.[134] Other characteristics associated with increased risk for T2DM are: advancing age, lifestyle (diet,

physical inactivity and smoking), ethnicity, and a family history of diabetes.[142-144] Morrisson et al showed that an elevated fasting glucose level of ≥5.6 mmol/L (IFG_{ADA}) in childhood increased the odds of remaining IFG or developing T2DM in adulthood almost four-fold.[98]

Magnussen et al showed that adolescent presence of the metabolic syndrome that remained into adulthood increased the risk for adult T2DM more than 12 times, compared with individuals who did not have the metabolic syndrome at either point in time. However, if the metabolic syndrome did not last into adulthood, the risk of T2DM was comparable to those who did not have the metabolic syndrome at either point in time.[145]

Genetic variants have been associated with future T2DM. However, they do not increase the prediction of diabetes over clinical risk factors.[140] Furthermore, maternal BMI seems to play an independent role in the risk for eventual T2DM in the offspring,[100] which may be mediated by the effect of maternal obesity on the epigenetic modulation of insulin sensitivity in the offspring.[146]

2.5.2.3 Consequences and Management

Early onset of T2DM has been shown to result in rapidly progressing diabetes-related comorbidities[147,148], and a markedly higher premature mortality rate than T1DM.[147,149] In adult high-risk individuals, T2DM can be prevented by lifestyle changes, including diet and exercise.[150] For young individuals who develop T2DM, the first line of therapy is a lifestyle modification program, including diet and exercise, and pharmacotherapy with metformin.[151,152] If needed, insulin therapy can be used in the initial treatment phase of the disease.[151,152] Pronounced and sustained weight loss, for example after obesity surgery, drastically reduces the need for T2DM medication, and for subjects with T2DM, a long period of remission can be obtained, especially if bariatric surgery is performed soon after T2DM diagnosis.[153]

2.6 DIFFERENTIAL DIAGNOSIS OF DYSGLYCEMIA AND TYPE 2 DIABETES

Previously, hyperglycemia in children was mostly found in T1DM subjects. However, since the prevalence of obese children and adolescents with IFG or T2DM is rising, it is important to identify the true origin of dysglycemia for each individual so that appropriate therapy can be initiated. The following are some other causes of hyperglycemia that should be ruled out.

2.6.1 Type 1 Diabetes Mellitus

Most children who develop T1DM are of normal weight and have a history of weight loss before diagnosis. However, certain HLA-variants increase the susceptibility to childhood development of T1DM with increasing BMI.[135] It is therefore important to also keep this diagnosis in mind if the child is obese, and to consider the whole clinical picture. If T1DM is suspected, β-cell autoantibodies should be evaluated.

2.6.2 MODY

Maturity Onset Diabetes of the Young (MODY) represents several single gene mutations and accounts for 1–2% of diabetes cases.[136] Three genes are responsible for the majority of MODY cases: GCK – mutation in the glucokinase gene (previously named MODY 2); HNF1α (previously

MODY 3), and HNF4α (previously MODY 1), which involves mutations in the gene hepatocyte nuclear factor 1 and 4.

GCK-MODY is the most common subtype of monogenic diabetes in the pediatric population and is characterized by modest hyperglycemia (5.5–8.5 mmol/L) but is otherwise asymptomatic. In GCK-MODY plasma glucose does not deteriorate over time, which rarely associates this subtype of MODY with microvascular or macrovascular complications of diabetes. Patients do not generally require any treatment.[154]

For HNF-MODY the prognosis is different, with a gradual increase in plasma glucose over time and about the same risk for diabetic complications as T1DM and T2DM, which is why treatment with plasma glucose lowering drugs is often initiated.[154]

2.6.3 LADA

Latent Autoimmune Diabetes in Adults (LADA) is probably a slowly progressing variation of T1DM that does not require immediate initiation of insulin treatment. The disease is often combined with insulin resistance and is generally discovered in adults. Therefore, LADA is easily misdiagnosed as T2DM. Compared with T2DM, LADA shows the presence of autoantibodies in the blood.[137] Hence, patients with LADA share a low insulin sensitivity with T2DM patients, but display a more severe defect in beta-cell capacity than patients with T2DM.[137]

As many as one third of young people with clinical T2DM have been reported to have autoantibodies present. This has raised the question of whether these might be classified as "LADY" (latent autoimmune diabetes in youth).[155]

3 AIMS

3.1 GENERAL AIMS

The overall aims of this thesis were to investigate the prevalence, risk groups, and consequences of impaired fasting glycaemia (IFG) in obese children and adolescents.

3.2 SPECIFIC AIMS

- To investigate the prevalence of IFG and fasting glucose levels among obese children in Sweden
- To study whether IFG and fasting glucose levels differ between regions and between northern European countries
- To investigate factors associated with the risk of IFG in children and adolescents suffering from obesity
- To investigate the predictive value of IFG for future T2DM in children and adolescents who have been treated for obesity
- To evaluate whether the degree of obesity in childhood impacts the risk of T2DM in the future
- To study whether childhood obesity treatment efficacy affects the risk of T2DM in the future
- To investigate whether IFG in the obese pediatric population affects completion of 12 or more years in school.

4 METHODS

4.1 STUDY DESIGN AND PARTICIPANTS

All studies included in this thesis contain data from the Swedish childhood obesity register – BarnObesitasRegistret i Sverige (BORIS). BORIS is a national quality register for obesity treatment in childhood and adolescence and was initiated in 2005. All studies in this thesis include subjects from BORIS. The basic purpose of the registry is to longitudinally follow the treatment of childhood obesity and provide working tools. Thus, the quality of childhood obesity care can be assured in Sweden.

BORIS has been supported by the Swedish National Board of Health and Welfare and the Swedish Association of Local Authorities and Regions. The register is a good source for epidemiological research since it allows for the study of a cohort of individuals with pediatric obesity over time and of various ages.

An overview of the four studies included in this thesis is presented in Table 1.

4.1.1 Study I

This study was implemented to investigate the prevalence of IFG in two nationwide cohorts of obese children and adolescents. Study I is a cross-sectional observational study that retrieved data from the APV register in Germany and the BORIS register in Sweden. Inclusion criteria were being 2-17.9 years of age, obese[156], with an eligible fasting glucose measurement. Subjects with diabetes or usage of metformin, syndromal or secondary obesity and endocrine disorders (except for controlled hypothyroidism) were excluded. The first registered fasting glucose measurement was used to define IFG. The total number of subjects included from the German cohort was n=32,907 and from the Swedish cohort n=2,726. In the German cohort, ~0.04% of the German population were included, and in the Swedish cohort, ~0.03% of the Swedish population were included. The prevalence of childhood obesity is approximately the same in Sweden and Germany.[6] Thus, the same proportion of obese subjects is recruited for both registers.

4.1.2 Study II

The aim of Study II was to compare fasting glucose levels in young obese children in Poland and Sweden. This cross-sectional observational study uses three groups of children: two obese groups and one cohort of normal weight. The subjects were obese children in the West Pomerania province of Poland who were receiving care for childhood obesity and matched children from BORIS. Inclusion criteria were 2-9.9 years of age, obese[156], with an eligible fasting glucose measurement. Subjects with diabetes or usage of metformin, syndromal or secondary obesity and endocrine disorders (except for controlled hypothyroidism) were excluded. Ten subjects from the BORIS registry were matched with each child from Poland. Matching variables were age, gender and degree of obesity. In addition to the two obese groups, a non-obese control group from Sweden was used. The non-obese subjects were originally involved in the STOPP-8 OM3 study at Karolinska Institutet, clinicaltrials.gov id: NCT01323283. In total, 109 obese children from Poland, 1,090 from Sweden, and 86 non-obese children were included.

Table 1. Overview of the studies included in the thesis.

	Study I	Study II	Study III	Study IV
Aim	To investigate the prevalence of IFG and to find factors that affect the risk of IFG in two nationwide cohorts of obese children.	To compare the levels of fasting glucose in obese young children in Poland and Sweden	To investigate the predictive value of IFG in obese children and adolescents whom have been undergoing obesity treatment for adulthood anti-diabetic drug usage.	To investigate if the completed educational level differs in young adults who have suffered obesity in childhood compared with the general population. Further, how IFG and childhood obesity treatment influences the educational level achieved.
Design	Observational cross-sectional	Observational cross-sectional	Prospective cohort study with a comparison group.	Prospective cohort study with a comparison group.
Subjects	Germany: n=32907, age 12.5 (SD 2.9) years, 52% girls Sweden: n=2726, age 11.4 (SD 3.4) years, 48% girls	Poland: n=109, age 7.7 (SD 1.6) years, 51% girls Sweden: n=1090, age 7.6 (SD 1.7) years, 51% girls Non-obese: n=86, age 8.5 (SD 0.3) years, 47% girls	Obese: n=1620, age 14.1 (2.4) years, 49% girls, mean follow-up time 8 years. Comparison group: n=8046	Obese: n=1465, age 14.2 (SD 2.5) Comparison group: (n=6979) All subjects were followed up beyond the age of 20 years.
Main outcome	Prevalence of IFG according to both ADA and WHO definition	The distribution of fasting glucose	Anti-diabetic drugs (ATC A10) usage and specifically non-insulins as a proxy for type 2 diabetes.	Completion of ≥12 years in school at the age of 20 years.
Covariates	Age, Gender, BMI SDS, ethnicity, regional data, socioeconomic risk,	Age gender, BMI SDS, serum insulin	Age, gender, BMI SDS, ethnicity, treatment efficacy	Age, gender, BMI SDS, ethnicity, treatment efficacy, treatment duration, medical ADHD treatment

4.1.3 Study III

Study III evaluated the predictive value of IFG in an obese pediatric population on adult T2DM. T2DM medication was used as a proxy for diagnosis. In this prospective study, the obese cohort consists of subjects from BORIS. 5-17.9 years of age and obese[156] at the first visit for obesity treatment at the pediatric clinic or pediatric care center where a fasting glucose measurement was documented. Exclusion criteria were obesity syndromes (such as LMBB, PWS), Mb Down or deceased before 18 years of age (start of follow-up). A flowchart is presented in Figure 4. A comparison group was matched based on gender, year of birth and geographic residence. Through a unique national personal identity number routinely assigned to each Swedish resident, a register link was initiated for the obese cohort and the matched general population group to data on collected prescribed medications. This study included 1,620 individuals from the obese cohort and 8,046 individuals in the comparison group, corresponding to five controls for each case.

4.1.4 Study IV

The aim of Study IV was to investigate the extent to which the obese pediatric population completed ≥12 years in school compared with a population-based comparison group. IFG was investigated as a risk factor for reduced school completion. Of the obese subjects included in Study IV, 877 (60%) had a documented fasting glucose measurement.

In this prospective cohort study, the source of the obese cohort was subjects included in BORIS. A comparison group consisting of five controls for each obese case was matched based on gender, year of birth and smallest available geographic residence providing similar socio-economic background, randomly selected from the Swedish Total Population Register. The inclusion criteria were: obese[156] and under 18 years of age at the first obesity treatment visit. At follow-up (2012-12-31): alive and age ≥20 years, which is at least one year after regular completion of the 12th grade in Sweden. The exclusion criteria in both groups were: diagnosed mental retardation, obesity syndromes (LMBB and PWS) and Mb Down. A flowchart is presented in Figure 5.

4.2 MEASUREMENTS AND DEFINITIONS

4.2.1.1 Obesity and Degree of Obesity (Study I–IV)

In all studies, obesity was defined according to the International Obesity Task Force (IOTF)[156] taking into account the age, BMI, and gender of the child. The IOTF references are based on large nationally representative cross-sectional surveys of growth data from six countries: Brazil, Great Britain, Hong Kong, Netherlands, USA, and Singapore.

In study I, two different BMI standardized age and sex-dependent deviation scores (BMI SDS) were used to define the degree of obesity. One reference was based on German children[157] and one was based on a sample of Swedish children.[158] The Swedish reference was also used in Study II–IV. Moderate obesity in Study III and Study IV was classified as BMI SDS up to 3.5 and severe obesity as BMI SDS of 3.5 or greater, which corresponds to a BMI of approximately 35 in an 18-year-old adult.

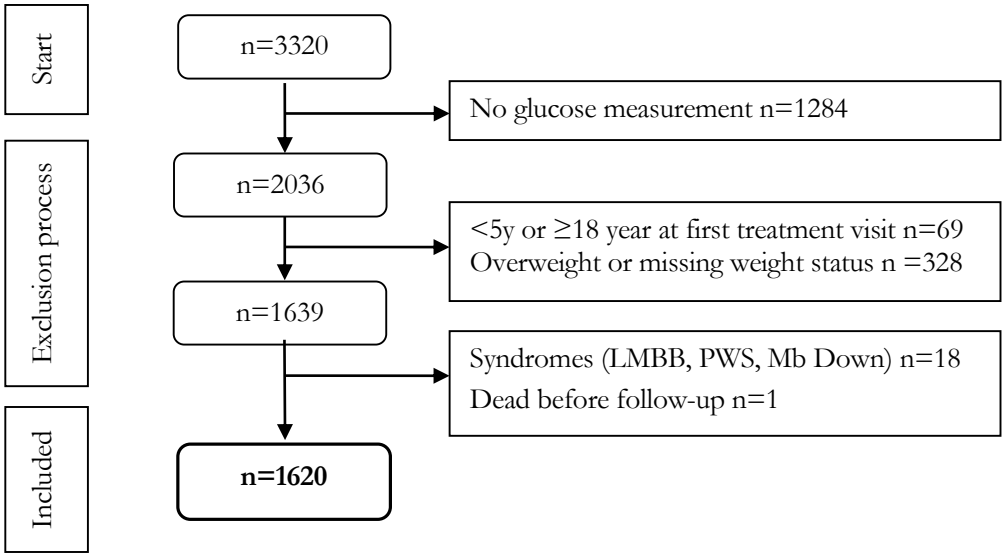


Figure 4. Flow-chart of exclusion process in Study III

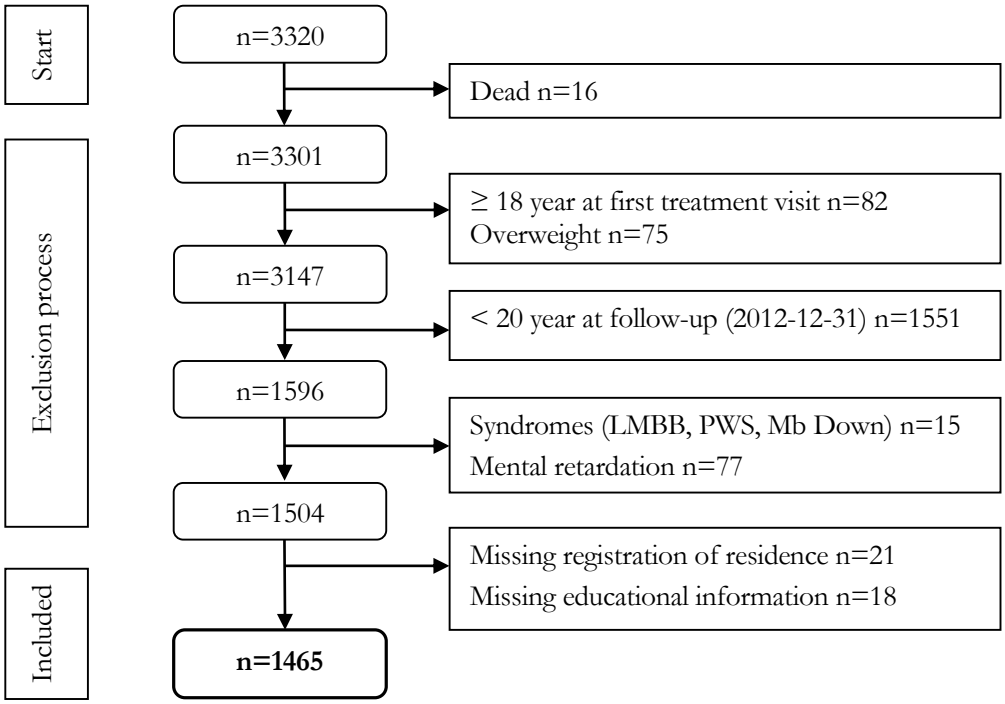


Figure 5. Flowchart of exclusion process in Study IV.

4.2.1.2 Impaired Fasting Glycaemia (Study I–IV)

The first registered fasting glucose measurement in BORIS was used to identify impaired fasting glycaemia (IFG) using both the ADA definition of ≥5.6 mmol/L[76] and the WHO definition of ≥6.1 mmol/L.[77] Normal fasting glycaemia (NFG) was defined as ≤5.5 mmol/L. In Study III, glucose levels were divided into three groups: Normal ≤5.5 mmol/L, isolated IFG ADA (i-ADA) 5.6-6.0 mmol/L, IFG WHO ≥6.1 mmol/L.

4.2.1.3 Regional Divisions (Study I)

In Study I, a Swedish national cohort was compared with a German national cohort of obese children. Germany was divided into four regions: north, south, west (old), and east (new). The Swedish cohort was divided into six regions corresponding to counties.

4.2.1.4 Socioeconomic Status (Study I)

In Study I, socioeconomic risk in the German cohort was based on unemployment, low level of education, and separated parents. Socioeconomic risk was not considered in the Swedish cohort due to lack of data.

4.2.1.5 Ethnicity (Study I, III, & IV)

In Study I, the impact of ethnicity on IFG was evaluated in the German cohort. Migration background was defined as one or both parents having been born outside Germany.

In Study III and Study IV, subjects were split into two groups based on the countries of birth of both the subjects and the parents: Scandinavian – subjects born in Scandinavia with one or two parents born in Scandinavia, and Non-Scandinavian – subjects born abroad or born in Scandinavia with two parents born abroad. Data was retrieved from Statistics Sweden.

4.2.1.6 Medical Treatment of Diabetes (Study III)

The date of the first collected prescribed anti-diabetic drug from 18 years of age was used to define the age of onset of diabetes. Groups of antidiabetic drugs were based on the Anatomic Therapeutic Chemical (ATC) classification system: all anti-diabetic drugs (ATC A10), subgroups, insulins and analogues (ATC A10A) and blood glucose lowering drugs, excluding insulins (ATC A10B). Data on collected medicines was retrieved from the Swedish Prescribed Drug Register, which started in July 2005.

4.2.1.7 The Swedish School System and Achieved Educational Level (Study IV)

In Sweden, children are required to attend school from the age of seven, corresponding to grade one. Compulsory school consists of nine years. Most compulsory schools in Sweden are run by the local municipality. All people in Sweden who have completed compulsory school are entitled to an additional three years of voluntary schooling. Education in grades 10-12 is tailored to either practical or theoretical studies, thus providing a good foundation for vocational activities and/or further studies. In Sweden, compulsory and voluntary school are both free; students also have access to free school health care and school lunches.

In study IV, a completed educational level of 12 or more years in school was evaluated. Data on the highest completed educational level was retrieved from the Swedish Register of Education, held by Statistics Sweden.

4.2.1.8 Prevalence of ADHD (Study IV)

Attention deficit disorders (ADHD/ADD) are known to affect school performance and are frequently investigated in Sweden. Therefore, registration of ADHD/ADD by international diagnosis classification (ICD-10 F90) in the National Patient Register, held by the National Board of Health and Welfare, and administration of psychostimulant drugs prescribed for ADHD/ADD (ATC N06B) from the Swedish Prescribed Drug Register were used as a proxy for ADHD/ADD.

4.2.1.9 Age at Start of Treatment (Study IV)

Age at start of treatment was defined as the first registered visit in BORIS. Subjects' ages at start of obesity treatment were divided into four groups: 5.0-9.9 years, 10.0-13.9 years, 14.0-15.9 years, and 16.0-17.9 years of age.

4.2.1.10 Obesity Treatment Efficacy (Study IV)

Treatment efficacy, based on change in relative weight (BMI SDS), was calculated by subtracting the first registered measurement from the last registered BMI SDS in BORIS. Children who stayed in treatment for less than 12 months were defined as early dropouts.

4.2.1.11 Obesity Treatment Duration (Study IV)

Data on obesity treatment duration was retrieved from BORIS. Time spent in treatment was divided into four categories: 1-2 years, 2-3 years, 3-5 years, and 5 years or more. As in the variable treatment efficacy, subjects who stayed in treatment for 12 months or less were defined as early dropouts.

4.3 STATISTICAL ANALYSIS

Table 2 presents the statistical methods used in the studies. In Study I, the statistical software Statistica, Version 10.0 (Stat Soft, Inc. Tulsa, OK, USA) was used. In Study I, II, III and IV SAS Version 9.3 and Version 9.4 (SAS Institute, Cary, NC, USA) was used. In addition, in the thesis, Kernel distribution and receiver operating characteristic (ROC) curve were used. The significance level was set at α=0.05, 95% confidence limits.

Table 2. Statistical methods used in each study.

	Study I	Study II	Study III	Study IV
Descriptive statistics	x	x	x	x
Linear regression	x	x		
Logistic regression	x			x
T-test independent samples		x		x
Chi square test			x	x
Cox regression			x	
Kaplan Meier curve			x	

4.4 ETHICAL APPROVAL

All ethical approvals were evaluated by the regional Ethics Committee Review Board in Stockholm, Sweden.

- Research within the BORIS registry, file no. 2014/381-31/5.
- The register linkage of BORIS to other national registers, file no. 2011/632-31/4.
- Collection of blood from normal weight children in Study II, file no. 2011/1176-31/2.

All ethical approvals were evaluated by the regional Ethics Committee Review Board in Ulm, Germany.

- Study of childhood obesity treatment in Germany, file no. 16/2005.
- Study of pediatric patients with T2DM in Germany, file no. 60/09.

5 RESULTS

5.1 PREVALENCE OF IFG (STUDY I AND II)

The overall prevalence in the Swedish cohort (n=2,726) of IFG_{ADA} was 17.1% and IFG_{WHO} 3.9%. The corresponding numbers in the German cohort (n=32,907) were 5.7% and 1.1%. Compared with Germany, the prevalence of IFG among obese children and adolescents in Sweden was three times higher according to ADA criteria and 3.5 times higher according to WHO criteria. When evaluating the unadjusted prevalence in subgroups (Table 3), the youngest age group had the lowest prevalence and subjects with the highest degree of obesity had the highest prevalence in both cohorts.

The combination of data on children <10 years from Study I and data from Study II revealed the prevalence of IFG_{ADA} to be 2.5 times higher in the Swedish obese population compared with both German and Polish obese populations. The differences were not as pronounced for IFG according to the WHO criteria. No subjects in the non-obese Swedish population had elevated fasting glucose levels. Table 4 presents the prevalence of IFG in children under 10 years of age.

Table 3. Prevalence of IFG by gender, age, and degree of obesity[158]

	Germany n=32,907		Sweden n=2,726	
	% IFG_{ADA}	% IFG_{WHO}	% IFG_{ADA}	% IFG_{WHO}
Overall	5.7	1.1	17.1	3.9
Girls	5.2	1.0	16.1	3.3
Boys	6.2	1.2	17.9	4.3
p	<0.001	0.28	0.22	0.16
Age				
Age 2 - <9 y	4.3	0.9	10.9	1.7
Age 9 - 12.9 y	6.0	1.2	18.7	3.4
Age 13 - 15.9 y	6.0	1.1	18.8	5.4
Age 16 - 18 y	5.3	0.8	21.5	5.5
p	<0.001	0.09	<0.001	<0.001
BMI SDS				
BMI SDS <2.5	5.0	1.0	11.8	2.6
BMI SDS 2.5- <3.0	5.3	1.0	14.1	3.0
BMI SDS 3.0 - <3.5	6.0	1.1	16.8	4.4
BMI SDS 3.5 - <4.0	6.7	1.2	20.4	5.0
BMI SDS 4.0 - <4.5	6.7	1.7	18.5	2.9
BMI SDS ≥4.5	6.1	1.4	22.2	2.8
p	<0.001	0.23	0.02	0.35

Adopted from Hagman et al. IJO 2014

Table 4. Prevalence of IFG among obese and non-obese children under 10 years.

	IFG ADA		IFG WHO	
	Study I	Study II	Study I	Study II
Sweden (obese)	10.9%	9.1%	1.7%	2.1%
Germany (obese)	4.3%		0.9%	
Poland (obese)		3.7%		1.8%
Sweden (non-obese)		0%		0%

Table 5. Odds ratios for IFG, adjusted for gender, age group, and BMI SDS[158], n=35,633.

	OR IFG ADA	(95% CI)	OR IFG WHO	(95% CI)
Age 2–<9 years	1.00	ref	1.00	ref
Age 9–12.9 years	**1.73**	**(1.48–2.02)**	**1.79**	**(1.27–2.51)**
Age 13–15.9 years	**1.77**	**(1.51–2.07)**	**1.93**	**(1.37–2.72)**
Age 16–18 years	**1.50**	**(1.24–1.81)**	1.33	(0.87–2.02)
BMI SDS 2.3–2.5	1.00	ref	1.00	ref
BMI SDS 2.5–<3.0	1.04	(0.94–1.14)	1.04	(0.80–1.34)
BMI SDS 3.0–<3.5	**1.20**	**(1.06–1.36)**	1.15	(0.85–1.54)
BMI SDS 3.5–<4.0	**1.44**	**(1.25–1.66)**	**1.39**	**(1.00–1.93)**
BMI SDS 4.0–<4.5	**1.54**	**(1.25–1.91)**	**1.74**	**(1.11–2.73)**
BMI SDS ≥4.5	**1.83**	**(1.36–2.47)**	1.79	(0.92–3.45)
Boys vs Girls (ref)	**1.13**	**(1.03–1.22)**	1.09	(0.91–1.31)
Sweden vs Germany	**3.40**	**(3.04–3.81)**	**3.66**	**(2.92–4.60)**

Bold numbers indicate statistically significant differences. Adopted from Hagman et al. IJO 2014

5.2 RISK FACTORS FOR IFG (STUDY I)

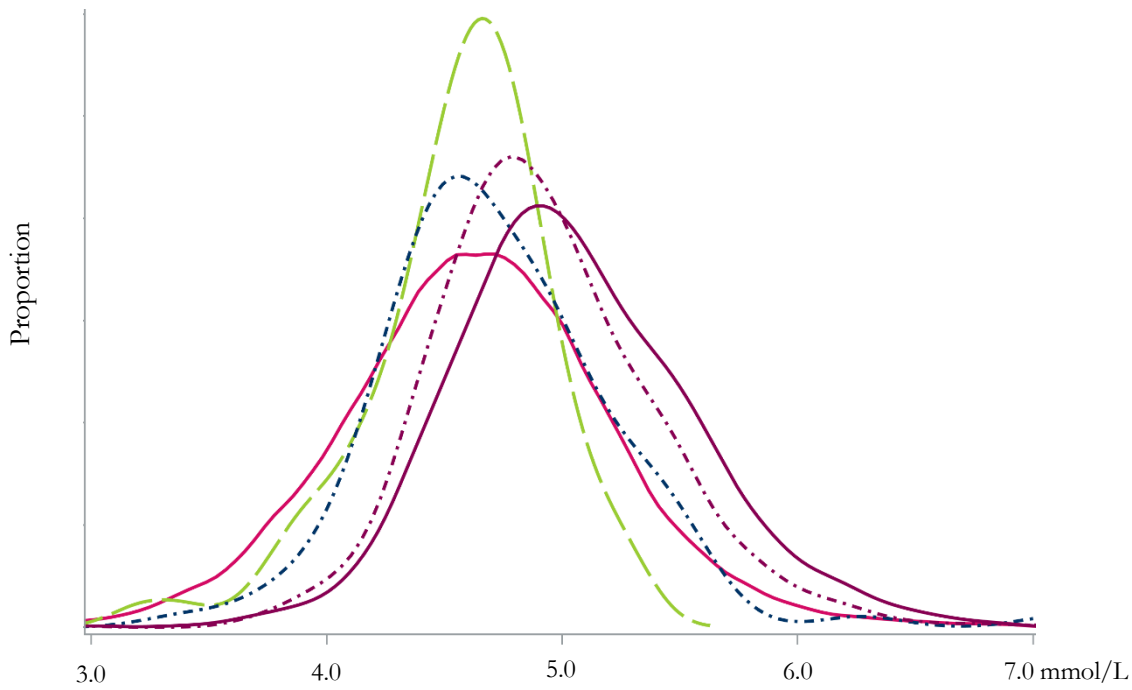
Risk factors for IFG in children and adolescents in Study I included higher age and more severe degree of obesity. Boys had a slightly higher risk of IFG_{ADA}. However, the largest independent risk factor investigated was living in Sweden, which had an adjusted odds ratio of 3.4 [3.0–3.8]. Table 5 presents adjusted odds ratios.

When investigating the impact of ethnicity in the German cohort, immigration background did not affect the risk of IFG. Regarding regional differences in each country, the risk of IFG_{ADA} was elevated in western Sweden (Västra Götaland) compared with most other regions. In Germany, eastern states had a higher risk of IFG than western states. No explanatory reasons for this association could be found.

5.3 DISTRIBUTION OF FASTING GLUCOSE LEVELS (STUDY I AND II)

In Study I, the two populations were between 2-18 years of age. Higher prevalence of IFG in the Swedish cohort compared with the German cohort was mirrored in a generally higher distribution of glucose levels, mean (SD) glucose level of 5.05 (0.52) mmol/L vs. 4.64 (0.58) mmol/L ($p<0.0001$), as illustrated in Figure 6. In Study II, the three populations were all under 10 years of age. The mean fasting glucose values of the Polish, Swedish and non-obese cohorts were 4.73 (0.51) mmol/L, 4.92 (0.50) mmol/L and 4.56 (0.39) mmol/L, respectively. After adjusting for variables affecting fasting glucose, the mean glucose value of the Swedish obese children was 0.20 mmol/L higher than that of Polish obese children ($p < 0.0001$) and 0.41 mmol/L higher than in non-obese controls ($p < 0.0001$).

A comparison of data from Study I and Study II revealed no differences between German obese children between 2-18 years of age and the Swedish sample of non-obese children under the age of 10 ($p=0.07$). Nor did the obese German cohort differ from the Polish subjects under the age of 10 ($p=0.1$). Figure 6 presents mean and SD.



	n	Mean glucose	SD
Germany 2-18y	32,907	4.64	0.58
Sweden 2-18y	2,726	5.05	0.52
Sweden <10y	1,090	4.93	0.49
Poland <10y	109	4.73	0.51
Non-obese <10y	86	4.56	0.39

Figure 6. Kernel-distribution and descriptive data of glucose levels in the cohorts in Study I and Study II.

5.4 PREDICTION OF ADULT TYPE 2 DIABETES MEDICATION (STUDY III)

5.4.1 Subjects Prescribed T2DM Medication

In total, 1,620 subjects from the obese cohort were eligible and matched with 8,046 subjects, henceforth called the comparison group. The proportion of subjects that collected specific T2DM medications (ATC A10B) were 3.2% (n=52) in the obese cohort and 0.1% (n=11) in the comparison group (p<0.0001). Figure 7 presents crude proportions of subjects collecting T2DM medications split into groups based on baseline glucose levels. Figure 8 presents Hazard Ratios (HR), adjusted for gender, degree of obesity, and ethnicity, for glucose levels, showing that the HR does not tend to increase before glucose levels above of 5.8 mmol/L, HR=3.0 [0.7-13.4] and is statistically different when glucose levels rise to 5.9 and above, HR=5.75 [1.63 – 20.26].

In Cox regression, adjusted for gender and ethnicity, the collection of T2DM medications differed considerably between the groups with a HR of 24.0 [12.5-46.0] (p<0.0001). When normoglycemic (<5.6 mmol/L) obese subjects were compared with the comparison group, adjusted HR was 18.49 [9.29–36.80] (p<0.0001). Neither gender nor ethnicity affects the risk of T2DM medication usage. Crude cumulative incidence of collection of T2DM medication is shown in Figure 9.

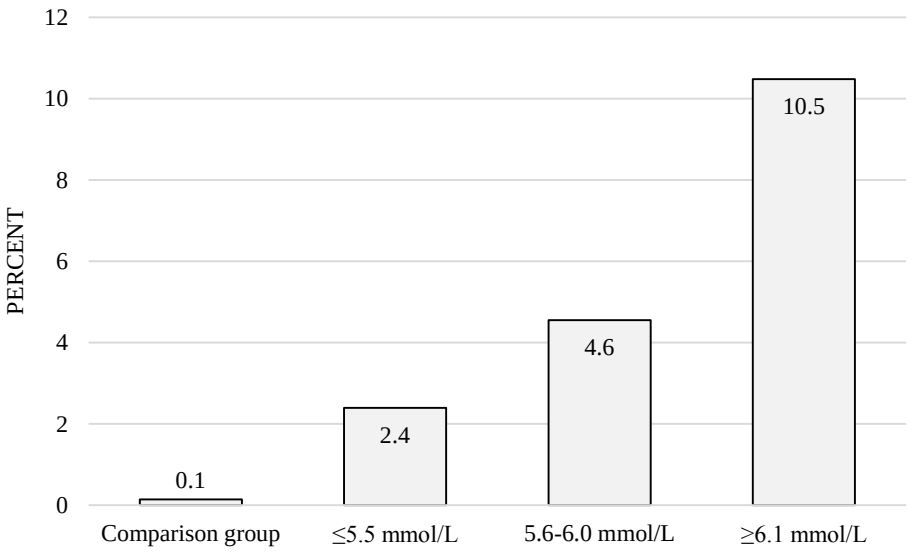


Figure 7. Proportion of type 2 diabetes medication usage in adulthood, divided into levels of glucose at childhood and a comparison group, matched based on gender, age, and living residence.

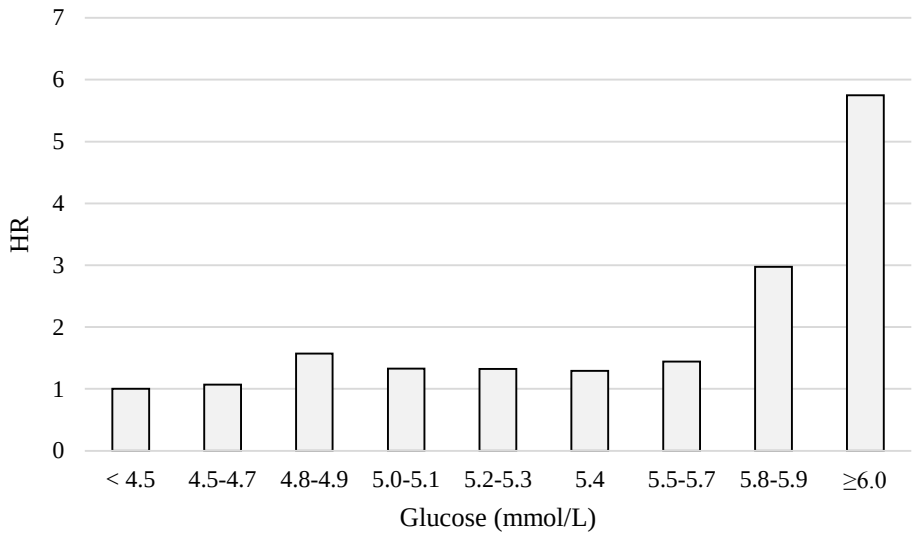


Figure 8. Hazard Ratios (HR) across glucose levels in the obese cohort, using <4.5 mmol/L as reference. The model is adjusted for gender, degree of obesity, and ethnicity.

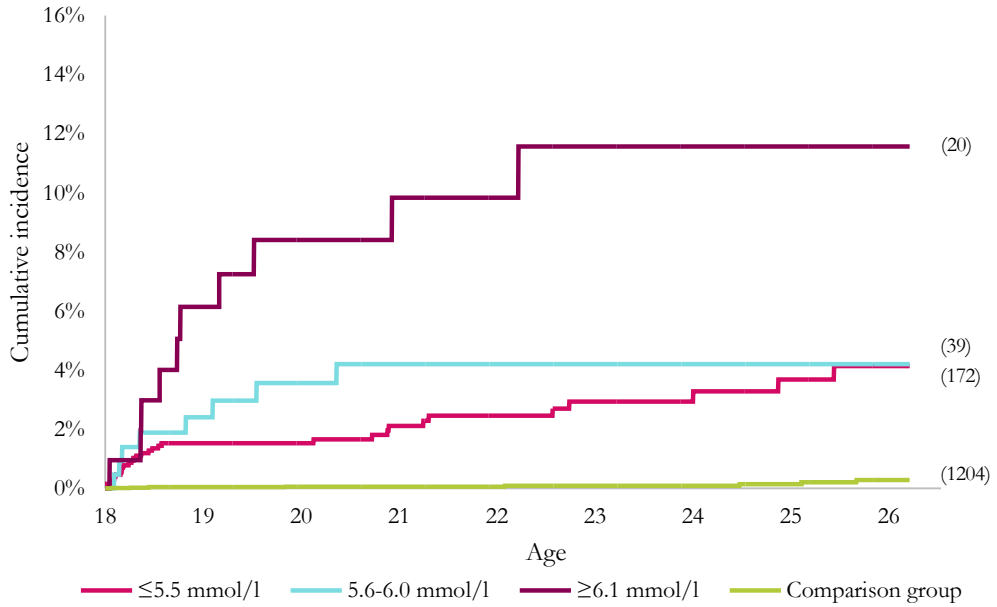


Figure 9. Cumulative incidence of type 2 diabetes medications (ATC A10B) in young adulthood among individuals who have been treated for obesity in childhood. They are divided based on fasting levels of glucose at childhood and compared with a group, matched on gender age and living area. Numbers to the right in brackets indicate numbers of individuals left in each strain at 26 years of age.

5.4.2 IFG According to ADA or WHO and Collected T2DM Medication

In adjusted models for collecting T2DM medications, HR for IFG_{ADA} was 1.67 [0.82 – 3.42] (p=0.16) and for IFG_{WHO} HR=3.84 [1.93–7.67] (p<0.0001). Women were twice as likely to collect prescribed T2DM medications, but an even greater impact was seen among those with a severe degree of obesity (≥3.5 BMI SDS). These subjects were more than twice as likely to collect T2DM medications. Ethnicity did not affect the outcome in any of the models. Hazard ratios are presented in Table 6.

Table 6. Hazard Ratios (HR) for collecting T2DM specific medications in the obese cohort n=1,620. The models are adjusted for IFG (i-ADA, 5.6-6.0 mmol/L, in Model 1 and WHO, ≥6.1 mmol/L, in Model 2), gender, ethnicity and severity of obesity.

	Model 1			Model 2		
	HR	95% CL	p	HR	95% CL	p
IFG (i-ADA) vs NFG	1.67	0.82 – 3.42	0.159			
IFG (WHO) vs NFG				**3.84**	**1.93 – 7.67**	**<0.0001**
Girls vs. Boys	**1.91**	**1.00 – 3.66**	**0.050**	**2.03**	**1.05 – 3.91**	**0.0349**
Non-Scandinavian vs. Scandinavian	1.24	0.62 – 2.49	0.537	0.93	0.46 – 1.90	0.842
Severe vs. Moderate degree of obesity	**2.37**	**1.26 – 4.47**	**0.008**	**2.66**	**1.42 – 4.97**	**0.002**

5.4.3 The Impact of Obesity Treatment on T2DM Incidence

Subjects who had a poorer obesity treatment effect during the pediatric years had higher incidence of collecting T2DM medications in adulthood, adjusted HR=2.37 [1.25–4.48] (p=0.008) for each 1 unit increase in BMI SDS. This is illustrated in Figure 10.

5.5 IFG AND SCHOOL COMPLETION (STUDY IV)

In the obese cohort, 55.4% completed ≥12 years in school, compared with 76.2% in the comparison group (p<0.0001). Subjects with severe obesity had a lower completion rate compared with subjects with moderate obesity. Girls were more likely to be completers in both cohorts, while being of non-Scandinavian origin marginally influenced the outcome in the obese cohort.

The proportion of IFG_{ADA} was 22.7%, and IFG_{WHO} was 7.1%. In crude models, IFG did not correlate significantly with school achievement. Completion of ≥12 years in school was 50.8% for those with i-IFG_{ADA}, and 48.4% for IFG_{WHO}, compared with 55.8% for non-IFG, see Figure 11. Adjusting for gender, age, degree of obesity, ethnicity, having non-IFG, IFG_{ADA}, or IFG_{WHO} did not significantly affect the possibility of completing ≥12 years in school (P for all analyses >0.09). When analyzing glucose as a continuous variable, a non-significant tendency on school completion could be seen (adjusted p=0.06).

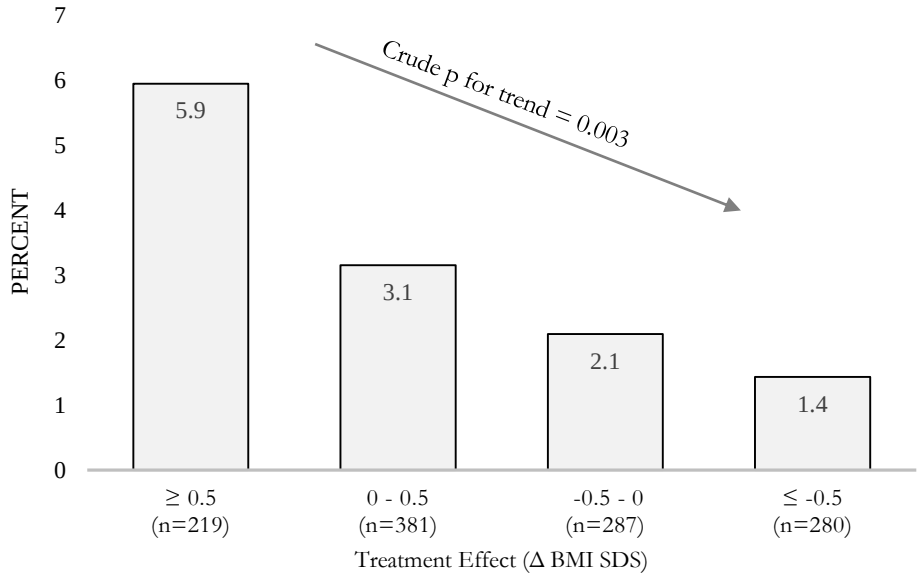


Figure 10. Percentage of individuals who have been treated for obesity in childhood collecting T2DM medications in young adulthood. They are divided into four groups depending on treatment effect based on BMI SDS change obtained from first to last registered BMI (n=1167).

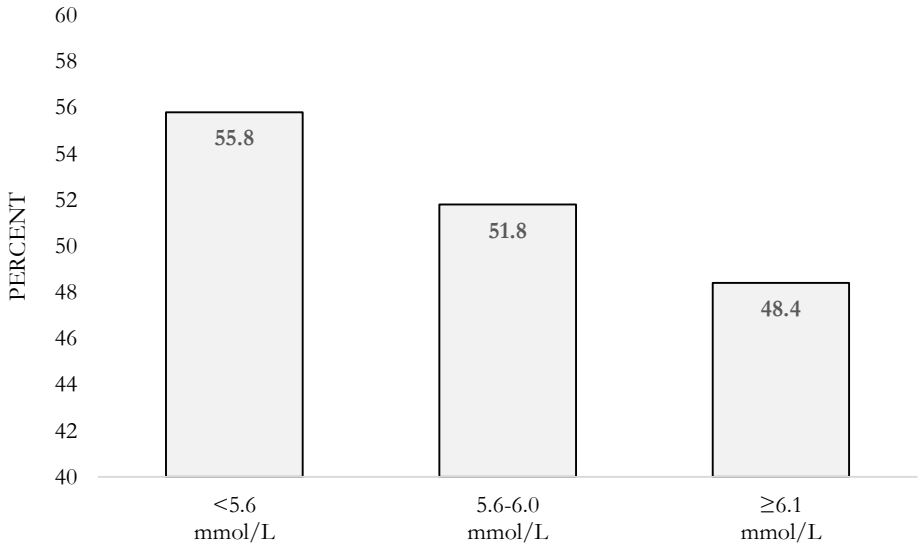


Figure 11. Crude percentage of subjects achieving ≥12 years in school. A non-significant trend towards lower proportion of schooling across fasting glucose levels.

6 DISCUSSION

6.1 MAIN FINDINGS

- IFG is highly prevalent among children and adolescents with obesity in Sweden. IFG_{ADA} (fasting glucose ≥5.6 mmol/L) affects more than 1 in 6 children with obesity in Sweden.
- Higher age and degree of obesity are risk factors for IFG. Boys are slightly more at risk for IFG.
- There are large international differences. After adjustments, children with obesity in Sweden, due to unknown reasons, have a 3 to 4-fold higher risk of having IFG than children in Germany, and a 2.4-fold higher risk compared with children in Poland.
- IFG_{WHO} (fasting glucose ≥6.1 mmol/L) but not the additional interval added by ADA (5.6-6.0 mmol/L) in the obese pediatric population is associated with increased risk for adult pharmacological treatment of T2DM.
- IFG in the obese pediatric population is not significantly associated with achieved educational level in Sweden, whereas severe obesity is associated with a markedly reduced educational level.

6.2 PREVALENCE OF IFG IN THE OBESE PEDIATRIC POPULATION

The prevalence of IFG in obese children and adolescents seems to vary considerably across different obese pediatric populations. Reported prevalence of IFG ranges from zero to almost 50%.[86,87] In Study I, the prevalence of IFG was compared in Germany and Sweden under similar conditions. Degree of obesity was calculated the same way and the source of the populations studied in Sweden and Germany were children seeking medical help for obesity. We also studied IFG defined both according to ADA and WHO. From this study, it is therefore possible to conclude that there are true international differences in IFG prevalence in the obese pediatric population. In Study II, a 2.4-fold higher prevalence of IFG was found among Swedish children compared with Polish children. Interestingly, data from another research group in Sweden recently reported marked differences in IFG prevalence in children suffering obesity between the town of Uppsala, Sweden and Salzburg, Austria[159], which seems to confirm our results.

6.3 THE USE OF ONLY ONE GLUCOSE MEASUREMENT

In epidemiological studies, diagnosis of diabetes is often made upon one test only. In clinical situations, a diabetes diagnosis has to be confirmed with a second test. It is known that IFG is a fickle condition, when normal fasting glucose levels may be present between periods of IFG.[77] In a sample of 60 overweight youth the mean absolute difference between two fasting glucose tests was 0.24 mmol/L, and ranged between 0-0.72 mmol/L.[160] The reproducibility of IFG in adults has been shown to have a κ-value of 0.44-0.56[161], which is considered to be a moderate agreement.[162] A high fasting glucose that is not reproduced could suggest that a tendency towards high fasting glucose might reflect a vulnerability of dysglycemia. With that argument, one fasting glucose measurement should be acceptable for larger epidemiological studies. However, repeated measurements probably enhance the precision of prediction of T2DM.

Another aspect that must be considered is that fasting glucose levels depend on living conditions the day before measurement. Both the degree of physical activity and food choices prior to blood sampling will affect overnight fasting glucose measurement.[163] Thus, ideally, food intake and physical activity should be standardized one to two days before the fasting glucose measurement. As this is not feasible, single fasting glucose levels should be handled with caution in individual cases.

6.4 ARE FASTING GLUCOSE CUT-OFF LEVELS REASONABLE?

Is the desire to categorize individuals as ill or healthy helping or hindering the clinical investigation of an individual? Cut-offs may be comfortable to use, but they do not take into account normal individual variability or risk susceptibility. Therefore, cut-off levels should be interpreted with caution on an individual level. There are also some downsides on the population level to be aware of. A cut-off for prediabetic stages assumes that the entire population, regardless of ethnicity, age, and gender, has the same relationship between early signs of dysglycemia and T2DM, which may not be the case, as I have shown here. A lower cut-off will of course increase the sensitivity, i.e., identifying the true positive rate that will develop T2DM, since the definition is more inclusive, but on the coast of specificity, i.e., the true negative rate.

Individuals from different ethnic backgrounds with the same BMI may have different body compositions and display different levels of vulnerability to metabolic comorbidities.[164] For example, Asians have a higher percentage of body fat than Caucasians with the same BMI.[165] This indicates that subjects with a similar degree of obesity based on BMI may be different when it comes to their degree of insulin sensitivity.[164,166-168] Furthermore, certain ethnic groups, i.e. African Americans, seem to be more susceptible to developing dysglycemia.[113] There are also large variations in the patterns of sexual maturation between boys and girls, which affect insulin resistance, within and between populations.[169,170]

The Bogalusa Heart study showed, with a community-based population, in normoglycemic (< 5.6 mmol/L) children that the optimal cut-off that predicts future diabetes is 4.8 mmol/L. Further Nguyen et al concluded that the risk of developing diabetes in adulthood was more than twice as high with a plasma glucose of 4.7 to 5.5 mmol/L compared to less than 4.7 mmol/L.[171] However, since no child had a fasting plasma glucose of 5.6 mmol/L or above, it is impossible to estimate from this study what IFG leads to. Nevertheless, it is rational to assume from this study that childhood dysglycemia might have a different prognostic impact than adult dysglycemia. When evaluating the population included in Study III, it is not possible to find a rational glucose cut-off for the prediction of future T2DM (Figure 13).

Further, one might speculate that those who are diagnosed with IFG and informed about it will adopt a healthier lifestyle. However, such evidence is not available to the best of my knowledge. Additionally, labeling subjects with a prestage of diabetes might affect the cost or possibility of health insurance in some countries.[172]

6.5 CONTINUOUS VALUES OF GLUCOSE

The glucose levels that define IFG are somewhat arbitrary, where 100 mg/dL (5.6 mmol/L) and 110 mg/dL (6.1 mmol/L) have been decided to use. A rise in glucose level over time follows a continuous pattern, and therefore, an increase in fasting glucose levels could be observed with caution regardless of which cut-off point is used. Some studies have even suggested lowering the threshold, since a level of 5.2 mmol/L, which is below both IFG definitions, has been shown to increase the risk for future diabetes.[173] Shaw et al demonstrate that the risk for future diabetes increases with increasing fasting glucose levels, which is logical. Furthermore, they show that 5.4-5.5 mmol/L is the optimal cut-off for predicting T2DM over a 5 year period, when sensitivity and specificity are optimized.[174]

Others have instead pointed out that by lowering the cut-off from 6.1 to 5.6 mmol/L results in a two to five-fold increase of IFG prevalence in most populations, and the public health aspects of that have yet to be evaluated.[175] Since IFG_{ADA} in adults not only increases the risk for T2DM, but also the risk for CVD and cancers[119,120] one could suggest that IFG_{ADA} should be treated or followed closely over time. In that case, it has to be shown that such actions reduce morbidity and the cost of such interventions both for the individual and for the society must be evaluated.

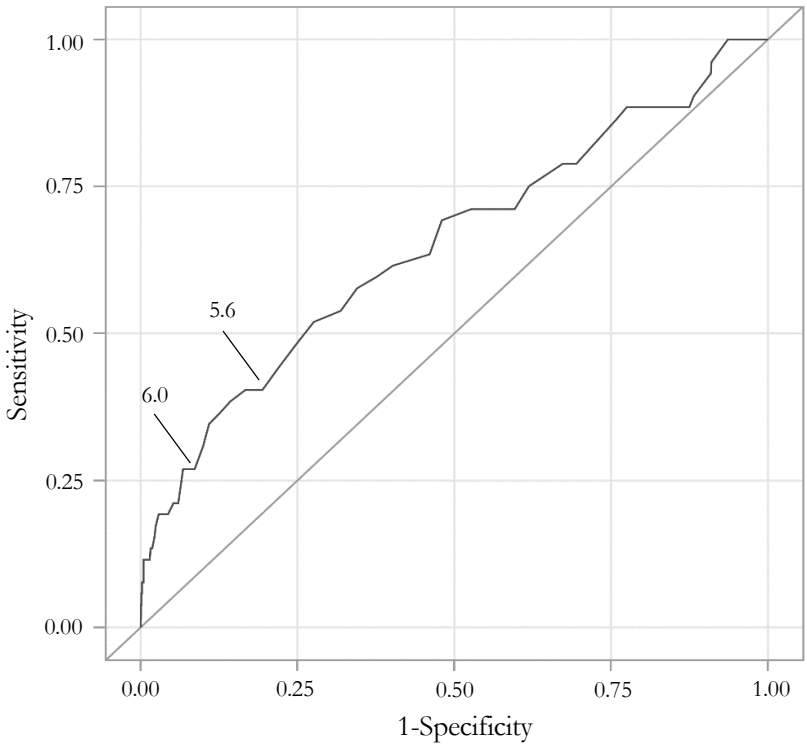


Figure 13. ROC Curve for the predictive value of pediatric fasting glucose and adult incidence of T2DM medication. Glucose value of 5.6 mmol/L and 6.0 are indicated in the graph. Area under the curve = 0.65.

6.6 CHALLENGES WHEN MEASURING GLUCOSE CONCENTRATION

Glucose concentration is usually measured in whole blood or in plasma. Plasma glucose is preferred over whole blood, due to its independence from hematocrit.[176] If glucose concentration is measured in whole blood, it could be recalculated to plasma glucose, at normal hematocrit, by the factor of 1.11.[177] Further venous plasma glucose is preferable over capillary tests[46]. Generally, whole blood samples display a lower fasting glucose concentration compared with venous plasma measurements, and a higher or equal concentration of 2h after an OGTT.[178,179] As an example: a venous plasma glucose of 6.1 mmol/L corresponds to a capillary whole blood concentration of 5.6 mmol/L, according to WHO.[179] The differences between venous and capillary whole blood are not as pronounced in the fasting state.[179,180] Physiological factors and pre-analytical factors, such as time from sampling to analysis, and the kind of glycolysis inhibitor used in the tubes can affect the glucose concentration.[181,182] Differences in glucose concentration due to pre-analytical factors can be up to 0.5 mmol/L.[180-182]

6.7 FACTORS AFFECTING THE RISK FOR DYSGLYCEMIA

6.7.1 The Impact of Degree of Obesity

It has been shown that children with overweight and obesity are at increased risk of IFG compared to normal weight peers.[86,91,94] Based on the assumption that IFG is a prediabetic stage, it is unlikely that IFG prevalence does not increase with increasing BMI, since T2DM is heavily dependent on obesity and insulin resistance, although T2D may also occur among subjects with BMI in the high normal range.[183]

To my knowledge, Study I of this thesis was the first study to show that not only obesity, but also the degree of obesity, increases the risk for IFG_{ADA} in children and adolescents. We observed that degree of obesity increased the risk for IFG_{ADA} but not IFG_{WHO}. Although it can be due to an insufficient power, as IFG_{WHO} has a lower prevalence, this might indicate that the highest levels of fasting glucose might be caused by genetic/epigenetic glucose homeostasis vulnerability in combination with obesity, rather than an effect of obesity per se.

6.7.2 The Impact of Age

In Study I, there was an increasing IFG prevalence with higher age in Sweden and Germany. In Study II, the IFG_{ADA} prevalence among Polish children under 10 years of age was 3.7%. Another study in older Polish children and adolescents displayed an IFG_{ADA} prevalence of 15.2%[184], which indicates that increasing age or pubertal progression is an important risk factor in the Polish obese pediatric population as well.

In Study I, the odds ratio for IFG appears to decrease in the highest age group (16-18 years) compared with the younger age group (13-16 years). This might be due to selection bias. It could be hypothesized that adolescents who start obesity treatment close to adulthood have not had obesity for as long and are therefore not as metabolically affected as the children and adolescents that receive obesity treatment at younger age.

Another reason might be that it reflects normal physiology to some extent. The insulin resistance induced by puberty[69] in combination with insulin resistance induced by obesity might increase the number of cases classified as IFG during that period of development. Assuming that obese adolescents also improve their insulin sensitivity, it could be mirrored by a lower prevalence of IFG among older adolescents who are more likely to have passed puberty. However, if IFG shall be considered a prediabetic stage, it is likely that the prevalence increases with age, as the risk of developing T2D also increases with age.

6.7.3 The Impact of Gender

In Study I, boys were shown to be more prone to have IFG, but the magnitude of gender differences for IFG risk was very small. The same pattern was seen when continuous fasting glucose levels were investigated in Study II, even though the population herein was under 10 years of age and most likely prepubertal. This is consistent with other published data on obese pediatric populations[88,92] , but not all.[89] However, in population-based samples the prevalence of IFG is estimated to be much higher in boys than in girls.[91,94]

Contrary to the higher prevalence of IFG in boys, women in Study III collected more T2DM medications than men, which is analogous to some[19,130], but not all earlier studies.[130] It remains unclear whether IFG is more dangerous or progresses faster in women, or if women are likelier to receive these kinds of medications for some other reason. In adults, undiagnosed T2DM is more common in men[185], and young women may seek medical help more often[186] due to family planning problems. Nevertheless, women seem to be more prone to developing T2DM independently of other risk factors such as BMI, physical activity, and SES.[187] Adherence to T2DM medication does not seem to differ between the genders.[188]

6.7.4 Genetic and Epigenetics

Genetic variations have been shown to affect fasting glucose levels in both healthy adults and children.[189-191] It was recently reported that a common variant of melatonin receptor 1B gene is associated with increased risk of IFG in children and adolescents with obesity.[192]

Epigenetics include methylation of DNA, which plays an important role in regulating gene expression. This is also the case for the expression of genes essential for the maintenance of normal glucose levels.[193] Environmental factors and natural physiological changes in childhood affect epigenetic patterns.[194,195] In T2DM, a defect in DNA methylation is suggested. It has been observed that S-adenosylmethionine (SAM), the main physiological donor of methyl groups, is decreased in erythrocytes of diabetic patients. In addition, decreased concentration of SAM in erythrocytes has been found to be associated with progression of the disease.[196] In a recent study of 11 individuals with IFG_{ADA} who developed T2DM, the authors suggest that epigenomic changes involving both hyper and hypomethylation may be involved in the progression from IFG to T2DM.[197]

Transgenerational epigenetic effects of diet may be passed on to future generations. In a study of historical records from Överkalix, a northern parish in Sweden, the grandsons of men who were well-nourished prior to puberty had an increased risk of developing T2DM.[193]

6.7.5 Does IFG Have Different Origins?

It is possible that IFG only to some extent in children and adolescents causes or is caused by factors associated with T2DM comorbidity. This seems plausible especially in the exclusive ADA range. Thus, we hypothesize that elevated glucose levels have two origins, one is caused by obesity and/or obesity comorbidities and one is caused by other environmental/epigenetic factors. We also hypothesize that the latter is not associated with obesity comorbidities or increased risk for T2DM.

There are several observations supporting these hypotheses. As seen in Study I and Study II, there are marked regional differences in IFG prevalence that persist after adjustment for possible confounders. We have no explanations for this regional variation. In addition we have no indication that T2DM prevalence in adolescents is higher in Sweden than in other countries despite very high IFG prevalence and silent diabetes seems to be unusual.[198] Furthermore, in Study III we cannot detect any association between IFG ADA in children and use of T2DM medication in young adults.

In theory, there are three possible explanations for the combination of high prevalence of IFG and low prevalence of T2DM 1) there are protective factors reducing the progress from prediabetes to T2DM in Swedish children resulting in low prevalence of T2DM despite high prevalence of IFG. However, this combination seems unlikely 2) the progress from prediabetes to T2DM is slower in children in general than in adults, as discussed in chapter 6.8, and 3) the high fasting glucose levels observed in Swedish children are, at least partly, not associated with obesity co-morbidity. This theory is supported by the observation that normal weight children in Study II had fasting blood sugar levels in the same range as obese children from Germany in Study I.

It has been shown that IFG in adults, even without progress to T2DM, is associated with increased co-morbidity.[120] However, the causal relationship between moderately elevated glucose levels and morbidity can be questioned as there are data form some populations indicating that high fasting glycemia in itself does not cause increased morbidity and mortality in adults. Subjects with mutations in the glucokinase gene (MODY 2) display elevated fasting glucose levels in the IFG and diabetes range without any increased risk for diabetes-related comorbidities. Therefore it is recommended that this type of diabetes should not be treated.[154] Thus, given that the clinical information obtained from patients with glucokinase mutations is relevant for IFG, it is not the elevated glucose levels per se, but factors causing it that are of importance as drivers of morbidity and mortality. If we hypothesize that IFG in some obese children in Sweden is caused by other factors, not associated with obesity co-morbidity, these children should not have increased risk for T2DM over normoglycemic obese children.

However, it is possible that other disease risks are associated with regional/cultural factors causing higher fasting glucose levels. T1DM is far more prevalent in Sweden than in Germany and Poland[199] and one might speculate that similar regional factors both affect the risk for IFG in obese children and the risk for T1DM.

6.7.6 Ethnic Differences

Ethnicity is a multifaceted concept for which consensus for the concept does not exist. Ethnicity includes several aspects, such as geographical, social, historical and cultural factors, linguistic background, lifestyle, and religion. Consequently, ethnicity is a highly changing factor that could be influenced by migration.[200] Further, migration studies might investigate ethnic characteristics caused by genetic disposition and what is controlled by environmental factors. A classic example is short stature among Japanese compared with Caucasians, which was long perceived as a genetic condition, but since the second-generation Japanese people in the United States have the same average height as Americans in general, it is clear that the short stature is conditioned by environmental factors.[201] This demonstrates that ethnicity and genetics should be considered separately in epidemiological research.

In Study I, we investigated the impact of ethnicity in the German cohort and found no difference in IFG prevalence between Germans and immigrants. Other studies have been able to evaluate the impact of different origins within the same country more closely. Van Vliet and colleagues found differences in the prevalence of IFG among children with overweight or obesity living in the Netherlands with different country backgrounds. They found that children of Moroccan origin had an IFG prevalence of 25%, Turkish origin 20%, and native Dutch 12% IFG.[90] Other studies have also shown marked ethnic differences of IFG in obese children[96,97], while another could not detect differences in fasting glucose in obese pediatric subjects across different ethnic origins.[86,94] It is possible that there are ethnic differences in both Sweden and Germany that we could not see due to the wide definition used. However, not seeing the ethnic impact on IFG risk but regional differences, such as those between former east and west Germany, might indicate that other factors, such as degree of obesity or eating habits, are of greater importance. Interestingly, environmental factors rather than ethnicity seem to explain the prevalence of type 1 diabetes in Sweden.[202]

Both Study I and Study II and new data from another research group[159] lead us to believe that glucose levels are generally higher in Sweden than in middle European countries. It is noteworthy that non-obese children in Sweden do not have lower fasting glucose levels than obese children and adolescents in Germany and Poland, raising a theory that non-obese children in Sweden have higher fasting glucose levels than non-obese children in middle Europe. However, this is still to be investigated.

Causes which could contribute to prevalence differences within or between populations might include differences in early dietary patterns, physical activity, levels of vitamin D[203], genetics[108,204], as well as viral infections linked to obesity and insulin resistance.[12]

6.8 IS IFG IN THE OBESE PEDIATRIC POPULATION REALLY A PRESTAGE OF T2DM?

In Study III of this thesis there was demonstrated a 24-fold increased risk for T2DM medication in the obese cohort compared with the comparison group. Further, subjects with severe obesity had more than twice the risk compared with subjects with moderate obesity. It has also been shown that not only obese, but also overweight adolescents without any apparent metabolic disturbance have a 6-fold increased risk of T2DM more than 20 years later, compared with normal weight controls.[25] Thus, these data clearly demonstrate that the degree of overweight and obesity is of importance for later development of T2DM.

The association between fasting glucose levels and future risk of T2DM seems to be more complex. In Study III, obese children and adolescents with fasting glucose within the exclusive IFG_{ADA} range (5.6-6.0 mmol/L) did not have significantly higher incidence of collecting prescribed T2DM medication in adulthood than obese children who were normoglycemic. It has previously been shown in a pediatric population that increased fasting glucose levels already within the normoglycemic range (<5.6 mmol/L) are a risk factor for future T2DM.[171] However, these results are probably not contradictory, as Study II shows that obese children in general have slightly higher fasting glucose levels than normal weight children. It is therefore possible that obesity and overweight contribute to the difference in T2DM risk observed in the population-based study of fasting glucose and future diabetes risk.[171] In Study III of this thesis, it was observed a risk 18.5 times higher for T2DM medication among obese children and adolescents with normoglycemia than in the population-based comparison group. When the HR were studied across the span of glucose concentration, no clear-cut increase could be observed before 5.8 mmol/L (Figure 8). Additionally, no clear-cut protective effect could be observed in the low glucose range. Thus, our data indicate that IFG_{ADA} is not a prediabetic stage in children and adolescents with obesity.

Assuming that all subjects in the comparison group who collected T2DM medication (0.14%) are obese, and assuming the same proportion of obese subjects collect T2DM medication (3.21%), it would correspond to an obesity prevalence in the comparison group of 4.3%, which is about the general obesity prevalence in children and adolescents.[4]

In 2014 in Sweden, only 52 youths (up to 18 years of age) were diagnosed with T2DM[205] and in the age group of 18-35 year-olds, 2,460 individuals were diagnosed with T2DM.[139] Since Study I reveals that 17% of obese children have IFG_{ADA} and approximately 5% of children in Sweden are obese[1], the number of T2DM diagnoses in adolescents are not as frequent as what one would expect. Taken together, this might indicate that the progress from IFG to T2DM in adolescents is slow – provided that IFG_{ADA} truly is a prediabetic stage! On the other hand, IGT in severely obese adolescents has been shown to have a rather fast progression to T2DM.[134] With the additional knowledge from the present study, we can conclude that the progression from IFG in the obese pediatric population to adult T2DM is not as pronounced as from IGT in childhood or IFG in adults.

Although the progression of IFG to T2DM may be slow in adolescents, the progress of the severity of the disease is very fast, as well as the progress of T2DM-related comorbidities. The Swedish DISS-study showed that 16% of 30-year-old subjects who have developed T2DM also developed renal complications.[148]

6.8.1 Children are not Small Adults

In the adult population, IFG results in a yearly incidence of T2DM of approximately 3.5% for IFG ADA[206] and up to 34% for IFG WHO%.[112,127] In Study III of this thesis, the incidence is much lower. This may indicate that IFG in children and adolescents is associated to a lesser extent with high risk of T2D development than in adults. There are several potential causes for that and the reasons are most likely multifactorial.

Insulin resistance is a part of T2DM progression, and onset of this syndrome may occur already in childhood.[13,207] However, children normally experience transient insulin resistance during puberty. In normal weight children, an immediate increase in insulin resistance occurs at the onset of puberty (Tanner stage 2), to return to near prepubertal levels by the end of puberty (Tanner stage 5). The physiologically normal insulin resistance peak occurs at mid-puberty (Tanner stage 3) in both boys and girls. However, girls seems to be more insulin resistant than boys at all Tanner stages.[208] Insulin sensitivity is also affected by puberty in the obese pediatric population.[13]

Another possible reason for prolonged progression from IFG to T2DM might include an adaptation to metabolic changes induced by early obesity. In children, microvascular changes related to obesity have been inversely associated with the duration of obesity[209], which indicates a possible adaptation to early cardiovascular changes. One can hypothesize that this is also the case for dysglycemic progression, which, in that case, could explain the prolonged progression to T2DM.

One must also keep in mind that T2DM was originally a disease of the elderly. Epidemiological evidence shows that psychosocial stress, primarily linked to adult life, such as work-related stress, is linked to T2DM.[210,211] Other attributes, such as tobacco consumption, which has also been linked to T2DM incidence[212,213], are adult related cues.

6.8.2 Medication as a Proxy for Disease

A prescription is needed in Sweden for all T2DM medication. The prescribed drug register, which is used in Study III, has information about medications that have been prescribed by a medical doctor *and* collected by the patient. It remains unknown whether the patient has taken the medicine or if there is a satisfying adherence during the time treated. Furthermore, there is no guarantee that the T2DM medication has not been prescribed for other implications than T2DM, such as PCOS or prediabetes, although prediabetes is not a disease.

6.9 IFG AND SCHOOL COMPLETION

In Study IV, pediatric obesity was associated with a strikingly lower achieved educational level in early adulthood compared to a population-based matched comparison group. In the obese cohort, IFG was investigated as a metabolic perturbation hypothesized to affect cognition or executive functions, and thereby school performance. The differences between the groups did not depend on gender, ethnicity, or living area. The causes for these results are most likely multifactorial and the relationships between obesity and the mediating factors, such as stigmatization and impaired cognitive function, are complex (Figure 12).

Obesity-related comorbidities such as the metabolic syndrome[214,215] and T2DM[126,216] have been associated with school performance, cognition, memory, and brain structure alterations, such as reduction in grey matter density and volume of the hippocampus and frontal lobes. In animal models, insulin resistance and other obesity-related comorbidities have been shown to affect cognitive performance.[217]

In Study IV of this thesis, the prediabetic condition IFG was not associated with lower school performance in the obese cohort. However, there was a non-significant tendency (p=0.06) towards lower risk for school completion. There might be a power issue for detecting statistical significance and for a fair evaluation on the impact of elevated glucose levels for school performance, larger studies are needed. However, the relative importance of IFG for school performance seems to be limited compared with the effects of the degree of obesity.

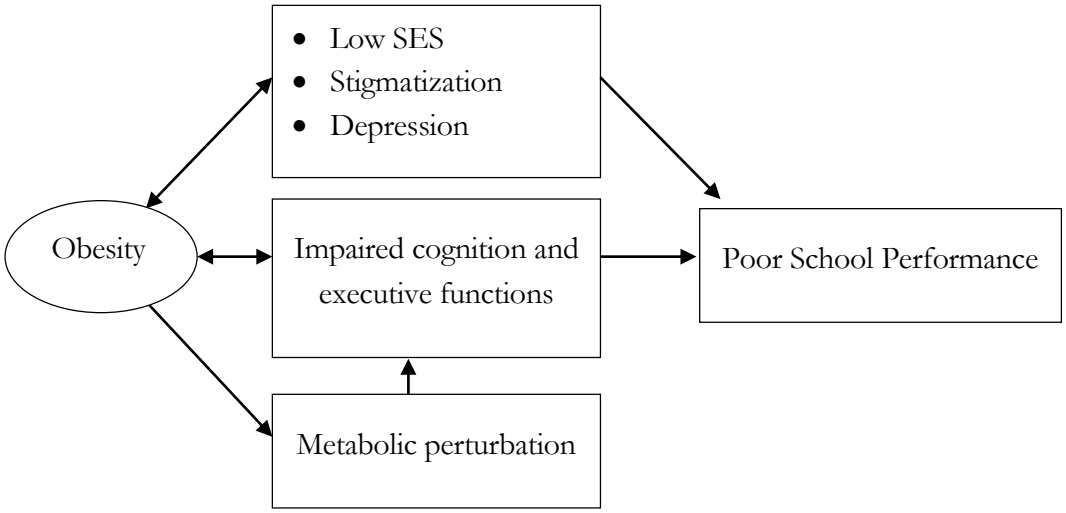


Figure 12. The relationship between obesity and poor school performance is complex and multifactorial.

6.10 REGISTER LINKAGE IN SWEDEN

All residents of Sweden are assigned a personal identification number that is used by all national authorities. This makes it possible to extract a large amount of data that is not possible to collect in a clinical setting. It also makes it possible to investigate scientific hypothesis that are difficult or impossible to assess in other research settings.

In the register linkage that Study III and Study IV are based on, 35 (1%) individuals from the BORIS registry were reported to have a non-valid personal identification number. For a quality register as young as BORIS, that is a fairly low number. Further, inaccuracies in the national registries occur. Potential pitfalls in register linkage studies include re-used or incorrect PINs among Swedish residents. However, this is extremely rare.[218]

6.11 STATISTICAL CONSIDERATIONS

6.11.1 The Nature of Statistics

The statistical methods used in this thesis are quite straightforward for epidemiological research. However, it is important to remember that statistical methods and approaches are the way to describe or explain associations, and in the end, to reject a H_0 hypothesis. Since statistical methods interpret the reality, and not vice versa, one has to remember that α or β errors might occur.

6.11.2 Other Statistical Methods to Consider

The basis of all parametric testing is that the sample is normally distributed, and that the association investigated would be the same over the distributed variable. If we suppose there is a different association across the distribution, neither parametric nor non-parametric tests will pick up those inter-variable differences. In that case, quantile regression would be more suitable since the method is based on the median instead of the mean, and has the potential to evaluate the association at any given percentile of the variable.

6.12 LIMITATIONS

I would like to acknowledge a number of limitations within the studies included in this thesis. Many have already been mentioned above. For Study I and Study II there were no cross validation of glucose measures between the countries. Further, no repeated glucose measurements were used. Another issue is that all data is reported from a large number of clinical settings and entered in registries. Hence, data is not from devoted research settings. Consequently, the accuracy in reported data might be affected. It is not possible to trace back to which method and analyzing kits that have been used for analysis. Further, it is impossible to assure that all samples were obtained during fasting conditions. However, all data collection was of clinical importance, and therefore in the interest of the child and the parents to obtain an accurate clinical picture. We did our best to test whether there were differences between small clinics and university settings with trained staff and laboratories or if some clinics systematically had higher or lower glucose levels but no such differences were found. Thus, although the accuracy is a potential error no such errors were identified.

Other general limitations involve the lack of data regarding genetics, autoantibodies for T1DM, family SES, parental education and health status, i.e. T2DM diagnosis.

Regarding the longitudinal studies, no anthropometric data, i.e. BMI, at follow-up were available. Further, the treatment data is based on change in BMI SDS, but it was not possible to identify the type of treatment received (group, individual, multidisciplinary team etc).

In Study III T2DM medication as a proxy has been used for T2DM. Hence, subjects diagnosed with T2DM and solely treated with behavioral modification are not identified. This group is reported to represent approximately 23% in this age group in Sweden. T2DM in the young obese population is occasionally silent, and such patients are not identified in the present study either. In addition, it is possible that medical treatment has been initiated for prediabetes in some individuals, although no national guidelines advise such practice.

In Study IV, there are no data related to some potential confounding factors that have been implicated in affecting school achievement, such as parental education level and SES. However, the comparison subjects were matched according to the geographical area at baseline, which most probably reduced the risk of marked differences in socioeconomic status. The ethnic similarity between groups also supports the assertion that the groups were comparable. The matched comparison group consists of a normal population, meaning that overweight and obese subjects are included. Neither baseline data nor reliable epidemiological estimates of obesity prevalence are available for this group. Therefore, we cannot exclude an underestimation of the effects of obesity on both achieved educational level and risk for future use of T2DM medication. As thoroughly discussed above, causality of our observed results has yet to be determined.

7 CONCLUSION

This thesis has shown that IFG is prevalent in both Germany and Sweden among children and adolescents with obesity. However, there are striking differences between these countries as Swedish obese children and adolescents have three times higher prevalence for unknown reasons. Young children with obesity in Sweden also have higher glucose levels and risk of IFG compared with a corresponding population in Poland.

Provided that IFG in the obese pediatric population, as in adults, is a predictor for increased risk for future comorbidities, the results indicate that IFG is a prevalent threat of early development of obesity-related co-morbidities in young obese subjects, especially in Sweden. However, the results presented in this thesis seem to indicate that this is not the case. Glucose levels of ≥6.1 mmol/L (IFG WHO) markedly increased the risk but glucose levels between 5.6 and 6.0 mmol/L (the additional IFG span of ADA) were not associated with increased use of T2DM medication compared with obese normoglycemic children and adolescents.

Children and adolescents treated for obesity had, despite normal glucose levels (<5.6 mmol/L), 18 times higher risk to use T2DM medication in young adulthood than a population-based comparison group. Severe obesity increased the risk of T2DM medication.

Being obese in childhood and adolescence was associated with a lower achieved educational level in early adulthood and, particularly for individuals with severe obesity, the differences were substantial. The mechanisms are unclear but most probably both socioeconomic and neurological factors contribute. However, IFG did not significantly increase the risk for low education in obese individuals.

8 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all those who have inspired, supported and encouraged me during my years of doctoral studies. Especially, I would like to thank:

Claude Marcus, my deepest sense of gratitude to you for taking me on this journey and your endless encouragement. You have been a great source of inspiration. Your guidance and supervision have made a researcher of me and for that I am forever grateful. To work with a visionary like you has not always been easy, but through hard working years, slight disagreements, and very much fun, have I now taken my driver's license in science and I could not have wished for a better supervision!

Pernilla Danielsson, my partner in crime and lately even co-supervisor. You have taught me so much and working together with you have been a blast! Let's make it a habit! I am looking forward to conduct more research with you!

Ricard Nergårdh, thank you for believing in me and my potential, and for appreciated discussions during the thesis writing.

Anders Ekbom, you are such an inspiring person! Discussions with you are always fruitful. Thank you for letting me take part of your knowledge, ideas, and honesty. And thank you for introducing me to Lena.

Lena Brandt, it has been so exciting to learn statistics and syntax from you! Thank you so much for your devoted guidance. You have opened a whole new and amazing world to me – the SAS-world – that I still love to experience.

Thank you **Tomas Reinehr and Reinhard W Holl,** for the inspiring international collaboration with data from Germany. As the rookie I was, it was both nervous and honoring to write my very first publication with experienced researchers as you.

Thank you **Elżbieta Petriczko** and **Perna Ighani Arani** for the valuable work we did together and for letting me take part of the Polish data.

Thank you **Jan Kowalski** for your statistical expertise in Study I.

Thank you **Louise Lindberg, Lisbeth Sjödin, Maria Staiger, Isabel Climent-Johansson, and Natalie von Zipel,** current and former administrative staff, for your well-organized help and always being available for a nice chat.

To the research group at B62, and especially **Viktoria Svensson** for your knowledge and support, **Markus Brissman** for laughs and challenging discussions, **Pernilla Hedvall Kallerman** for all work we have done together in the FOCUS-study, and all current and former colleagues at B62; **Veroniqa Lundbäck**, **Anna Ek**, **Anna E Ek**, **Julia Xiu**, **Paulina Nowicka**, **Kerstin Ekbom**, **Karin Nordin, Linnea Bergqvist**, **Michaela Forssén**, **Jonna Nyman**, **Elin Johansson**, **Maria Hagströmer**, **Maria Westerståhl**, **Aziz Elgadi**, **Helén Zemack**, and **Eva Flygare-Wallén** for stimulating collaboration, discussions, and everyday life at the lab.

I like to thank **all pediatric clinical centers** for reporting their obesity treatment efforts. Without your participation, none of the studies in this theses would have been possible to conduct.

I also like to thank the **BORIS steering committee**: Malin Agn, Jovanna Dahlgren, Pernilla Danielsson, Anders Ekbom, Carl-Erik Flodmark, Eva Gronowitz, Sven Klaesson, Claude Marcus, Martin Neovius, John Ryberg, , and Viktoria Svensson for the establishment and maintenance of the BORIS register.

My dear friend and external mentor **Rebecka Hornburg**. You have encourage me in all possible ways and in all possible times. You have been there through fail and foul, but also in happiness and joy. Thank you for your invaluable support and of course for designing the cover illustration of this theses. Also a huge thank you to your lovely man **Matt** for all great moments. I am so grateful to have you.

My dear friend **Anna Franzén** that I have known for more than 25 years, friends as you are rare. Always being wise and honest, and never doubting a second of inviting me to your home. A huge thank you and your enchanting man **Ante** for cheerful moments, glasses of wine, and for always being there!

Tina Jansson, my cooking Uppsala-friend! It is always a pleasure to visit you and your dear **Carl**. There is always something coming up and I am looking forward to many more adventures and funny dinners with you.

Thank you **Lisa Eriksson Marklund** for sporadic, but great lunches, talking about all those important things in life.

Ewa Björkeson, you are such an amazing and inspiring person. The way you mix science with natural medicine and never stop fighting for what you believe in is very encouraging. And always with a smile! I am so thankful for having you as a friend.

Hedvig Eleonora Chamber choir – singing has always been and still is a big part of my life. Thank you everybody for great musicianship, tours, and laughs. Thank you **Pär Friberg** for being a very understanding leader for my sporadic absences for work and health. I am looking forward to share much more music in the future.

Mamma and **Pappa**, you have been trusting and encouraging me when I have made *my* choices in life, which not always have been the straight forward ones. My interest in physiology started early and even though none of you mastered the area, you promoted my devoted interest. Thank You!!

Malin and **Oscar**, thank you for being supportive and being there for me throughout.

Sist och minst - solstrålarna i mitt liv: **Alice** and **Clemens**, det är fantastiskt att få vara er faster. Ni fyller mig med glädje och liv. Ni ger mig perspektiv när det är motigt och påminner mig det viktiga i livet. Tack!

8.1 FINANCIAL CONTRIBUTIONS

I have had the great honor of receiving generous part time grants throughout my PhD-studies from **the Stockholm Freemasons' Foundation for Children's Welfare** and **the Swedish Heart- and Lung Foundation**. Additionally, in 2014 I received the prestigious Rolf Zetterström research scholarship by the **Solstickan Foundation**.

I am also very grateful for financial support for the studies in this thesis and for myself during PhD-studies are with gratitude also received from: **The Swedish Childhood Diabetes Foundation, the Foundation of Signe and Olof Wallenius, the Foundation of Olle Engkvist Byggmästare, the Foundation of Wera Ekström, the Jerring Foundation, the Samariten Foundation, the Foundation of Anna-Brita & Bo Castegrens Memory**, and last but not least **the Foundation of HRH Crown-princess Lovisa**.

A moment of gratitude. The year was 2014, and I thankfully received a scholarship from the Stockholm Freemasons' Foundation for Children's Welfare, which was provided by Her Majesty the Queen.

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