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Glucose Intolerance in Children and Adolescents with Obesity

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Abstract

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Background: The increasing prevalence of childhood obesity worldwide is leading to a decrease in the age of onset of obesity complications such as type 2 diabetes and its early manifestations, also described as glucose intolerance.

Objective: To investigate the prevalence of and identify risk factors associated with glucose intolerance in children with obesity, and to investigate pharmaceutical treatment with metformin for childhood obesity and glucose intolerance.

Subjects: The study is based on paediatric obesity cohorts from three different countries: the Uppsala Longitudinal Study of Childhood Obesity (ULSCO) cohort, from which even participants for the Metformin Intervention in children with obesity (MINT) study were recruited, a Sri Lankan cohort consisting of 357 children with obesity, and the Beta-cell function in Juvenile Diabetes and Obesity (Beta-JUDO) cohort that includes subjects from ULSCO and Paracelsus Medical University Hospital (Salzburg, Austria).

Methods: Blood samples were collected after an overnight fast and glucose tolerance tests (OGTT) conducted. A parallel, three-armed, randomized, 6 months multi-center study, MINT, was conducted with an extended-release metformin formulation combined with lifestyle intervention (XR+L), an immediate-release metformin formulation combined with lifestyle intervention (IR+L), or lifestyle intervention alone (LA).

Results and conclusions: The total prevalence of all forms of glucose intolerance was high in all included study populations, but particularly in Swedish children and adolescents with obesity with 72%. The prevalence was lower at follow-up visits compared with baseline both in Uppsala and Salzburg patients. Risk factors for glucose intolerance included a family history of type 2 diabetes, Swedish origin (within the Swedish cohort) and higher educational status of the father for the Sri Lankan cohort. Elevated histamine-N-methyltransferase (HNMT) was identified as a potential risk factor among the markers associated with mast cell activity, showing significantly higher levels in patients with obesity than in controls and an independent association with fasting proinsulin. Metformin treatment led to a clinically significant BMI-SDS reduction in children and adolescents with obesity compared to lifestyle alone and was shown to be safe and generally well tolerated from the age of 7 years.

Keywords: Childhood Obesity, Glucose Tolerance, Inflammation, Treatment, Metformin

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Ciba I, Warnakulasuriya LS, Adikaram AVN, Bergsten P, Dahlbom M, Fernando MAM, Rytter E, Samaranayake DL, Ruchira Silva KDR, Wickramasinghe VP, Forslund AH. Prevalence of different states of glucose intolerance in Sri Lankan children and adolescents with obesity and its relation to other comorbidities. *Pediatr Diabetes.* 2021;22(2):168-181. doi:10.1111/pedi.13145 [1]

II. Ciba I, Dahlbom M, Manell H, Mörwald K, Roomp K, Weghuber D, Bergsten P, Forslund A. Studies in children with obesity in two European treatment centres show a high prevalence of impaired glucose metabolism in the Swedish cohort. *Acta Paediatr*. 2024;113(2):286-295. doi:10.1111/apa.17030 [2]

III. Iris Ciba, Hannes Manell, Elin Klintman, Banu Aydin, Peter Bergsten, Thomas Lundqvist, Katharina Mörwald, Kirsten Roomp, Rasmus Stenlid, Daniel Weghuber, Kjell Alving, Anders Forslund. Histamine-N-methyltransferase as a Novel Indicator of Beta-Cell Stress in Pediatric Obesity. *Submitted to Journal of Diabetes*. [3]

IV. Iris Ciba, Hannes Manell, Kerstin Andersson, Banu Aydin, Peter Bergsten, Klara Danielsson, Pia Kalm-Stephens, Mats Karlsson, Maria Kjellsson, Malte Lidström, Björn Persson, Rasmus Stenlid, Helena Vilén, Anders Forslund. Metformin extended or immediate release for the treatment of pediatric obesity – a randomized trial. *Manuscript* . [4]

Contents

Abbreviations

2h-G	2 hours glucose
2h-Ins	2 hours insulin
ADA	American Diabetes Association
AE	Adverse event
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUCm	Area under the metformin concentration-time curve
Beta-JUDO	Beta-cell function in Juvenile Diabetes and Obesity
BIA	Bioelectrical impedance assay
BMI	Body mass index
BMI-SDS	Body mass index standard deviation score
BP	Blood pressure
CL	Clearance
comb IFG+IGT	Combined impaired fasting glucose and impaired glucose tolerance
CRP	C-reactive protein
DAO	Diamine oxidase
FG	Fasting glucose
FAS	Full analysis set
FI	Fasting insulin
FM	Body fat mass
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
GLP-1RA	Glucagon-like peptide-1 receptor agonists
HbA1c	Haemoglobin A1c
HC	Hip circumference
HDL	High density lipoprotein cholesterol
HNMT	Histamine-N-methyltransferase
HOMA-IR	Homeostatic model assessment for insulin resistance
hs-CRP	Highly sensitive C-reactive protein

IFG	Impaired fasting glucose
IFN-gamma	Interferon gamma
IgE	Immunoglobulin E
IGT	Impaired glucose tolerance
Il-6	Interleukin 6
IMP	Investigational medicinal product
IR	Immediate release
IR+L	Immediate-release metformin formulation (Glucophage®) plus lifestyle
iso-IFG	Isolated impaired fasting glucose
iso-IGT	Isolated impaired glucose tolerance
LD	Lactate dehydrogenase
LDL	Low density lipoprotein cholesterol
MINT	Metformin Intervention in children and adolescents with obesity
MAFLD	Metabolic dysfunction-associated fatty liver disease
NCD	Non-communicable disease
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PedsQLTM	Pediatric Quality of Life Inventory TM 4.0. Generic Core Scales
PPS	Per protocol set
SAD	Sagittal abdominal diameter
SAE	Serious adverse event
SAS	Safety analysis set
SDS	Standard deviation score
TNF-alfa	Tumor necrosis factor alpha
TC	Total Cholesterol
TG	Triglycerides
ULSCO	Uppsala Longitudinal Study of Childhood Obesity
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist to hip ratio
WHtR	Waist to height ratio
XR	Extended release
XR+L	Extended-release metformin formulation (Glucophage® SR) plus lifestyle

Introduction

Childhood Obesity

Overweight and obesity have grown to epidemic proportions in both adults and children over the last decades and continue to grow, with over 5 million people dying each year as a result of having overweight or obesity according to the global burden of disease initiative and the World Health Organisation (WHO). [5] Overweight and obesity during childhood have consistently been reported to show a high risk of persistence into adulthood. [6]

Obesity is also described as one component of the double burden of malnutrition, and today more people are obese than underweight in every region except sub-Saharan Africa and Asia. [7] From 1975 to 2016, the prevalence of overweight and obesity in children and adolescents aged 5–19 years increased more than four-fold from 4% to 18% globally. The rate of increase has been more than 30% higher in developing countries than in the most developed countries, [8] and the majority of children and adolescents with overweight or obesity live now in developing countries. [7]

Like many other developing countries, Sri Lanka has been subject to rapid socioeconomic changes over the last decades. The country developed from a low-income country in the 1950s to a middle-income country by the beginning of the new millennium, with parallel improvement in health and social indicators. [9] Although the nutritional status of children under 5 years of age has improved gradually, the prevalence of underweight, wasting and stunting have almost plateaued since the turn of the century. Meanwhile, overweight rates have kept increasing, and with it the prevalence of non-communicable diseases (NCDs). [10] In 2016, 12,9% of 5–19-year-old Sri Lankan children and adolescents had overweight or obesity. Corresponding rates were 18% globally, 26,2% for Europe and 23,7% in Sweden. The prevalence of obesity in Sri Lankan children of this age group was 4.8%, compared to 8,6% in Europe and 6,7% in Sweden. [11] Data from the Colombo municipal area showed a prevalence of overweight and obesity about 14% among 8-to-12-year-old school children (obesity 4,3% in boys and 3,1% in girls), [12] and in the Colombo district about 13% among 5-to-15-year-old children (obesity 3,5%). [13]

Obesity complications

The increasing prevalence of childhood obesity worldwide leads to an increase in the prevalence of obesity-related complications such as type 2 diabetes, metabolic dysfunction-associated fatty liver disease (MAFLD), hypertension, and other NCDs, and to a decrease in the age of onset of these complications. Even certain malignancies, autoimmune disorders, asthma morbidity, and psychiatric disorders have been described to be associated with childhood obesity. [14] A report that the World Obesity Federation released in 2016 estimated the likely numbers of children in 2025 with obesity-related comorbidities such as type 2 diabetes (4 million) and its early manifestation, impaired glucose tolerance (IGT, 12 million). [15]

Glucose intolerance

Amongst obesity complications, type 2 diabetes has an important impact on global health and health economics. [16,17] Impaired glucose tolerance (IGT), in essence elevated levels of plasma glucose after two hours during an oral glucose tolerance test (OGTT), and impaired fasting glucose (IFG) have been identified as early manifestations of type 2 diabetes, also summarized as prediabetes or glucose intolerance, and are common among children and adolescents with obesity. [18]

The prevalence of type 2 diabetes and its early manifestations among children and adolescents has been investigated in a number of studies with varying results.[19] High prevalence rates of glucose intolerance (including both IGT and IFG) in children and adolescents with obesity have been reported from the USA with around 25%,[20,21] and India,[22] Bangladesh[23,24] and Sri Lanka[25,26] with over 20%. In Europe, prevalence rates of 3-6% in Italy,[27,28] Germany[29] and the Netherlands,[30] and 15-19% in Spain,[31] Montenegro[32] and Sweden[29] have been reported. The highest prevalence of over 50% has been reported from a cohort of children and adolescents with severe obesity in Sweden. [33]

Glucose intolerance in South Asian children and adolescents with obesity

There are only sparse data regarding the prevalence of glucose intolerance in South Asian and especially in Sri Lankan children with obesity, but one study in 202 children with obesity from Colombo reported a prevalence of 11.4% for IGT and 10.9% for IFG, according to the American Diabetes Association (ADA) criteria. [25] Among Sri Lankan adults, the prevalence of type 2 diabetes was about 10.3% in a study conducted 2005-2006, and some form of

dysglycemia was present in 21.8% of the participants. [34] The same study claimed a projected diabetes prevalence of 13.9% in Sri Lanka for the year 2030. Compared with many other ethnic groups, South Asian populations are, due to their fast economic growth and genetic predisposition, prone to develop many adverse metabolic consequences at an earlier degree of obesity, including insulin resistance, [35] glucose intolerance and type 2 diabetes. [36] A WHO expert consultation concluded that the proportion of Asian people with a high risk of developing type 2 diabetes and cardiovascular disease is substantial at BMI values lower than the existing WHO cut-off point for overweight (> 25 kg/m^2). [37] In line with this, people born in the Indian sub-continent who had migrated to England and Wales were found to have higher mortality from both ischemic heart disease and cerebrovascular disease than the national average. [38] Another study compared the relationship between obesity and prevalent diabetes across ethnic groups in the UK Biobank cohort and found that for the equivalent prevalence of diabetes at 30 kg/m^2 in white participants, BMI equated to 22.0 kg/m^2 in South Asians. [39]

The overall observation is that South Asian adults have a higher prevalence of comorbidities at the same BMI compared with other ethnic groups. Whether this also is true for Sri Lankan children with obesity compared to children with obesity of other origins has rarely been investigated. One previous study examined whether British South Asian children differ in insulin resistance, adiposity, and cardiovascular risk profile from white children, and found that the tendency to develop insulin resistance observed in British South Asian adults was also apparent in children. [40] Another study in 6-11-year-old randomly selected South Indian children with varying weight status showed an overall prevalence of prediabetes or diabetes of 3.7%, with the highest prevalence of 12.7% in girls with abdominal obesity. [22] A recent study in 5-15-year-old randomly selected Sri Lankan children from the Colombo district found that insulin resistance among Sri Lankan children was high in all groups of weight status, even if many children were able to control glucose levels within normal limits. [41] Even if these studies suggest a relatively high prevalence of glucose intolerance in South Indian and Sri Lankan children with obesity, only one of the mentioned studies was conducted in Sri Lankan children, and the data has not been compared to data from children with obesity of other ethnic origins.

Glucose intolerance in Swedish and Austrian children and adolescents with obesity

A high prevalence of glucose intolerance with over 50% in children and adolescents with obesity has been reported from Sweden, [33] and another study found that the prevalence of IFG was higher in Swedish children with obesity

compared with German and Austrian children with obesity. [29] These findings have led to concerns that the risk of developing type 2 diabetes in Swedish children and adolescents with obesity might be particularly high and result in a high prevalence of type 2 diabetes in future adult generations in Sweden.

As the prevalence of type 2 diabetes in adults is comparable between Austria with 6% and Sweden with 6.9%, [42] it is unclear whether the high prevalence of glucose intolerance found in Swedish children and adolescents will track into adulthood and eventually lead to an increasing prevalence of type 2 diabetes even in Swedish adults in future generations, or if glucose tolerance normalizes during adolescence or early adulthood, as described in a longitudinal multicentre analysis of children with obesity from a German registry. [43] A comparison of a Swedish adult cohort with an Austrian adult cohort showed higher mean levels of fasting glucose in Swedish adults at the age of 30-60 years, indicating a possible difference in the prevalence of type 2 diabetes and its early manifestations even in the adult population. [44]

Not much is known about the causes leading to the observed differences in the prevalence of glucose intolerance between children with obesity in Sweden and other countries, and their implications for the future development of diabetes prevalence in Sweden need further investigation and discussion.

The role of inflammation for the risk of developing glucose intolerance

Besides other well-known risk factors, the role of chronic, low-grade inflammation in white adipose tissue has gained emerging interest in research investigating the pathophysiology of obesity and type 2 diabetes. [45] In obesity, the balance between different types of immune cells is disrupted. Increases are seen in neutrophils, macrophages, mast cells, and cluster of differentiation 8+ T-cells, while eosinophils, T helper 2-cells, and regulatory T-cells decrease. [46] These changes contribute to a pro-inflammatory environment, which has been argued to play a significant role in the development of insulin resistance and consecutive hyperinsulinemia. [47,48] Elevated levels of inflammatory cytokines originating from macrophages such as interleukin 6 (Il-6) and tumor necrosis factor alpha (TNF-alfa), or inducing macrophage activity such as C-reactive protein (CRP) and interferon gamma (IFN-gamma), have been shown to be associated with an increased risk of glucose intolerance and type 2 diabetes. [49–52] The role of mast cell activity in the pathogenesis of type 2 diabetes has been less investigated. Mast cells have been shown to be increased in the islets of Langerhans in adult patients with diabetes, [53,54] and studies in mice [55,56] and adult human populations [57] indicate a higher abundance of mast cells in the

visceral adipose tissue in obesity. Mast cell activity can be estimated measuring levels of inflammatory mediators that are released from mast cells upon the exposure of a triggering factor. These mediators include tryptase and histamine. [58,59] Elevated levels of tryptase have been shown to be associated in adults with higher BMI, diabetes, and age, suggesting a possible link between mast cell degranulation and metabolic disturbances. [60–62] These associations could not be confirmed in a paediatric study that compared tryptase levels between children with healthy weight, overweight and obesity, where serum tryptase levels were not increased in children with overweight or obesity. [63] Histamine, a central inflammatory mediator released by mast cells, plays a complex role in metabolic regulation. Histamine is metabolized by two key enzymes: diamine oxidase (DAO) and histamine-N-methyltransferase (HNMT). DAO is primarily expressed in the gastrointestinal tract and regulates histamine from dietary sources, while HNMT is more broadly expressed in tissues and manages systemic histamine levels. [64–66] HNMT expression is often upregulated in response to high histamine levels, a mechanism observed in inflammatory conditions such as allergic asthma, where increased histamine turnover leads to elevated HNMT levels. [67] This adaptive upregulation suggests that HNMT acts as a protective mechanism against prolonged histamine-induced inflammation.

In summary, studies in human adults and animal models have indicated possible correlations between mast cell activity, obesity and type 2 diabetes. However, the role of mast cell activation and its impact on glucose metabolism in children and adolescents remains largely unexplored.

Pharmacological treatment of childhood obesity and glucose intolerance

Much effort is being put into finding ways to counteract the discussed rise in childhood obesity and its complications including type 2 diabetes and glucose intolerance. Lifestyle modification interventions, including behavioral treatment, diet modification and physical activity, are the cornerstones of treatment of pediatric obesity. [68] However, such interventions have shown relatively limited success among children and adolescents with severe obesity in either reduction of body weight or improvement of metabolic outcomes.[68–70] As a result, there is considerable interest in combining lifestyle modification with more intensive strategies, including pharmacotherapy, to ameliorate pediatric obesity.[71] The introduction of glucagon-like peptide-1 receptor agonists (GLP-1RA) in obesity management has been paradigm shifting, and GLP-1RA are increasingly prescribed. [72] Exenatide was the first approved GLP-

1RA and has subsequently been investigated for the treatment of obesity both in adults [73,74] and in adolescents. [75–77] However, no long term safety data for the use of GLP-1RA for pediatric obesity is available, and weight regain is to be expected following discontinuation of treatment. [78] Moreover, the high costs of GLP-1-RA lead to restricted availability in many low- and middle-income countries and to health inequities between socioeconomic groups, as long as funding through national health economic budgets is not sorted out.

In comparison, metformin is a well evaluated, safe, easily accessible, and cheap alternative for the treatment of type 2 diabetes and obesity. Metformin has been described to have many different beneficial effects such as reducing appetite and insulin resistance, and has therefore an established role in managing hyperinsulinemia and insulin resistance. [79] The antihyperglycemic action of metformin has been ascribed to reducing hepatic glucose output and improving insulin sensitivity, and to some extent also to direct effects on the insulin-producing cells. [80] However, metformin has many other effects such as increasing satiety [81,82] and influencing gastrointestinal flora. [83,84] There is evidence for the beneficial effect of metformin in the treatment of childhood obesity, [85–88] but one limitation of existing studies is that differences in body weight usually have not been taken into account. Pastor-Villaescusa et al. found that metformin had better treatment effects in prepubertal children than in pubertal children. [85] However, their study concluded that further investigations of the observed differences are necessary, as all children received the same dose of metformin irrespective of their age and body weight, which resulted in a significantly higher dose per kg body weight for the prepubertal children, potentially leading to higher plasma concentrations.

The Metformin Intervention in children and adolescents with obesity (MINT) study was a parallel, randomized, 6 months multi-center study that investigated metformin as a treatment option for childhood obesity. In this study, study subjects were allocated to three different weight groups, receiving three different dosages of metformin. The aim of this grouping was to get as equal dosage per kg body weight as possible, and thereby as equal plasma concentration of metformin as possible for all included subjects. Unlike in other studies, this was monitored by measurements of plasma concentrations of metformin at certain time-points during the treatment period.

Another aspect distinguishing the MINT study from other pediatric metformin studies was its objective to investigate the treatment effect, safety, and tolerability of both metformin in an extended release (XR) form and metformin immediate release (IR) in children and adolescents with obesity. Studies with metformin in pediatric obesity generally report that metformin is well tolerated, with diarrhea being the most common adverse event in both

metformin and placebo groups, and no significant differences in any safety parameters between metformin and placebo groups. [86,87] However, one study in adults with type 2 diabetes showed that metformin XR had better effect and less gastrointestinal side effects than the widely used metformin IR. [89] Metformin XR has even been investigated in adolescents from 13 years of age, [90] but no studies with metformin XR have been conducted in younger children with obesity yet.

Aims

The aims of the reported studies were:

I. To investigate the prevalence of different states of glucose intolerance in Sri Lankan children and adolescents with obesity and its relation to other metabolic and anthropometric parameters. Furthermore, to compare body composition and prevalence of obesity comorbidities among Sri Lankan and Swedish children with the same degree of obesity.

II. To investigate the prevalence of glucose intolerance in children and adolescents from two childhood obesity cohorts in Uppsala, Sweden and Salzburg, Austria. Moreover, to compare independent risk factors associated with glucose intolerance at baseline between the Swedish and Austrian cohorts. In addition, to investigate the longitudinal course of glucose intolerance and identify risk factors associated with glucose intolerance at follow-up visits.

III. To evaluate potential differences in biomarkers associated with mast cell activity, in essence DAO, HNMT, and tryptase, between children and adolescents with obesity and normal weight controls. Additionally, to investigate possible associations between these markers and indicators of glucose metabolism in children and adolescents with obesity.

IV. The primary objective of the MINT study was to investigate whether metformin XR in combination with lifestyle intervention reduces obesity more than lifestyle intervention alone. Secondary objectives were to investigate whether metformin IR in combination with lifestyle intervention reduces obesity more than lifestyle intervention alone, and to compare the effect of treatment on obesity comorbidities such as glucose intolerance and other cardiovascular risk factors between the treatment groups. Moreover, the study investigated the effect of age and metformin concentration on the treatment.

Materials and Methods

Study Populations and Settings

ULSCO (Paper I-III)

All Swedish subjects included in the presented studies were recruited from the Uppsala Longitudinal Study of Childhood Obesity (ULSCO) cohort. [91] The ULSCO cohort includes children and adolescents aged 4-18 years with obesity, as well as normal weight controls. Inclusion criteria for patients with obesity is defined as body mass index standard deviation score (BMI-SDS) ≥ 2.0. Normal weight controls with BMI-SDS between -2 and +1 have been enrolled through collaboration with local schools as well as through advertisement. The ULSCO cohort was founded in 2012 and since then over 400 patients with obesity and 70 normal weight controls have been included.

Sri Lanka (Paper I)

In a cross-sectional survey carried out in Sri Lankan schools in the city of Negombo, all children identified as having obesity (BMI-SDS ≥ 2.0 according to WHO criteria, 2007) were invited to the Diabetes Screening and Vocational Training Centre of the Lions Club of Negombo Host for further examination and possible treatment. [92] Out of 13 688 children participating in the school screening, 667 (4.8%) were identified as having obesity at screening, and the 500 that were 7 years or older were invited to the Diabetes Screening Centre. In the analysis, 357 children with complete OGTT and fulfilling the WHO criteria for obesity (BMI-SDS ≥ 2.0) were included (**Paper I, Figure 1**). Children with chronic diseases, secondary causes for obesity or on long term medication were excluded according to judgement of the clinical examiner. The original school screening was conducted between July 2013 and March 2014 in eight schools in the Negombo educational zone of the Western Province of Sri Lanka. The baseline assessments of children with obesity at the Diabetes Screening and Vocational Training Centre of the Lions Club of Negombo Host were carried out between July and September 2014. For comparison of amount and distribution of body fat as well as metabolic and lifestyle parameters, Sri Lankan subjects were matched for sex and BMI-SDS (to the first

decimal) as well as for approximate age (± one year) with Swedish subjects from the ULSCO cohort. The matching procedure resulted in a study population of 167 (95 boys) Sri Lankan and 167 (95 boys) Swedish subjects. Although 45% of the ULSCO subjects included for comparison had at least one parent born in another country than Sweden, only 1.75% (n=7 subjects) had a parent with South Asian origin, none of them Sri Lankan. In the Sri Lankan study population, subjects of other than Sri Lankan origin, or who had not been living in Sri Lanka during the last five years, were excluded. Different ethnic groups within the Sri Lankan population (Singhalese, Tamils, Burghers/Eurasian, Moors/Muslims) were represented in the study population.

Beta-JUDO (Paper II)

All analyses presented in paper II are based on the Beta-cell function in Juvenile Diabetes and Obesity cohort (Beta-JUDO cohort, FP7-HEALTH-2011-two-stage, project number: 279153), a dual-centre study that was carried out at Uppsala University Children`s Hospital in Uppsala, Sweden and Paracelsus Medical University Hospital in Salzburg, Austria. The Beta-JUDO cohort includes children and adolescents aged 4-18 years with overweight and obesity (BMI-SDS ≥ 1.0) as well as normal weight control subjects (BMI-SDS < 1.0) [93]. All subjects at both study sites were screened for participation in the study at clinical baseline visits. However, all Swedish participants in the Beta-JUDO cohort are also part of the ULSCO cohort, where patients with obesity are referred from other caregivers and thereby likely to have received previous examination and treatment for obesity, whereas subjects with overweight or obesity in Salzburg are referred without previous examination or treatment. Normal weight control subjects were recruited through advertising in local media and in cooperation with local schools. Inclusion criteria were signed informed consent and ability to comply with the oral glucose tolerance test (OGTT) protocols. Exclusion criteria at baseline were syndromic obesity, total or partial gastric or small intestine resection, diabetes mellitus type 1, hypothyroidism or hyperthyroidism unless under stable treatment, malignancies, other severe chronic disease as judged by the clinical examinator, or concomitant medication influencing weight status or blood glucose. After applying all inclusion and exclusion criteria, the cross-sectional study cohort consisted of 732 subjects (**Paper II, Figure 1**). For the longitudinal part of the study, all subjects who had completed at least two OGTTs were included in a subgroup analysis. Patients receiving standard lifestyle treatment during the follow-up period were included, whereas patients receiving pharmaceutical treatment for obesity during the follow-up period were excluded from the analysis. After applying all inclusion and exclusion criteria, the longitudinal study cohort

consisted of 188 subjects (**Paper II, Figure 1**). Baseline visits were conducted between 2012 and 2019 and follow-up visits between 2012 and 2021. All examinations were harmonized between study centres.

Sub-cohort recruited from the ULSCO cohort (Paper III)

For the study presented in paper III, inclusion criteria for all subjects were signed informed consent and registered measurements of markers associated with mast cell activity, as well as complete data from an OGTT. After applying all inclusion criteria, the final study cohort consisted of 184 subjects, of which 154 were patients with obesity and 30 were normal weight controls (**Paper III, Figure 1**).

MINT (Paper IV)

The Metformin Intervention in children and adolescents with obesity (MINT) study was a parallel, three-armed, randomized, 6 months multi-center study conducted at Uppsala University Children's Hospital as the principal and coordinating investigator site, and Falun Regional Hospital as the second site.

The study population included children and adolescents between 6 years and 17 years and 3 months old (at date of screening) with obesity according to WHO criteria (BMI-SDS >2.0),[94] who were referred as patients to the pediatric obesity clinics at the different study sites. Inclusion criteria were signed informed consent, body weight ≥ 40 kg, obesity (BMI-SDS >2.0) according to WHO, stable body weight during the last 90 days before the screening visit (less than 5kg measured or self-reported weight change), and for females of childbearing potential not being sexually active or the usage of adequate anticonception and having negative pregnancy tests. Exclusion criteria were: syndromic obesity, pregnancy or lactation, severe gastrointestinal disease, total or partial gastric or small intestine resection, type 1 diabetes mellitus, acute or chronic kidney disease or renal dysfunction defined as glomerular filtration rate (GFR) <60ml/min/1.73m^2, hypo- or hyperthyroidism (unless under stable treatment), severe depression, severe anxiety or other psychiatric disorder referred to or undergoing special treatment, severe sleep apnea, or other chronic disease, all as judged by the investigator, metformin treatment within 3 months prior to screening or concomitant medication influencing blood glucose, other parameters of the metabolic syndrome or interfering with the investigational medicinal product, steroid treatment, antidepressants that can lead to weight gain, unstable treatment and/or treatment started within 3 months prior to screening visit for neuropsychiatric disorders, known hypersensitivity to metformin or any of the excipients, language difficulties,

impaired mental ability or not being willing to understand or comply with the study procedures, participation in another clinical study involving an investigational medicinal product within three months prior to screening, and another subject from the same household participating in this trial.

The study was conducted between September 2021 and May 2024. The total duration of participation in the study was approximately 29 weeks for each participant, starting with a screening period of up to 3 weeks and followed by a treatment period of 24 weeks plus a follow up period of 2 weeks. After completing the treatment and follow up period, participants were asked for participation in a follow-up visit after one additional year (18 months from baseline) and informed consent was obtained for this additional visit (data not included). The study consisted of 11 visits, of which six were conducted on site and five via phone calls (**Paper IV, Figure 1**). After the initial screening period, eligible participants were randomized 1:1:1 to treatment with either an extended-release metformin formulation (Glucophage® SR) combined with lifestyle intervention (XR+L), an immediate-release metformin formulation (Glucophage®) combined with lifestyle intervention (IR+L), or lifestyle alone (LA), based on a centralized computer-generated randomization scheme (permuted blocks, stratified by site, with block size unknown to the researchers).

Baseline assessments and anthropometry

Sri Lanka (Paper I)

Baseline assessments at the Diabetes Screening and Vocational Training Centre of the Lions Club of Negombo Host were conducted after an overnight fast. Height, weight,[95] waist circumference (WC)[96] and blood pressure were measured by trained research assistants using a standardized protocol. To limit the impact of inter-operator-variability, the same six assistants who had undergone the same training conducted all the measurements. Height (cm) was assessed using a standardized, calibrated stadiometer and weight (kg) was assessed using a standardized, calibrated scale with the patient wearing light clothing and no shoes. WC (cm) was measured with a flexible tape midway between the superior border of the iliac crest and the lowest rib on the standing patient. BMI was calculated as weight in kilograms divided by the square of height in meters. BMI-SDS was calculated with Microsoft Excel add-in LMS Growth version 2.76 using WHO 2007 growth reference. The same software was used to calculate SDS for both systolic and diastolic blood pressure. Waist to height ratio (WHtR) was calculated by dividing WC(cm)/Height(cm). Pubertal staging was assessed using visual charts,[97] and wherever subject and parents were not certain of the staging, with consent, the examiner assessed

the correct pubertal stage. In boys, testis size was assessed by the examiner using a Prader orchidometer.[98]

ULSCO (Paper I-III), Beta-JUDO (Paper II) and MINT (Paper IV)

All measurements were performed according to established standard operating procedures. Weight (kg) was measured to the nearest 0.1 kg with the patient wearing light clothing and no shoes, by using a standardized, calibrated scale (Uppsala: SECA model 704; Salzburg: SECA model 801, Hamburg, Germany), and 0.5 kg was subtracted from the value to account for clothing. Height (cm) was measured twice using a stadiometer, and the mean result to the nearest 0.1 cm was recorded (Uppsala: Ulmer stadiometer, Busse, Elchingen, Germany; Salzburg: SECA, model 222 stadiometer, Hamburg, Germany). BMI was calculated as weight in kilograms divided by the square of height in meters. BMI-SDS was calculated according to WHO Growth Reference Charts. [94] WC in cm was measured with a flexible tape midway between the superior border of the iliac crest and the lowest rib on the standing patient (rounded to the nearest 0,5 cm), and hip circumference (HC) in cm was measured at the greatest circumference of the hip (rounded to the nearest 0,5 cm). Waist to hip ratio (WHR) was calculated by dividing WC/HC and WHtR was calculated by dividing WC/height. Sagittal abdominal diameter (SAD) was measured by an abdominal caliper including a ruler, to the nearest 0.1 cm with the subject lying flat on a hard surface with knees bent and feet down. Measurement was done using a ruler at the level of iliac crest (L4-5) from the highest point to the surface below after a normal expiration. Area of measurement is without clothes, allowing the measurement arm to touch the abdomen slightly but without compression. Systolic and diastolic blood pressure (mmHg) were assessed by the mean of two measurements with a standardized clinical aneroid sphygmomanometer with appropriate cuffs (Uppsala: CAS 740, CAS Medical Systems, Inc, Branford, CT, USA; Salzburg: Carescape V100, Dinamap Technology/GE, Vienna, Austria) in sitting position, right arm after 5 minutes of quiet rest. The heart rate was measured simultaneously with the same equipment. The Microsoft Excel add-in LMS Growth version 2.76 using WHO 2007 growth reference was used to calculate standard deviation score (SDS) for both systolic and diastolic blood pressure. Physical examination included assessment of general condition, skin (including striae, acanthosis nigricans, hairiness), lymph nodes, thyroid gland, mouth and throat, heart, lungs, abdomen, neurology, and pubertal development according to Tanner stage.

Body Composition

Body composition and body fat mass (FM) were assessed by bioelectrical impedance assay (BIA) on a fasting subject who was instructed to empty the bladder before the examination. In Sri Lanka, a platform-type, eight electrode In-Body 230® instrument (InBody® Biospace, South Korea) which has been validated against locally developed BIA prediction equations was used. [99] In Uppsala, analysis was conducted using a Tanita MC980 bioimpedance device, and in Salzburg a Tanita BC415 bioimpedance device was used. Where no data from a Tanita bioimpedance device was available, data was obtained from some Uppsala subjects using an InBody S20 bioimpedance device (Biospace, Seoul, Korea). Percentage of body fat mass (% FM) was expressed as a fraction of total body weight.

Questionnaires

In Sri Lanka (paper I), the subjects and their parents were asked to complete a questionnaire about their medical history, socioeconomic background, and family situation during baseline assessments at the Diabetes Screening and Vocational Training Centre of the Lions Club of Negombo Host. One of the questions estimated the parents` educational level using a scale from 1 (did not attend school) to 8 (post graduate training), where the options 1-4 were considered as lower educational level ("did not attend school" up to "Grade 6-10") and the options 5-8 as higher educational level ("O-level=more than 10 years of school" up to "post graduate training"). Data regarding medical family history, physical activity and nutritional habits were obtained from another questionnaire that was completed at the original school screening.

Within the ULSCO/Beta-JUDO cohort (Paper I-III), all subjects and legal guardians were at baseline visits asked to answer questionnaires regarding medical history, family history, nutrition, physical activity, sleeping habits, screentime, well-being, stress, relationships, and socioeconomic information such as parents' educational level, smoking habits, diseases and BMI. [91] For Paper II, information from the questionnaires was compared between the groups (**Paper II, Table 1**) and used as independent variables in univariate and multivariate regression models (**Paper II, Table 2**). For Paper III, information about subjects' medical history regarding asthma diagnosis was obtained from medical records in addition to the questionnaires.

For the MINT study (Paper IV), growth charts including height and weight to current date from birth or as far back as possible were collected to evaluate the duration of obesity. Lifestyle-related questionnaires were answered by subjects and, if applicable, their caregivers, and consisted of a food frequency

questionnaire, a questionnaire addressing the regularity of meals during the day, a self-measured picture tick-box questionnaire estimating portion size, a questionnaire regarding physical activity and sedentary activities, bedtime and sleep duration weekdays and weekend, and the Pediatric Quality of Life Inventory TM 4.0. Generic Core Scales (PedsQLTM) questionnaire for the assessment of quality of life.

Blood Sampling

All examination procedures, including sample collection and storage, were conducted according to established standard operating procedures. Blood was sampled after a 10 hour overnight fast using an intravenous stationary catheter and analysed locally at the respective centre. Samples were analysed for total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), creatinine, cystatin C/GFR, highly sensitive C-reactive protein (hs-CRP), insulin, proinsulin, haemoglobin A1c (HbA1c), albumin and 25OH-vitamin D. For the MINT study (Paper IV), lactate dehydrogenase (LD), bilirubin, and urine sample Protein HC were analyzed in addition. In Sri Lanka (Paper I), serum was separated immediately and stored at -20°C until analysis was conducted in batches at the biochemical laboratory of the same centre. Within the beta-JUDO project (Paper II), validation of analyses was performed between the laboratories in Uppsala and Salzburg using reference blood samples. In Uppsala, glucose was analysed using an Architect c8000 instrument (Abbott Diagnostics, Solna, Sweden) and in Salzburg using a Glucoquant Glucose-Kit (Roche Diagnostics, Mannheim, Germany). For Paper III, markers associated with mast cell activity were analysed non fasting (at 90 minutes during OGTT, due to practical reasons) as serum tryptase (in μg/L), DAO (in U/ml) and HNMT (in NPX=Normalized Protein Expression according to Olink). [100] Specific immunoglobulin E (IgE) was defined as the sum of Phadiatop and fx5 (all in kU_A/L). Analysis of tryptase, Phadiatop and fx5 was conducted with the immunofluorometric assay ImmunoCAP (Thermo Fisher Diagnostics AB, Uppsala, Sweden). The enzymatic activity of DAO was analysed with DAO REA (ALPCO, Salem, NH), and HNMT, IL-6, TNF-alfa and IFN-gamma were analysed by a proximity extension multiplex immunoassay (Target 96 Inflammation and Target 96 Immune Response; Olink Proteomics, Uppsala, Sweden). [101,102]

OGTT

Oral glucose tolerance test (OGTT) was performed as previously described after an overnight fasting period of at least 10 hours. [91] In short, subjects were instructed to drink a solution containing 1.75 g glucose per kilogram body weight up to a maximum of 75 g glucose within approximately five minutes. Blood was drawn at fasting and repeatedly for up to 180 minutes (at -15 and -5 minutes before dose and 30, 60, 90, 120 and 180 minutes after dose intake) for measurements of plasma glucose and serum insulin. In the Sri Lankan study population (Paper I), samples were taken at fasting and 120 minutes only. The subjects were advised to avoid dramatic change in dietary habits and physical exercise habits at least 3 days prior to OGTT. In addition, subjects were informed that they should not take any medications in the morning of blood sampling, unless necessary. During the OGTT, the subjects were forbidden to eat or smoke and had to remain sedentary throughout the procedure.

Validation of glucose tolerance and metabolic derangements

Impaired fasting glucose (IFG) was defined as fasting glucose (FG) 5.6-6.9 mmol/l (100-125 mg/dl) according to American Diabetes Association (ADA) criteria.[103] Impaired glucose tolerance (IGT) was defined as 2 hours glucose (2h-G) between 7.8-11.0 mmol/l (140-199 mg/dl). Type 2 diabetes was defined as FG ≥ 7.0 mmol/l (126 mg/dl) and/or 2h-G ≥ 11.1 mmol/l (200 mg/dl). Based on levels of FG and 2h-G, subjects were divided into five groups, representing states of glucose intolerance, as having normal glucose tolerance (NGT), isolated impaired fasting glucose (iso-IFG), isolated impaired glucose tolerance (iso-IGT), combined IFG+IGT (comb IFG+IGT) or type 2 diabetes. Prediabetes was defined as the sum of iso-IFG, iso-IGT and comb IFG +IGT, whereas the term glucose intolerance was used for the sum of all forms of prediabetes plus type 2 diabetes. To assess insulin resistance, HOMA-IR was calculated as (FG x FI)/22.5 (FG in mmol/L, FI in μIU/ml), [104] and HOMA-IR >2.5 was used as cut-off value.[105,106]

Elevated blood pressure was defined as ≥ 2 SDS for both systolic and diastolic blood pressure. [107] According to local reference values, other metabolic derangements for the Sri Lankan cohort (paper I) were identified as: FI ≥ 12 μIU/ml,[108] TC ≥ 200 mg/dl (≥ 5.17 mmol/L), LDL ≥ 130 mg/dl (≥ 3.36 mmol/L), HDL < 40 mg/dl (< 1.03 mmol/L), TG ≥ 150 mg/dl (≥ 1.69 mmol/L),[109] AST > 40 IU/L, ALT > 40 IU/L,[110] hs-CRP > 1 mg/dl. [111] For the ULSCO cohort (Paper II), other metabolic derangements were identified as:

FI > 25 µIU/ml (173.6 pmol/l), [112] LDL > 2.6 mmol/l (girls 0-8 years) or > 2.8 mmol/l (boys 0-8 years) or > 5.0 mmol/l (girls 9-17 years) or > 3.6 mmol/l (boys 9-17 years), [113] TG > 2.7 mmol/l, [113] ALT > 0.29 µkat/l (boys 0-8 years) or > 0.39 µkat/l (girls 0-8 years) or > 0.51 µkat/l (girls and boys 9-17 years), [114] albumin < 38 g/l (0-11 years) or < 41 g/l (12-17 years), [114] (25OH)Vitamin D < 50 nmol/l. [115]

For comparison of glucose values at the two Beta-JUDO sites (Paper II), a correction factor generated from a study that investigated comparability of glucose values in the Beta-JUDO cohort was applied. [116] According to this study, original Salzburg values measured at the Salzburg site were corrected using the following calculation (all glucose values in mmol/l): (Salzburg value corrected to Uppsala value) = [1.0153*(original Salzburg value)] + 0.2489. For comparison of insulin values from both sites, local insulin measurements were re-analysed in the smaller subgroup of patients who had centrally analysed insulin measurements available. Central analysis was conducted from frozen plasma samples from both sites at the department of medical cell biology at Uppsala University, Sweden.

Pharmacokinetic parameters of metformin (Paper IV)

For participants randomized to treatment with either metformin XR or metformin IR within the MINT study (Paper IV), blood samples were collected before the morning administration of metformin at visit 7 and 10 in fasting condition to determine plasma metformin concentrations. In connection to the OGTT at visit 10, additional blood samples were collected at 60, 120 and 180 minutes after glucose solution intake and metformin dosing. Furthermore, metformin urine concentrations were measured at visit 10 at the same post-dose time points as the blood samples were collected (i.e., 60, 120 and 180 minutes after glucose intake). Participants were instructed to empty their bladder at the start of the OGTT. Metformin concentrations in plasma and urine were analyzed with an ultra-performance liquid chromatography tandem mass spectrometric (UPLC-MS/MS) method performed on ACQUITYT UPLCTM coupled to a XevoTM TQ-XS Tandem Quadrupole Mass Spectrometer system (Waters Corporation, Milford, MA, USA) by ArcticMass Ltd., Reykjavik, Iceland. Pharmacokinetic parameters, i.e., clearance (CL) and area under the metformin concentration-time curve (AUC_m) were determined based on the plasma and urine metformin concentrations, assuming that metformin is fully renally eliminated.

Liver ultrasound (Paper I)

In the Sri Lankan cohort (paper I), ultrasound scan of the abdomen was conducted by an experienced radiologist using a Siemens Acuson X300, to detect and grade different stages of MAFLD. Results were reported as normal echogenicity or hepatic steatosis categorized from grade 1-3.[117]

Treatment within the MINT study (Paper IV)

Pharmacological treatment

Subjects who were randomized to a treatment arm including pharmacological treatment with either metformin XR + lifestyle or metformin IR + lifestyle were instructed to take the metformin tablets orally together with a meal and that the tablets should not be crushed or chewed. To minimize differences in dosage per kg body weight that might imitate differences in effects of treatment between prepubertal and pubertal children, the participants in this study were divided into three different dosage groups according to their body weight: children with a body weight of 40-60 kg escalated their dose up to 500 mg twice daily, children with a body weight of over 60 kg up to 80 kg escalated their dose up to 1000 mg twice daily and children with a body weight of over 80 kg escalated their dose up to 1500 mg twice daily. The treatment period started with a three-week dose escalation period during which the subjects gradually increased the dose of metformin weekly to improve gastrointestinal tolerability, until maximum tolerated dose or planned target dose was reached. A maintenance period of 21 weeks followed until the treatment period was completed at 24 weeks. The medication was handed out and drug accountability was performed at each site visit.

Lifestyle Intervention

All three study groups received lifestyle intervention during the treatment period. The lifestyle intervention was conducted by study personnel such as dietitian, psychologist, study nurse, counsellor, or physician, and consisted of counselling in healthy lifestyle, focusing on the three key areas: nutrition, physical activity and sleep. The individual dietary advice was based on the current Nordic Nutrition Recommendations. Counselling also covered media consumption and quality of life. The overall intervention was characterized by a salutogenic approach to lifestyle changes, meaning that health is in focus, rather than illness is avoided. The intervention was individualized,

continuously evaluated, and adapted to the participants' individual needs during the study.

Safety and tolerability outcomes within the MINT study (Paper IV)

Safety and tolerability outcomes included vital signs (blood pressure and heart rate), the change in physical examination from baseline to follow-up and clinical laboratory evaluations (FG, ASAT, ALAT, GGT, LD, bilirubin, creatinine, cystatin C/GFR, sodium (Na), potassium (K), albumin, lactate, hematology, hs-CRP and urine Protein HC). Urine pregnancy tests were performed at screening and at visit 2, 7, 9, 10 and 11 for female subjects with childbearing potential. Furthermore, the number of treatment-emergent adverse events (AEs) was registered, and AEs were classified into standardized medical terminology from the Investigator term using the Medical Dictionary for Regulatory Activities and presented by preferred term nested within System Organ Class. AEs were summarized by presenting the incidence for each treatment group.

Ethical clearances

All protocols and examinations performed on subjects within the ULSCO and Beta-JUDO cohort studies were approved by the Uppsala Regional Ethics Committee (registration numbers 2010/036 and 2012/318) and the Salzburg Ethics Committee (EK1544/2012). Informed and written consent was obtained from legal guardians, and for subjects ≥12 years of age, written consent was also obtained from the subjects themselves. Participation in the cohorts is voluntary, and consent can be withdrawn at any time by subjects and legal guardians without having to state a reason. [91]

Ethics clearance for the screening of Sri Lankan school children`s nutritional status in Negombo (Paper II) was obtained from the Ethical Review Committee of the Sri Lanka College of Paediatricians. Ethical approval for the following metabolic screening of children with obesity was obtained from the Ethics Review Committee of Faculty of Medicine, University of Colombo (EC-13-143). Only subjects with informed and written consent were included in the study. [118]

The MINT study (Paper IV) was approved by the Swedish Ethical Review Authority (Etikprövningsmyndigheten, Registration No.: 2019-05910, EudraCT No: 2019-003940-61). The study was performed, and essential study

documentation archived in compliance with the EU Clinical Trials Directive 2001/20/EC and in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization (ICH, Appendix 19.6), Good Clinical Practice and applicable regulatory requirements. All participants and their caregivers received full and adequate oral and written information about the nature, purpose, possible risks, and benefits of the study, including the information that participation in the study was voluntary and that participants could withdraw their consent at any time. Age adapted informed consent was provided to the children and adolescents. For adolescents from 15 years old, the consent was signed by both the adolescents themselves and their caregivers. No study-specific procedures were performed before the informed consent had been signed and dated by the subject, the subject's caregivers, and the investigator.

Statistical Analysis

Paper I-II

Statistical analysis was performed using the software IBM SPSS statistics version 25. Continuous variables are presented as mean values with standard deviation for normally distributed variables and median and interquartile range for non-normally distributed variables. For comparison of two sample means, Student`s independent t-test was used when test criteria for parametric testing was fulfilled, otherwise the non-parametric Independent-Samples Mann-Whitney-U-Test was performed. For comparison of mean values between more than two groups, one-way analysis of variance (ANOVA) with post-hoc analysis was used when test criteria for parametric testing was fulfilled, otherwise the non-parametric Kruskal-Wallis test was performed. In paper II, differences between groups were calculated on log-transformed data where the criteria of normality were not fulfilled. In descriptive tables, all data are presented as non-transformed values. Correlations between parameters were calculated with Pearson bivariate correlation analysis and correlation coefficient along with the p-value is presented. Univariate logistic regression was used in Paper I to study relation between the dependent variable (IGT/type 2 diabetes) and independent variables. A multivariate logistic regression model was then used to calculate the Odds ratios of different covariates regarding to the risk of having IGT/type 2 diabetes or IFG (Paper I). In Paper II, univariate logistic regression was used for the cross-sectional part of the study to investigate the relation between the dependent variable IGT and all variables presented in **Table 1** (Paper II) as independent variables. Only significant variables were included in a backward stepwise multivariate logistic regression model. Level

of significance for elimination was 0.05. From the logistic regression models, odds ratios and 95% confidence intervals were calculated. In the cross-sectional part of the study, all multivariate logistic regression models were adjusted for age, sex, BMI-SDS, ethnic origin in another country than Austria or Sweden and family history of diabetes, and analysis was performed for each site separately. For the longitudinal part of the study, univariate and multivariate logistic regression models were performed as described above with the dependent variable impaired glucose metabolism/prediabetes (IFG, IGT or both) at the follow-up visit and all variables presented in **Table 1** (Paper II) at baseline as well as sex, ethnic origin in another country than Austria or Sweden, family history of diabetes, change in BMI-SDS during the follow-up period and duration of the follow-up period as independent variables. Multivariate logistic regression models within the longitudinal part of the study were adjusted for duration of the follow-up period. To evaluate the relative strength of association of different covariates with impaired glucose metabolism at the follow-up visit, the Wald x2 minus the predictor degrees of freedom was plotted (Paper II, **Figure 4**). This statistic allows comparison of predictors in the same model with different number of parameters, with a higher number indicating a stronger association. Analysis was based on available data only; no imputation of missing data was performed. P values <0.05 were considered statistically significant

Paper III

The Kolmogorov-Smirnov test and Shapiro-Wilk test as well as visual analysis of histograms were used to investigate whether variables were normally distributed or skewed. For skewed variables (specific IgE, DAO, FI, 2h-Ins and fasting proinsulin), the regression analyses were performed on log10-transformed data. However, for the sake of interpretation, original values are presented for these variables in descriptive tables (Paper III, **Table 1**). Analysis of HNMT, Il-6, TNF-alfa and IFN-gamma was performed on log2-transformed data according to the Olink NPX scale. [119] As most of the variables were skewed, the non-parametric Mann-Whitney-U test was used for comparison of medians between the patient group and the control group (Paper III, **Table 1**). Sex distribution between the groups was compared using Chi-square test. Bivariate correlations between markers associated with mast cell activity were analyzed using Spearman`s rank correlation test. Simple linear regression models in the patient group were conducted with each marker of glucose metabolism as dependent variable and all markers associated with mast cell activity as independent variables (Paper III, **Table 2**). All models were then adjusted for age and sex in multiple linear regression model 1 and for age, sex

and BMI-SDS in model 2. Phadiatop and fx5 were summarized as specific IgE and included as an independent variable in addition to age, sex and BMI-SDS in multiple linear regression model 3, in order to adjust for adaptive activation of mast cells due to allergic sensitization. Furthermore, hs-CRP, IL-6, TNF-alfa, and IFN-gamma were included in separate models as independent variable in addition to the variables included in model 3, to adjust for inflammation mechanisms related to macrophage activity (Paper III, **Table 3**). Analysis was based on available data only; no imputation of missing data was performed. P values <0.05 were considered statistically significant. All regression models were corrected for multiple testing according to the Bonferroni method. Level of significance was thereby set to 0.017 by dividing 0.05 with the number of mast cell markers. All statistical analyses were performed using the software IBM SPSS Statistics 29.

Paper IV

Demography and baseline characteristics are presented in terms of descriptive statistics by treatment group. Continuous variables are presented with sample size, mean, standard deviation, median, min and max. Categorical variables are presented as frequency and percentages. The primary and secondary objectives analyses were performed on the Full-Analysis-Set (FAS), which consisted of all participants who were randomized and had received at least one dose of study medication (for participants randomized to metformin treatment) and provided at least one efficacy baseline assessment and at least one corresponding post-baseline efficacy assessment. The Per-Protocol Set (PPS) consisted of all FAS participants who completed the full 6 months of treatment with no major protocol violations or deviations. Safety evaluation was performed on the Safety Analysis Set (SAS), which consisted of all randomized participants who received at least one dose of study medication. If participants had missing data for the primary endpoint, these data points were imputed using the worst observation carried forward method. The analysis of the primary and secondary efficacy endpoints was performed using Analysis of Covariance (ANCOVA) models. The ANCOVA models for the primary efficacy endpoint and for the difference between the treatments included treatment as a fixed effect and baseline as a covariate. The ANCOVA model for the effect of age, sex, puberty, and AUC_m at visit 10 included treatment as a fixed effect and the baseline age, sex, puberty, and AUC_m at visit 10 as covariates. The ANCOVA models for change from baseline for the continuous endpoints glucose and insulin metabolism, cardiovascular risk factors, renal and liver function factors and inflammation factors included treatment as a fixed effect and

baseline as covariate. The results are presented as mean change in the FAS and the estimated treatment effect from the ANCOVA models. The hypothesis testing for all endpoints used a 5% significance level (α=0.05). Outliers have been included in summary tables and listings and were not handled separately in any analyses. All statistical analyses were performed using SAS Version 9.4 (SAS institute, Cary, NC).

For power calculations, a mean treatment difference in BMI-SDS change from baseline to the 6 months visit of -0.2 was considered as a clinically relevant improvement in this study and used as the minimum detectable effect for power calculations. The anticipated standard deviation of 0.21 was confirmed in previous studies.[120] Using these assumptions and a two-sided test, a sample of 25 participants in each arm would provide 90% power to detect the assumed effect at a 5% significance level. Since a relatively high dropout rate of approximately 16% was anticipated, 30 participants per arm (90 participants in total) were planned to be randomized to have data from 75 participants available for the primary analysis.

Results and Discussion

Prevalence of different states of glucose intolerance in different study populations

Sri Lanka (Paper I)

In the Sri Lankan study population, 51.8% (n=185) of the 357 included subjects were boys and 48.2% (n=172) were girls. Mean age was 11.9 years and mean BMI-SDS was 2.6 (Paper I, **Table1**). According to OGTT results, 77.5% (n=276) of the Sri Lankan subjects had normal glucose tolerance (NGT). Isolated impaired fasting glucose (iso-IFG) was present in 9.0% (n=32) and isolated impaired glucose tolerance (iso-IGT) in 8.4% (n=30) of the Sri Lankan subjects. Combined IFG+IGT was present in 3.1% (n=11) and type 2 diabetes in 2.0% (n=7) of the subjects (Paper I, **Figure 2a**). One of the subjects fulfilled diabetes criteria defined only by elevated FG but did not fulfil diabetes criteria defined by 2h-G. Out of the other six subjects with type 2 diabetes, three had IFG and three NFG. All states of glucose intolerance were more common in pubertal and post-pubertal subjects than in pre-pubertal subjects (Paper I, **Figure 2b**). Furthermore, there was an increase in mean age between the five groups in the mentioned order (Paper I, **Figure 3a**), indicating a possible progression during childhood and adolescence from NGT over the isolated and then combined forms of IFG and IGT to type 2 diabetes.

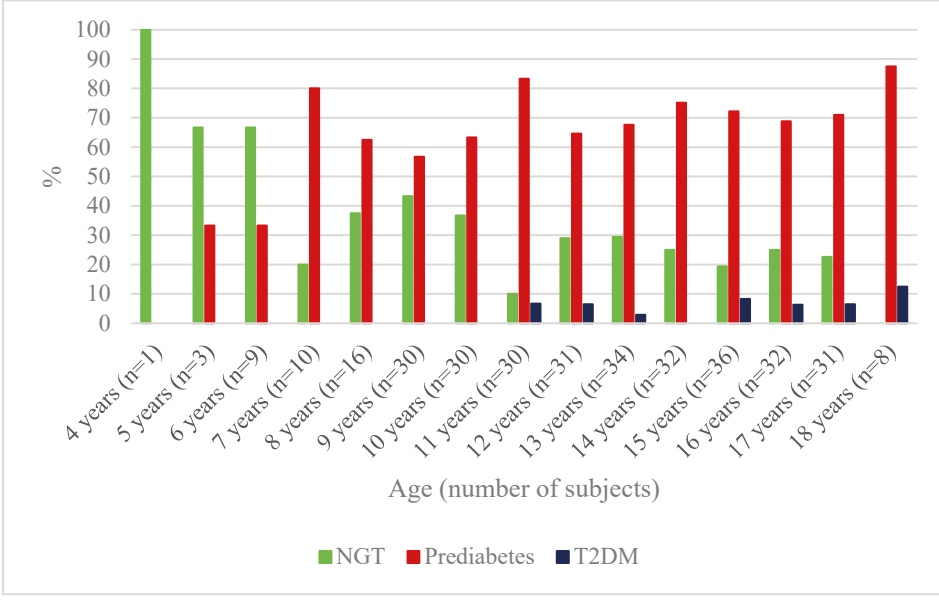
ULSCO/Beta-JUDO (Paper II)

Out of the 732 subjects enrolled in total from the Beta-JUDO cohort, the Uppsala cohort consisted of 333 patients and 43 controls (Paper II, **Figure 1**). Within the Uppsala part of the cohort 28% of the patients with obesity had NGT at baseline, whereas 72% had glucose intolerance with either iso-IFG (30%), iso-IGT (13%), comb IFG+IGT (26%) or type 2 diabetes (4%) (Paper II, **Figure 2**). Amongst Uppsala control subjects, 70% had NGT, and 30% had glucose intolerance (iso-IFG 21%, iso-IGT 5%, comb IFG+IGT 5% and type 2 diabetes 0%). When Uppsala patients and controls were sorted according to age and glucose tolerance, the number of Uppsala patients with NGT gradually decreased from 100% at 4 years to 0% at 18 years. Reciprocally,

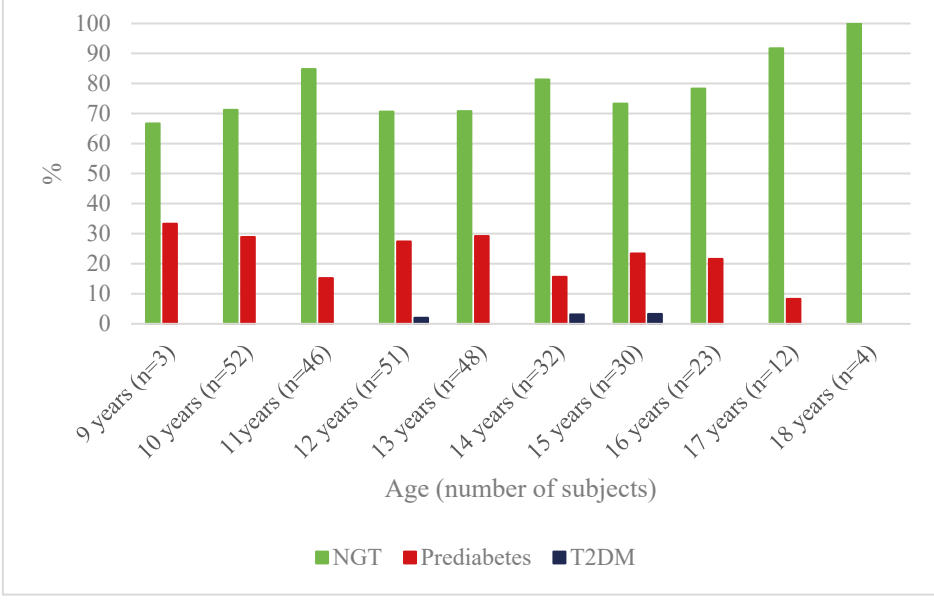
prediabetes gradually increased from 33.3% at 5 years to 87.5% at 18 years and type 2 diabetes from 6.7% at 11 years to 12.5% at 18 years (**Figure i.a**). Corresponding proportions of subjects with NGT, prediabetes and type 2 diabetes per age group are shown for Uppsala control subjects in **Figure i.c.** Out of 16 patients with type 2 diabetes in the whole cohort, 13 patients were from Uppsala and three from Salzburg. Six patients with type 2 diabetes were diagnosed based on 2h-G levels alone, three patients on FG levels alone, and seven patients had both FG and 2h-G levels above cut-off levels for type 2 diabetes.

Salzburg/Beta-JUDO (Paper II)

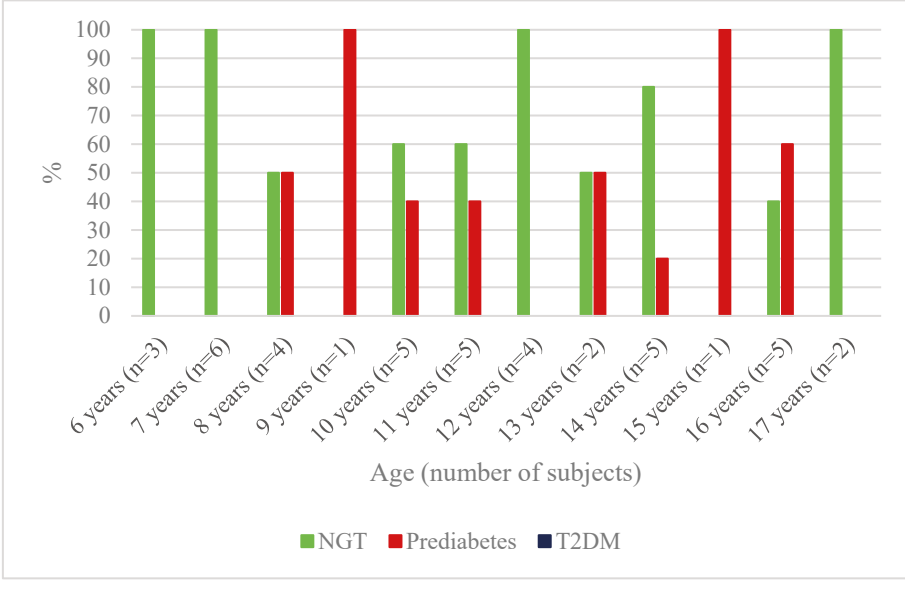
Out of the 732 subjects enrolled in total from the Beta-JUDO cohort, the Salzburg cohort consisted of 301 patients and 55 controls (Paper II, **Figure 1)**. In Salzburg patients with obesity, 76% had NGT, and 24% had glucose intolerance (iso-IFG 8%, iso-IGT 11%, comb IFG+IGT 4% and type 2 diabetes 1%). In Salzburg control subjects, 87% had NGT, and 13% had glucose intolerance (iso-IFG 9%, iso-IGT 2%, comb IFG+IGT 2% and type 2 diabetes 0%). Proportions of subjects with NGT, prediabetes and type 2 diabetes per age group are shown for Salzburg patients in **Figure i.b** and for Salzburg control subjects in **Figure i.d**. Out of 16 patients with type 2 diabetes in the whole cohort, 13 patients were from Uppsala and three from Salzburg.



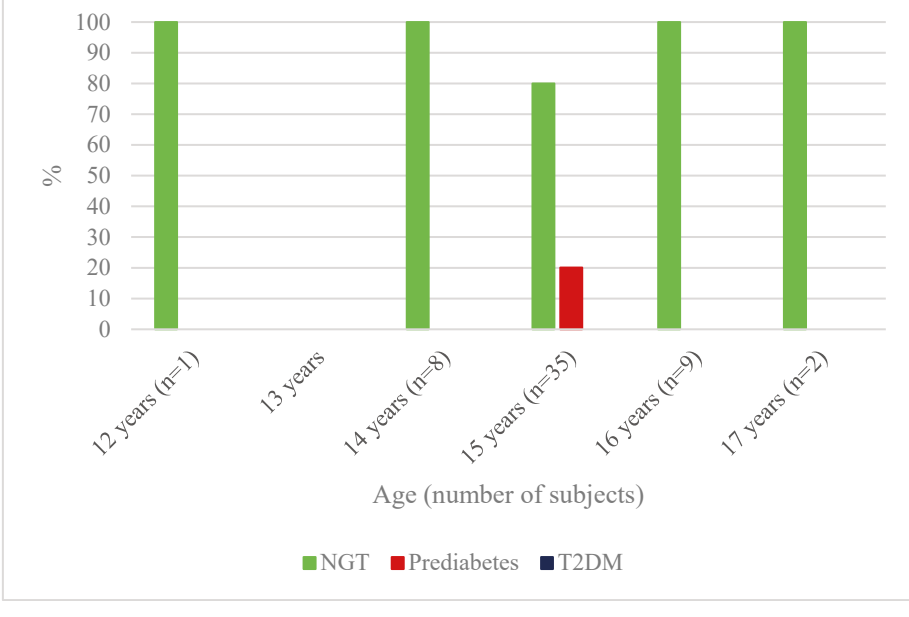
a) Uppsala patients with obesity



b) Salzburg patients with obesity



c) Uppsala control subjects



d) Salzburg control subjects

Figure i. Glucose tolerance according to age by group

(Prediabetes = iso-IFG, iso-IGT and comb IFG+IGT, T2DM=type 2 diabetes)

Discussion of prevalence in different cohorts

The study presented in paper I shows that Sri Lankan children with obesity have relatively high prevalence of glucose intolerance, and thereby confirms the findings from another study in Sri Lankan children and adolescents with obesity. [25] The prevalence of IFG in the Sri Lankan study population was 12.1% according to ADA criteria and the prevalence of IGT was 11.5%. These numbers are comparable to prevalence rates reported from European and other industrialized countries, but also to findings from previous studies in South Asian children and adolescents. [40,22] At the same time, the prevalence of childhood overweight and obesity is still lower in Sri Lanka compared to most industrialized countries. [11] A possible explanation for the finding that Sri Lankan children with obesity have a comparable prevalence of comorbidities at a lower mean degree of obesity could be that they have a higher vulnerability to obesity related complications compared with other ethnic groups.

The study presented in Paper II shows that the prevalence of glucose intolerance was extraordinarily high in Swedish children and adolescents with obesity from the ULSCO/beta-JUDO cohort. The findings not only corroborate the previously reported high prevalence of above 50% of glucose intolerance in Swedish children with obesity,[33] but report 72% of glucose intolerance among these children. The results also confirm previous findings that prevalence of IFG was higher in Swedish children with obesity compared with German and Austrian children with obesity,[29] and show that a similar difference in prevalence exists even for IGT and type 2 diabetes.

Comparison of different study populations

Comparison of the Sri Lankan with a Swedish study population (Paper I)

For comparison of fat distribution and risk profile at the same degree of obesity, 167 of the Sri Lankan subjects were matched for sex, BMI-SDS and approximate age with 167 Swedish subjects from the ULSCO cohort. At both sites, the study population included for comparison consisted of 95 boys and 72 girls. There was no significant difference in mean BMI-SDS or mean age between the Sri Lankan and the Swedish subjects included for comparison. The group of 167 Sri Lankan subjects had significantly higher amount of body fat in %, but lower WHtR compared with 167 Swedish subjects with similar BMI-SDS, age and sex distribution. The Sri Lankan subjects also had higher TC, LDL, HDL, and TG, but there was no significant difference in TC/HDL-ratio or LDL/HDL-ratio between the two groups. Sri Lankan subjects had lower glucose and insulin levels both fasting and two hours after glucose load during OGTT, and lower HOMA-IR. Acanthosis nigricans was more common in Sri Lankan subjects than in Swedish subjects (Paper I, **Table 2**).

Discussion of comparison between the Sri Lankan and the Swedish cohort

These results confirm findings from previous studies claiming that Sri Lankan children with obesity have higher amount of body fat compared with children of other origin with the same degree of obesity. [121] The fact that Sri Lankan children in the study presented in Paper I not only had higher percentage of total body fat than Swedish children with the same degree of obesity, but lower WHtR at the same time indicates that they have a different fat distribution with lower amount of abdominal fat. Compared with Swedish children, Sri Lankan children with obesity had higher levels of TC, LDL and TG, but even HDL. They also had lower HOMA-IR, which indicates a lower degree of insulin resistance in Sri Lankan children compared with Swedish children. Caution should be exerted in interpreting these results, as Swedish subjects included in the ULSCO cohort are referred from other caregivers and thereby likely to have received previous treatment for obesity, whereas subjects at the Sri Lankan study site were included from a school screening without any previous treatment for obesity. Furthermore, it should be considered that blood samples were taken at two different centres and analysed under different conditions. Also, the use of different BIA devices for measuring body composition is a potential limitation of the study, as it might lead to inaccuracy in the comparison of subjects from the two different sites. Further studies under more

standardized conditions are needed to explain which of the factors testing conditions, referral routines, different lifestyle parameters or biological factors that have the strongest impact on the observed differences in body composition and prevalence of comorbidities between Sri Lankan and Swedish children and adolescents with obesity.

Nevertheless, differences in blood indicators of comorbidities between South Asian and European children have been described in other studies. One previous study compared British children of South Asian origin with white children and found that mean WC and WHR were similar, but South Asian children had higher triglycerides and insulin levels (both fasting and after glucose load), though glucose concentrations were similar. TC, LDL and HDL levels were similar in the two groups in the same study. [40] The results of the present study only partly confirm these findings and other findings from previous literature comparing South Asian children with children of other ethnic origin, as Sri Lankan children in this study showed higher amount of total body fat and higher levels of blood lipids, but lower degree of abdominal obesity and insulin resistance compared with Swedish children. Discrepancies in results from studies comparing prevalence of different comorbidities in children with obesity and different ethnic backgrounds indicate a need of future multicentre studies with well harmonized study settings.

Comparison of Uppsala patients with Salzburg patients with obesity (Paper II)

There were no significant differences in age or sex distribution between Uppsala patients and Salzburg patients. BMI-SDS was significantly higher in Uppsala patients than in Salzburg patients. Other anthropometric measures such as WC, WHR, WHtR and percentage of body fat measured by BIA followed BMI-SDS distribution with higher values in Uppsala patients compared with Salzburg patients. FG and 2h-G, FI and 2h-Ins as well as HOMA-IR were significantly higher in Uppsala patients than in Salzburg patients. Whereas there was no significant difference in HbA1c, triglycerides or TC between the groups, lipid distribution differed significantly with higher LDL and lower HDL values in Uppsala patients. Furthermore, Uppsala patients had higher levels of the liver enzymes AST and ALT and lower albumin. Diastolic blood pressure (BP) SDS was higher in Uppsala patients compared with Salzburg patients. Birth weight SDS was significantly higher in Uppsala patients compared with Salzburg patients, whereas birth length SDS did not differ significantly between the groups. Uppsala patients reported shorter sleep duration and more screen time than Salzburg patients. Whereas actual BMI of the parents did not differ significantly between Uppsala and Salzburg patients,

highest BMI during lifetime (max BMI) of both the father and the mother was higher in Uppsala patients than in Salzburg patients. The proportion of subjects who were born abroad or had at least one parent who was born abroad was significantly higher in Salzburg patients with 49% compared to Uppsala patients with 33%. There were no significant differences in the proportion of patients that had a parent with type 2 diabetes between Uppsala patients and Salzburg patients. In summary, Uppsala patients scored significantly worse compared to Salzburg patients on most and equal on some metabolic parameters measured (Paper II, **Table 1**).

Comparison of Uppsala control subjects with Salzburg control subjects (Paper II)

Control subjects were older in Salzburg compared with Uppsala. Uppsala control subjects had significantly higher FG, lower HDL, lower albumin, and higher diastolic BP than Salzburg control subjects. There were no significant differences in sex distribution, BMI-SDS, WHR, WHtR, percentage of body fat measured by BIA, 2h-G, FI, 2h-Ins or HbA1c between Uppsala control subjects and Salzburg control subjects. In summary, Uppsala controls scored significantly worse compared to Salzburg controls on some and equal on the rest of metabolic parameters measured (Paper II, **Table 1**).

Comparison of patients with control subjects at both Beta-JUDO sites (Paper II)

Both Uppsala and Salzburg control subjects had lower BMI-SDS, WHR, WHtR and percentage of body fat measured by BIA compared with Uppsala and Salzburg patients. Control subjects also had lower FI, 2h-Ins, TG, ALT, GGT and hs-CRP and lower systolic BP SDS compared with patients at both sites. Both actual BMI and highest BMI during lifetime (max BMI) of the parents were significantly higher for Uppsala patients and Salzburg patients compared with Uppsala controls (Paper II, **Table 1**). Percentage of subjects who were born abroad or had at least one parent who was born abroad was lower in Uppsala control subjects with 25% than in both Uppsala patients (33%) and Salzburg patients (49%), whereas there was no information on country of birth for Salzburg control subjects.

Discussion of comparison between Beta-JUDO groups

The study presented in Paper II confirms previous findings that the prevalence of IFG was higher in Swedish children with obesity compared with German

and Austrian children with obesity,[29,122] and shows that a similar difference in prevalence exists even for IGT and type 2 diabetes. Furthermore, also the Uppsala control subjects had higher FG, higher diastolic BP, lower HDL and lower albumin than the Salzburg control subjects. These findings indicate that even normal weight Swedish children might be in a poorer metabolic state compared with normal weight Austrian children.

Underlying environmental causes for the observed differences in prevalence have been discussed, such as the consumption of milk cereal drinks in Swedish infants[123] or elevated levels of per- and polyfluoroalkyl substances delivered by Uppsala drinking water.[124,125] Another factor of interest is physical activity, as Swedish children and adolescents with obesity have been shown to be less physically active than normal weight Swedish children,[126] and cardiorespiratory fitness of Swedish children with and without obesity has worsened over time.[127, 128] Also, analysis of the Beta-JUDO study showed that both a lower degree of moderate to vigorous physical activity and a higher degree of sedentary time were associated with the metabolic syndrome independently of each other.[129] In the present study, neither physical activity nor any other of the investigated lifestyle or socioeconomic parameters could explain the observed differences in the prevalence of glucose intolerance between the sites. Uppsala patients reported more screen time and shorter sleep duration compared with Salzburg patients, whereas self-reported physical activity was comparable between the two groups. Nevertheless, in multivariate regression analysis none of the investigated lifestyle parameters showed any significant associations with glucose intolerance neither at baseline nor at the follow-up visit.

Longitudinal data from a subgroup analysis (Paper II)

The longitudinal analysis was conducted in a subgroup of the Beta-JUDO cohort consisting of 188 subjects (Paper II, **Figure 1**). Mean follow-up time was 2.1 years (0.1-8.3 years). Mean age at baseline in the subgroup was 12.6 years (4.9-19.0 years) and mean age at the follow-up visit was 14.7 years (7.7-19.9 years). Mean change in BMI-SDS was -0.1 (-1.3-0.8). The prevalence of different forms of glucose intolerance at baseline and at the follow-up visits is presented in **Figure 3** (Paper II) for each site separately. For each subject, glucose metabolism at baseline was compared with glucose metabolism at the follow-up visit and categorised to have improved, worsened or to be unchanged. Categorization was based on the assumption that the different states of glucose intolerance from NGT over the isolated forms and then combined IFG and IGT to type 2 diabetes represent disease progression in the mentioned

order. [1] Out of 188 patients, n=58 (30.9%) had improved glucose metabolism and n=34 (18.1%) had worsened glucose metabolism at the follow-up visit, while glucose metabolism was unchanged in n=96 patients (51.1%).

Discussion of longitudinal data

The prevalence of glucose intolerance was lower at the follow-up visit compared with baseline at both sites, besides minor changes in BMI-SDS. This corroborates previous results from a longitudinal multicentre analysis of children with obesity from a German registry, showing that glucose metabolism normalised during adolescence or early adulthood. [43] As the prevalence of type 2 diabetes in adults is comparable between Austria with 6% and Sweden with 6.9%,[42] it is unclear whether the high prevalence of glucose intolerance found in Swedish children will track into adulthood and eventually lead to increasing prevalence of type 2 diabetes even in Swedish adults in future generations. To detect a possible ongoing rise in the prevalence of type 2 diabetes in Sweden, prevalence data from young adults specifically and longitudinal data from childhood obesity cohorts are of major interest. However, even if the prevalence of glucose intolerance was lower at the follow-up visit compared with baseline, it was still alarmingly high particularly in Uppsala patients, and less Uppsala patients showed regress to NGT at the follow-up visit compared with Salzburg patients. Irrespective of possible explanations, these findings call for strong actions including both primary and secondary prevention in children of the general population and with particular focus on children and adolescents already affected by obesity.

Analysis of risk factors associated with glucose intolerance (Paper I-III)

Risk factors at baseline in cross-sectional settings (Paper I-II)

In the Sri Lankan study population (Paper I), bivariate correlation analysis showed that 2h-G correlated positively with age, puberty, WHtR, FG and 2h-Ins, but also with family history of diabetes and educational status of the father. 2h-G did not correlate with FI, liver enzymes or blood lipids, and neither with BMI-SDS, total body fat mass or WHR. There was no significant correlation between grade of MAFLD and glucose intolerance. In a multivariate logistic regression model, three variables (FG, 2h-Ins, and educational status of the father) independently increased the Odds ratio to have IGT or type 2 diabetes. When corrected for age, puberty, sex, WHtR and family history of diabetes, FG increased the Odds ratio to have IGT or type 2 diabetes with

OR=1.09 (95% CI: 1.05-1.14, p<0.0001), 2h-Ins with OR=1.01 (95% CI: 1.01-1.02, p<0.0001) and educational status of the father with OR=5.60 (95% CI: 2.21-14.18, p<0.0001). Out of the five factors adjusted for (age, puberty, sex, WHtR and family history of diabetes), age had the strongest impact on development of IGT or type 2 diabetes with OR 1.23 (95% CI: 1.01-1.50, p<0.05). Pubertal stage did not significantly increase the Odds ratio for IGT or type 2 diabetes. Comparison of means showed that subjects whose father had higher educational status had significantly higher mean 2h-G (122.7 mg/dl ± 34.0 SD) during OGTT compared with subjects whose father had lower educational status (112.2 mg/dl ± 21.0 SD, p=0.001). There was no significant difference in mean BMI-SDS between these two groups.

To assess the risk for IGT at baseline within the Beta-JUDO cohort, all laboratory, anthropometric and lifestyle parameters presented in **Table 1** (Paper II), and additionally sex, family history of diabetes and origin in another country than Austria or Sweden were included in logistic regression analysis as described above. For the Uppsala cohort, independently significant variables associated with the outcome IGT at baseline in a multivariate logistic regression model were FG (OR 3.08, 95% CI 1.41-6.77, p=0.005), HbA1c (OR 1.15, 95% CI 1.03-1.29, p=0.015), 2h-Ins (OR 1.00, 95% CI 1.002-1.004, p<0.005), FI (OR 0.99, 95% CI 0.99-1.00, p<0.005) and Swedish origin (OR 2.78, 95% CI 1.37-5.64, p=0.005). For the Salzburg cohort, significant variables were 2h-Ins (OR 1.00, 95% CI 1.002-1,004, p<0.005) and FI (OR 0.99, 95% CI 0.98-1.00, p=0.032). There were no changes in significant variables when the models were corrected for pubertal status instead of age. None of the other anthropometric, laboratory or lifestyle parameters presented in **Table 1** (Paper II) showed any independent significant associations with IGT in the multivariate logistic regression models at baseline.

Risk factors at follow-up in the longitudinal subgroup analysis (Paper II)

Variables significantly associated with the outcome glucose intolerance (IFG, IGT or both) at the follow-up visit in univariate logistic regression models including the whole subgroup of the Beta-JUDO follow-up cohort are presented in **Table 2a** (Paper II). None of the other baseline variables presented in **Table 1** (Paper II) showed any significant associations with glucose intolerance at the follow-up visit, and neither did sex or origin from another country than Austria or Sweden. In a multivariate logistic regression model including the whole follow-up cohort and adjusted for duration of the follow-up period, belonging to the centre in Uppsala showed the strongest association with the outcome glucose intolerance at the follow-up visit, followed by family

history of diabetes (Paper II, **Figure 4**). Other significant variables were 2h-G at baseline and ALT at baseline (Paper II, **Table 2b**).

Risk factors associated with mast cell activity and inflammation (Paper III)

Biomarkers associated with mast cell activity in children with obesity and normal weight controls

Clinical characteristics and biomarkers associated with glucose metabolism and mast cell activity in each group as well as in the whole population are presented in Paper III, **Table 1** and **Figure 2**. HNMT was significantly higher in the patient group than in the control group, whereas both DAO and tryptase were numerically higher in patients with obesity than normal weight controls, although not significant. Analysis of bivariate correlations showed a significant but weak correlation between HNMT and tryptase ($R=0.26$, $p<0.001$), but no other correlations between markers associated with mast cell activity. Out of the 154 patients with obesity, 30 reported a medical history of asthma or allergies, whereas none of the normal weight controls had such a medical history. Median values of HNMT did not differ between patients with and without a medical history of asthma or allergies. The criteria for IFG were met by 55.4 % and the criteria for IGT by 33.7 % of the whole population. Four subjects (2.2 %) met the fasting glucose criteria for type 2 diabetes, and five subjects (2.7 %) met the criteria for type 2 diabetes by elevated 2h-glucose.

Associations between biomarkers associated with mast cell activity and markers of insulin and glucose metabolism

Simple linear regression models in the patient group showed a significant association after adjustment for multiple testing ($p<0.017$) only for HNMT as an independent variable in relation to fasting proinsulin as the dependent variable (Paper III, **Table 2**). This association was still significant when two outliers with HNMT values above 11 (both male patients with obesity and one of them with a medical history of asthma) were excluded ($\beta=0.176$, $p<0.001$). Also, when additional four individuals with HNMT values over 10 were excluded (all male patients with obesity, of which two had a medical history of asthma and two did not), the association was still significant ($\beta=0.213$, $p<0.001$). When the same models were adjusted in a stepwise multiple regression model for sex and age in model 1, BMI-SDS in addition in model 2, specific IgE in further addition in model 3, and hs-CRP in further addition in model 4, the association between HNMT as an independent variable in relation to fasting proinsulin as the dependent variable remained significant after

correction for multiple testing (Paper III, **Table 3**). When model 1 and 2 were adjusted for pubertal state instead of age, HNMT was still significantly associated with fasting proinsulin. When model 3 was adjusted for a medical history of asthma instead of specific IgE in order to adjust for adaptive activation of mast cells due to allergic sensitization, this did not change the results. When model 4 was adjusted for IL-6, TNF-alfa, or IFN-gamma instead of hs-CRP to adjust for inflammatory mechanisms related to macrophage activity, the association between HNMT and fasting proinsulin still remained significant after correction for multiple testing (data not shown).

Discussion of the role of different risk factors for glucose intolerance

In the Sri Lankan study population (Paper I), the risk to have come further in the progress of disease and to present a more advanced state of glucose intolerance was not only determined by age, but also by educational status of the father. Previous studies have found that overweight and obesity are more prevalent among lower socioeconomic groups in industrialized countries, whereas the prevalence of overweight and obesity is higher in higher socioeconomic groups in developing countries. [130] In the study presented in Paper I, there was no significant difference in the prevalence of obesity itself between different socioeconomic groups, but the level of education of the father correlated positively with 2h-G. Furthermore, the risk of having IGT or type diabetes was higher for children to parents with higher educational level compared with those with lower educational level. These findings indicate that the risk for certain metabolic complications of obesity is higher for individuals belonging to higher socioeconomic groups in Sri Lanka, as well as in other developing countries.

The only difference between the two Beta-JUDO sites (Paper II) in significant risk factors for glucose intolerance was that ethnic origin in another country than Sweden showed a negative correlation with 2h-G in both linear and logistic regression models for the Uppsala cohort. This correlation was only found when adjusted for all mentioned correction factors in multivariate regression models. It must therefore be interpreted with caution, but it might represent a trend indicating that Swedish origin is a risk factor for glucose intolerance independent of preanalytical factors and thereby pointing into the same direction as the finding that prevalence of glucose intolerance was higher in the Uppsala cohort compared with the Salzburg cohort. Besides this, the comparison between Uppsala and Salzburg corroborates the observation that study site, i.e. growing up in Uppsala, is the strongest risk factor for developing obesity-related glucose intolerance.

Variables identified as common risk factors for glucose intolerance in all three studied populations described in Paper I and II (Sri Lanka, Beta-JUDO/ULSCO Uppsala and Beta-JUDO Salzburg) were FG and 2h-Ins, which are already widely used for the diagnosis of glucose intolerance in children and adolescents with obesity. Besides this, the fact that other variables such as ethnic origin, family history of diabetes and educational level of the father had greater impact on the risk of developing IGT or diabetes in this study than age and BMI-SDS illustrates the multifactorial genesis of obesity itself and its comorbidities. It also explains the present difficulties in defining simple and easily accessible fasting parameters for the selection of subjects that should undergo the more elaborate OGTT to screen for glucose intolerance.

Besides belonging to the study site Uppsala, having a parent with type 2 diabetes was the strongest risk factor for developing glucose intolerance during the follow-up period within the longitudinal subgroup analysis presented in Paper II. Besides baseline parameters such as glucose values and ALT, family history of type 2 diabetes should be considered when assessing the risk of children and adolescents with obesity to develop glucose intolerance.

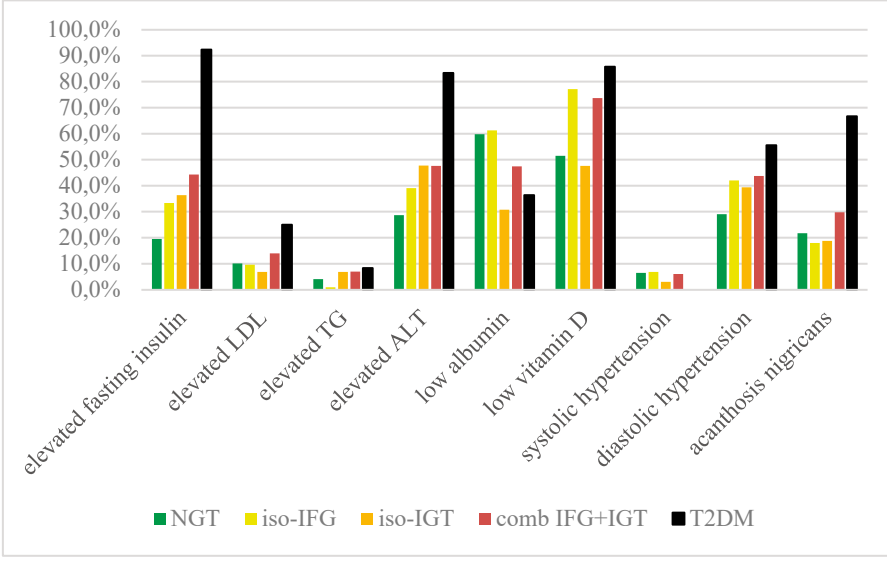
The key findings of the analysis of inflammation related risk factors presented in Paper III were that children and adolescents with obesity had higher levels of plasma HNMT, a marker associated with mast cell activity, than normal weight controls, and that HNMT was associated with fasting proinsulin as a marker of beta-cell stress in these children, independently of BMI, allergic sensitization, and monocyte/macrophage-associated inflammatory markers. There were no differences in median values of DAO, another putative marker of mast cell activity, between children and adolescents with obesity and normal weight controls, and DAO did not correlate with any marker of glucose or insulin metabolism. This is possibly due to the fact that DAO is almost exclusively expressed in the gastrointestinal tract and responsible primarily for the metabolism of exogenous histamine, whereas HNMT is expressed in many organs as well as in blood cells. [64–66] Furthermore, DAO is involved in the metabolism of many different substrates, whereas HNMT specifically targets histamine. Thus, it is more plausible that an increased presence of histamine leads to an upregulation of HNMT than DAO. The levels of CRP, IL-6 and TNF-alfa were higher in patients with obesity than in normal weight controls, indicating elevated monocyte/macrophage activity in children and adolescents with obesity, as would be expected. No association between HNMT and other markers of glucose intolerance was found in the study. Even if it cannot be assumed that the observed mast cell activity specifically has its origin in the white adipose tissue or the pancreatic beta-cells, the fact that the association between HNMT and proinsulin was significant even when

adjusted for specific IgE indicates that this association is independent of allergic sensitization. Moreover, the association remained significant when adjusted for CRP, IL-6, TNF-alfa or IFN-gamma as markers of monocyte/macrophage-related inflammation. The mechanisms behind the observed association between HNMT and proinsulin remain unknown. Possible explanations include mast cell-related inflammation, where the binding of histamine released from mast cells to H1 and H2 receptors on adipocytes, hepatocytes, and pancreatic beta-cells activates inflammatory signalling pathways that interfere with insulin receptor signalling. [131]

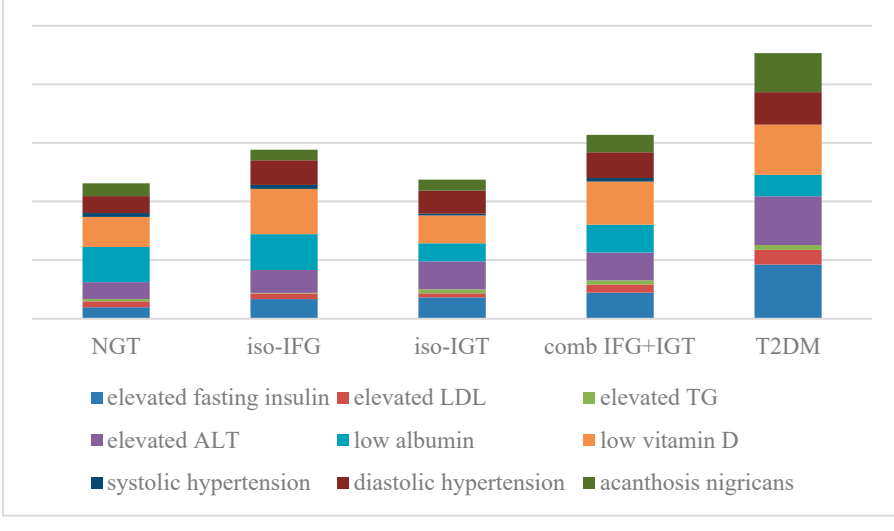
Prevalence of other obesity comorbidities according to state of glucose intolerance (Paper I)

The most common comorbidity in the Sri Lankan study population was acanthosis nigricans with a prevalence of 85.7% in the whole cohort and quite equal distribution between the different states of glucose intolerance (Paper I, **Figure 5a**). Other comorbidities with high prevalence were insulin resistance defined by elevated HOMA-IR with a prevalence of 52.9% in the whole Sri Lankan cohort (highest prevalence in the iso-IGT group with 85.2%) and elevated TC with a prevalence of 29.2% in the whole Sri Lankan cohort (highest prevalence in the comb IFG+IGT group with 45.5%). Whereas 34.5% of the subjects in the Sri Lankan study population showed increased echogenicity determined by ultrasound scan of the liver, only 17.7% had elevated values of the liver enzyme ALT. This indicates a discrepancy in the estimation of liver steatosis depending on the method chosen. The cumulative prevalence of all assessed obesity comorbidities appeared to increase from NGT over iso-IFG, iso-IGT, comb IFG+IGT to type 2 diabetes (Paper I, **Figure 5b**).

Even in the ULSCO cohort, the total prevalence of different comorbidities in addition seemed to increase in the groups comb IFG+IGT and type 2 diabetes compared with the NGT group (**Figure ii**).



a) Prevalence of each condition/comorbidity in percent within each group (state of glucose intolerance)



b) Cumulative prevalence for all conditions/comorbidities in addition for each of the five groups

Figure ii. Prevalence of comorbidities according to state of glucose intolerance (Uppsala patients and controls)

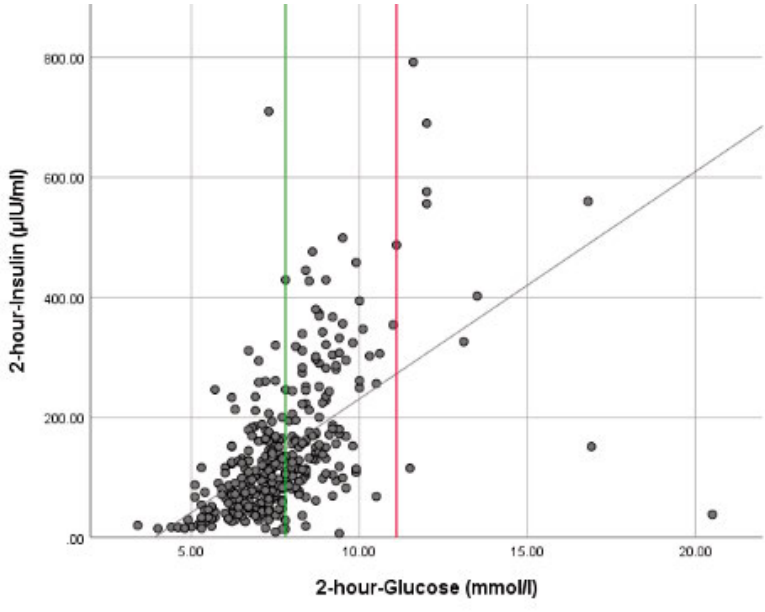
Discussion of associations with other obesity comorbidities

The finding that the cumulative prevalence of different obesity comorbidities seems to increase from NGT over iso-IFG, iso-IGT, comb IFG+IGT to type 2 diabetes in both study populations is another indicator that these five states of glucose intolerance in the mentioned order might reflect a timeline of disease progression from NGT to overt type 2 diabetes with increasing prevalence of other obesity comorbidities in the same order. However, prevalence rates of different comorbidities should be compared with caution, taking into account that different reference values are used in different countries and that reference values often are generated from studies in adults.

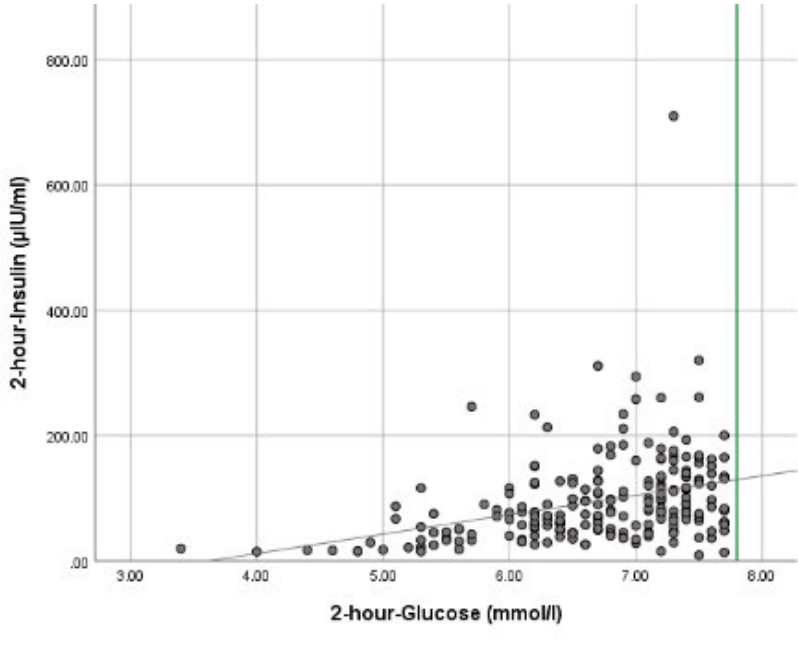
Correlations between 2h-Ins and 2h-G according to state of glucose intolerance

In the Sri Lankan study population (Paper I), a significant positive correlation between 2h-Ins and 2h-G was observed. When analysed only for the groups with normal 2h-G (NGT and iso-IFG), the positive correlation between 2h-Ins and 2h-G was still significant (Paper I, **Figure 4a**). In the groups with elevated 2h-G (iso-IGT, comb IFG+IGT, type 2 diabetes), there was no significant correlation between 2h-Ins and 2h-G, and individuals with the highest values of 2h-G tended to have lower values of 2h-Ins (Paper I, **Figure 4b**).

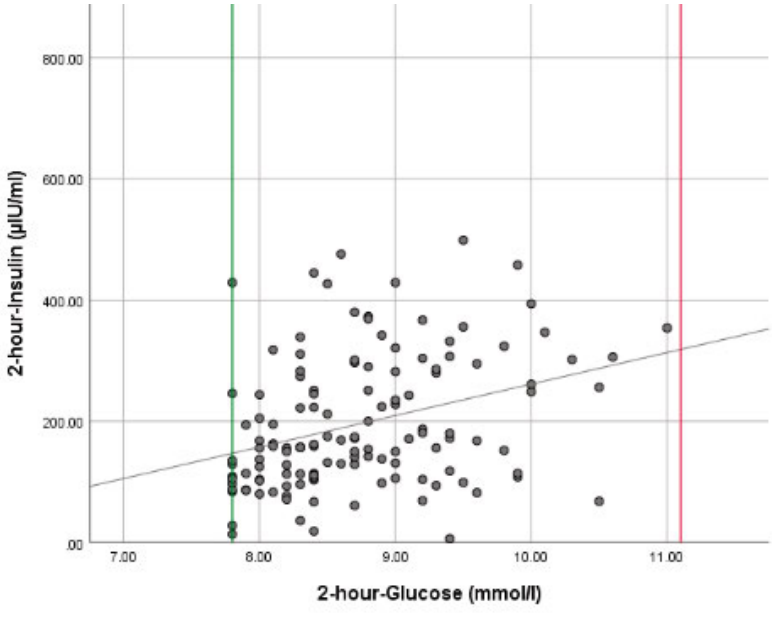
These findings could partly be reproduced in the ULSCO cohort with a significant positive correlation between 2h-G and 2h-Ins for the whole cohort (**Figure iii.a**). This positive correlation was also significant in the groups with normal 2h-G (NGT and iso-IFG, **Figure iii.b**) and the groups with IGT but not type 2 diabetes (iso-IGT and comb IFG+IGT, **Figure iii.c**). In subjects with type 2 diabetes and 2h-G≥11.1, there was no significant correlation, but a trend to a negative correlation showing decreasing levels of 2h-Ins with increasing levels of 2h-G (**Figure iii.d**).



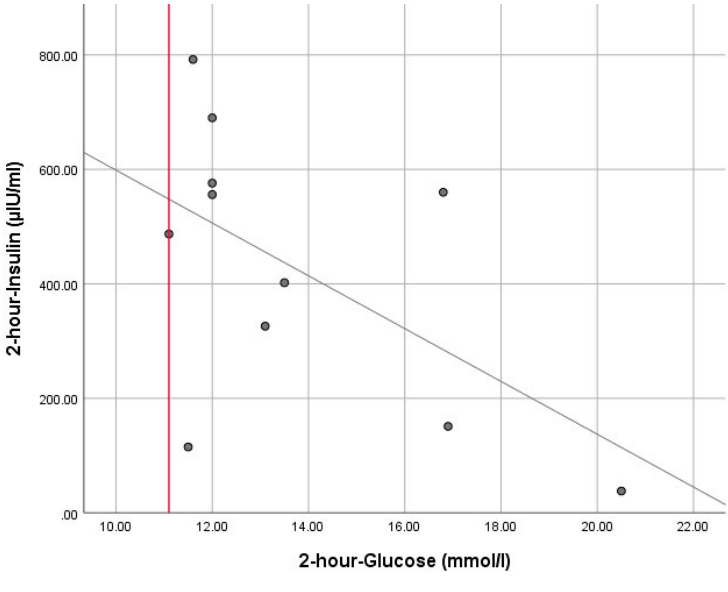
a) Whole Uppsala cohort: R=0.55, p<0.0001



b) Subjects with normal 2h-G (NGT and iso-IFG): R=0.34, p<0.0001



c) Subjects with 2h-G between 7.8-11.1 mmol/l (iso-IGT, comb IFG+IGT, some type 2 diabetes): R=0.36, p<0.0001



d) Subjects with 2h-G ≥ 11.1 mmol/l (type 2 diabetes): R= -0.39, p=0.067

Figure iii. Correlations between 2h-Ins and 2h-G in Uppsala patients and controls

Green line indicates 2h-G=7.8 mmol/l, red line indicates 2h-G=11.1 mmol/l.

Discussion of correlations between glucose and insulin during OGTT

In both study populations (Sri Lanka and ULSCO), a positive correlation between 2h-G and 2h-Ins was found in the groups representing earlier states of glucose intolerance. In subjects with type 2 diabetes, there was instead a trend to a negative correlation showing decreasing levels of 2h-Ins with increasing levels of 2h-G. One possible explanation for these findings might be that they reflect the capacity of the beta-cell to respond to increasing glucose levels with increased insulin production, showing that the beta-cell has efficient capacity to compensate for increasing levels of 2h-G in the lower states of glucose intolerance, whereas the capacity starts to decline in the more advanced states of glucose intolerance (mainly comb IFG+IGT and type 2 diabetes). This might confirm the hypothesis that the capacity of the beta-cell to compensate for increasing glucose levels with increased insulin production is failing with progression of glucose intolerance due to "pancreatic exhaustion". [132]

Metformin as a treatment option for childhood obesity and glucose intolerance – the MINT study

A total of 94 participants were screened in the MINT study (Paper IV, **Figure 2**). Five participants did not meet the inclusion/exclusion criteria and were thus excluded. The remaining 89 participants were randomized, of which 30 participants were randomized to XR+L, 29 participants to IR+L and 30 participants to LA. A total of 77 participants completed the study until visit 11 (two weeks follow-up). However, 78 participants completed the treatment part, since one participant terminated after visit 10 but before visit 11. Twelve participants terminated early from the study. In all 12 cases the participants themselves wished to discontinue. All 89 randomized participants were included in the FAS population, while 67 participants were included in the PPS population. In addition to the 11 participants who terminated the study prior to visit 10, another 11 participants were excluded from the PPS population due to poor adherence to treatment and major protocol deviations. A total of 33 participants (18 in the XR+L group and 15 in the IR+L group) reached the target dose and could maintain the dose during the 6-months treatment part of the study. Eight participants (three in the XR+L group and five in the IR+L group) did not reach the target dose during the dose escalation period but completed the study with a reduced dose (these were counted for in the statistical analysis in the PPS group). Thirteen participants (seven in the XR+L group and six in the IR+L group) were judged as non-adherent to treatment as they

did not reach the adherence threshold of ≥80%, after tablet count of returned tablets from the participants, as described above, or did early terminate the study prior to visit 10.

Baseline characteristics and demographics for the randomized subjects in the MINT study are presented in Paper IV, **Table 1**. In the FAS population, the group treated with XR+L had a mean age of 12.0 years (SD 2.4, range 7-16 years), the group treated with IR+L had a mean age of 12.0 years (SD 2.7, range 7-17 years) and the group randomized to LA had a mean age of 11.7 years (SD 3.0, range 7-17 years). In the group treated with XR+L and in the group treated with IR+L, 56.7% (17/30) and 51.7% (15/29) were females, respectively, whereas in the LA group 43.3% (13/30) were females (Paper IV, **Table 1**).

The effect of metformin XR and metformin IR on BMI-SDS

The reduction in BMI-SDS mean value after 6 months treatment was 0.13 units larger in the group treated with XR+L compared with the group who received treatment with LA (p=0.0185, Paper IV, **Table 2**). In the XR+L group, the change from baseline in BMI-SDS was -0.281, a reduction of 9.2% (p=0.006), while the LA group showed a change of -0.126 BMI-SDS, a reduction of 3.9% (p=0.359). The treatment effect was more pronounced in the PPS population with a BMI-SDS reduction of -0.290 or 9.5% (p=0.008) in the XR+L group (n=21) and -0.126 BMI-SDS or 3.9% (p=0.359) in the LA group (n=27).

The reduction in BMI-SDS mean value after 6 months treatment was 0.13 units larger in the group treated with IR+L compared with the group who received treatment with LA (p=0.0264, Paper IV, **Table 2**). In the IR+L group, the change from baseline was -0.280 SD or 9.2 % (p= 0.009). The treatment effect was similar between the IR+L group and the XR+L group (-0.002, p=0.9757).

Change in BMI-SDS was also studied at the individual level (Paper IV, **Figure 3**). In all groups, a majority of the participants, but not all, decreased their BMI-SDS. In the metformin treatment groups, 60% and 62% of individuals achieved ≥5% BMI-SDS reduction (XR+L and IR+L, respectively), compared to 30% for the LA group (Paper IV, **Figure 3D**). To analyze whether the BMI-SDS change at visit 7 could predict having at least a 5% reduction in BMI-SDS at visit 10, ROC-curves for the combined IR+XR groups were analyzed and optimal cut-offs by Youden's index calculated. A BMI-SDS reduction of ≥-0.1 at visit 7 (six weeks treatment) could predict a 5% reduction in BMI SDS at visit 10 with 81% sensitivity and 82% specificity. That is, our estimate is that among participants started on metformin who have *not* reduced

BMI-SDS by at least 0.1 units after six weeks, only 19% will receive at least a 5% reduction by 24 weeks.

The effect of metformin XR and metformin IR on glucose metabolism

Reduction in plasma glucose was greater in the IR+L group than in the LA group at OGTT timepoint 180 min (-0.91, p<0.01). Reduction in serum insulin was seen in the IR+L group at end of study compared to baseline at OGTT timepoints 60 min (-54.50, p=0.038), 120 min (-55.42, p=0.03) and 180 min (-26.05, p=0.018). However, when reduction in insulin was compared between the IR+L group and the LA group, the differences did not reach statistical significance (Paper IV, **Table 2**). Reduction in HbA1c was greater in the XR+L group than in the LA group (-0.81, p=0.044, Paper IV, **Table 2**).

The effect of metformin XR and metformin IR on cardiovascular risk factors and inflammation

Mean values of TG, TC, HDL, LDL, systolic BP, diastolic BP, heart rate and hs-CRP showed normal values at baseline in all groups, and no changes in these parameters were observed at visit 10 compared to baseline in any treatment group. Also, no differences in the treatment effect between the groups were observed (Paper IV, **Table 2**).

The effect of age on the treatment effect

In all three treatment groups, younger participants (aged 7-11 years) had a greater reduction in their BMI-SDS than older participants (aged 12-17 years). However, the difference between age groups in mean BMI-SDS change was most pronounced and only statistically significant in the LA group (-0.14, p=0.002, Paper IV, **Figure 4**). Contrastingly, the treatment effect of XR+L and IR+L compared with LA was more pronounced in older participants (-0.15 and -0.14 BMI-SDS for XR+L and IR+L, respectively) than in younger participants (-0.06 and -0.12 BMI-SDS for XR+L and IR+L, respectively). The effect of XR+L (-0.133, p=0.021) and IR+L (-0.178, p=0.003) remained statistically significant also when accounting for age, sex and puberty.

The effect of metformin concentration on the treatment effect

Plasma AUC_m of metformin XR and metformin IR had no significant effect (-0.001, p=0.784) on change in BMI-SDS, from baseline to the 6 months visit.

The three dosage groups receiving a dose of 500 mg, 1000 mg, or 1500 mg metformin twice daily, respectively, had comparable plasma concentrations of metformin and there were no significant differences in plasma AUC_m between these groups. Linear regression analysis showed no significant correlation between age and metformin concentration (Paper IV, **Figure 5A**). Participants receiving metformin IR had lower basal concentrations and reached a higher peak plasma concentration of metformin earlier after dose compared with participants receiving metformin XR during the OGTT (Paper IV, **Figure 5B**).

Safety

There were in total 555 AEs reported during the treatment period of the study (Paper IV, **Table 3**). None of these were classified as a Serious Adverse Event (SAE). The majority (67%) were judged as not related to the investigational medicinal product (IMP), and the majority (81%) were reported as mild severity. No AE led to withdrawal from the study. For one participant, three AEs led to withdrawal of IMP. This participant continued in the study until visit 12. In some cases, the AE led to temporarily stopping of the IMP, or to reduction in the dosage. As expected, most AEs were related to gastrointestinal symptoms (200 of 555 events). The distribution of these AEs between groups were 92 (XR+L), 86 (IR+L) and 22 (LA). Safety laboratory values, vital signs and physical examination did not show clinically significant abnormalities leading to withdrawal of IMP or withdrawal from the study. All urine pregnancy tests were negative.

Discussion of the treatment effects of metformin XR and metformin IR

The MINT study investigated the treatment effect of metformin XR and metformin IR in combination with lifestyle modification interventions and lifestyle intervention treatment alone on childhood obesity. Treatment with metformin XR or IR, in combination with lifestyle intervention, equally led to a significant reduction in BMI-SDS compared to lifestyle alone. A reduction in insulin and glucose values during OGTT was seen only in the IR+L group. Whereas younger children reduced their BMI-SDS more than older children in all three treatment groups, the treatment effect of metformin XR and metformin IR compared with lifestyle intervention alone was more pronounced in older children. Plasma concentrations of metformin XR and metformin IR had no significant effect on change in BMI-SDS. The study showed that treatment with metformin XR or metformin IR in addition to lifestyle interventions is safe for children from the age of 7 years.

The treatment effect of metformin XR + lifestyle compared with lifestyle alone, which was the primary objective of the MINT study, was -0.132 BMI-SDS. Both groups receiving metformin treatment in the MINT study reduced their BMI-SDS by 0.28 SD or 9.2% after six months of treatment. These effect sizes are in line with those of previous studies.[86,88,90] While earlier pediatric studies with GLP-1RAs such as exenatide and liraglutide described a mean reduction in BMI-SDS of 0.14 SD,[75,77] some recent studies with GLP-1RAs found superior treatment effects, such as the STEP TEENS study, in which treatment with the GLP-1RA semaglutide once weekly in adolescents aged 12 to <18years led to a mean reduction in BMI-SDS of 1.1 SD.[133] Similarly promising results were recently published from the SCALE Kids trial, showing that treatment with liraglutide led to a mean reduction in BMI-SDS of 0.7 SD in children aged 6 to <12 years.[134] However, in adolescents aged 12 to <18 years of age, liraglutide led to a more modest reduction in BMI-SDS of 0.23 SD or 8.32 %.[78] In comparison to metformin, GLP-1RAs are a relatively new treatment option and their long-term benefit–risk profile for the treatment of childhood obesity still needs to be evaluated. Moreover, the high costs of GLP-1RAs limit their availability and contribute to health inequities, not only in low- and middle-income countries but also wherever funding through national health budgets has not been secured. In comparison, the advantages of metformin are that it is taken orally and easy to administer, has a beneficial adverse event profile and low costs. Furthermore, metformin has been described to have many different beneficial effects such as reducing appetite and insulin resistance, and has therefore an established role in managing hyperinsulinemia and insulin resistance.[79]

The MINT study adds knowledge about the effects of metformin on glucose homeostasis in children and adolescents with obesity as it includes postprandial measurements of glucose and insulin from an OGTT, whereas most studies only report parameters of glucose metabolism based on fasting values of glucose and insulin. A reduction in serum insulin at the OGTT timepoints 60 min, 120 min and 180 min and in plasma glucose at 180 min was seen in the IR+L group, again confirming that metformin IR in combination with lifestyle intervention has a beneficial effect on glucose and insulin metabolism. However, no significant changes in insulin or glucose values during OGTT were observed in the XR+L group. This may be due to the delayed absorption resulting in lower metformin concentration during OGTT compared to the IR+L group. There were furthermore no clear indications that metformin treatment had any clinically significant effect on cardiovascular risk factors, renal and liver functions, or inflammation parameters.

Previous studies have shown better treatment effects of metformin in younger children compared with adolescents.[85] However, these studies used a flat dose of metformin in all age groups, resulting in a higher dosage per kilogram body weight for the younger children, and thereby likely a higher exposure of metformin. The MINT study employed a weight-based target dosing, aiming for a similar exposure between groups. The comparison of plasma concentrations showed that the lowest dose, i.e. 500 mg twice daily, resulted in a rather lower exposure than the higher doses, although, there was no significant correlation between exposure of metformin and age, body weight, or BMI. This confirms that the study achieved its aim to provide a comparable dose of metformin per kg body weight for all included participants. Younger participants reduced their BMI-SDS more than older participants in all three treatment groups. This effect was most pronounced in the group receiving lifestyle alone, indicating that lifestyle interventions should be implemented as early as possible to achieve the best results, and thereby confirming previous studies.[135,136] However, the difference in BMI-SDS reduction between the groups receiving metformin treatment and the group receiving lifestyle alone was most pronounced in older participants. Potential conclusions for clinical practice include that early implementation of treatment for childhood obesity is important for all types of treatment, but especially for lifestyle interventions, whereas metformin treatment combined with lifestyle interventions should be offered more frequently to older children and adolescents. The fact that the dropout rate in the MINT study was somewhat lower than anticipated in all groups indicates that all treatment forms offered within the study were received positively by the participants and their families.

There is a recognized need to clarify the relationship between metformin plasma concentrations, efficacy and side effects.[120] The MINT study addresses this knowledge gap by monitoring of plasma concentrations of metformin at certain time-points during the treatment period. The results show that the chosen regimen, where metformin was administered based on body weight, resulted in plasma metformin exposures that were comparable across all age and BMI groups, and in general, lower than previously reported,[137] indicating that metformin treatment according to the weight-based target dosing utilized in the MINT study is safe even for younger children with a body weight as low as 44 kg. As expected, the profile of metformin concentrations after dose intake differed between the groups, with metformin XR showing higher basal concentrations and lower and delayed peak values compared with metformin IR. These profiles were reflected in the pattern of insulin values during OGTT, where an effect on serum insulin was seen only in the IR+L group, indicating that higher peak values of metformin contribute to the observed reduction in

insulin levels during OGTT. However, no correlation of the total plasma concentration of metformin described by AUC_m, and effect of metformin treatment on BMI-SDS could been found.

Safety and tolerability are crucial factors in the clinical use of any medication for the pediatric age group. Studies with metformin in pediatric obesity report that metformin is generally well tolerated, with diarrhea being the most common adverse event in both metformin and placebo groups, and no significant differences in any safety parameters between metformin and placebo groups.[86,87] In the MINT study, 555 AEs were reported, with 67% of them judged as not related to the study medication. Most AEs (81%) were mild in severity, and there were no serious or unexpected safety concerns. As expected, most AEs were related to gastrointestinal effects, such as abdominal pain, diarrhea, nausea, and vomiting. In line with a previous study by Derosa et al,[89] the frequency of nausea was lower in the XR+L group with 23.3% of the participants reporting nausea compared to 44.8% in the IR+L group, however not significant. In comparison, nausea was reported by 42.2% of the participants in the Liraglutide group in a study with the GLP-1RA Liraglutide in adolescents.[78] Gastrointestinal side effects are common and known to occur most frequently during initiation of metformin therapy and resolve spontaneously with continued treatment. The three-week dose escalation period employed in this study, designed to improve gastrointestinal tolerability, seemed to be a practical approach to minimize these effects. The laboratory safety parameters and physical examinations did not show clinically significant findings or abnormalities leading to withdrawal of metformin treatment or withdrawal from the study. However, it is worth noting that some children had difficulty swallowing the tablets, which could be a barrier for adherence to treatment in younger populations. Adaptation of the tablet size or exploring alternative formulations, such as liquid preparations, might be important steps to ensure better adherence to the treatment. In summary, the MINT study showed that treatment with metformin XR or metformin IR in addition to lifestyle interventions is generally well tolerated and safe for children from the age of 7 years.

Further discussion

Strengths and weaknesses

Besides potential differences in laboratory methods between the Sri Lankan and the Swedish study site, another weakness of the study presented in Paper I is that only subjects with BMI-SDS >2 were considered as having obesity, as recent data shows that lower cut-offs might be recommended for Sri Lankan children. [99] Furthermore, there was a time gap of up to 14 months between the school screening and the biochemical assessments at the centre. Even if anthropometric measurements were conducted at both events, lifestyle patterns such as physical activity and nutritional habits were only evaluated at screening and might have changed until the assessments at the centre, possibly even due to the knowledge of the child having obesity.

Nevertheless, this study is one of the first to present data on glucose intolerance in a bigger sample of Sri Lankan children and adolescents with obesity, and it provides detailed information not only on metabolic and anthropometric, but also on socioeconomic and lifestyle parameters. Furthermore, it adds knowledge about the metabolic risk profile of Sri Lankan children and adolescents in comparison with Swedish children and adolescents with the same degree of obesity.

The strength of the study presented in Paper II lies in its detailed characterization of not only laboratory and anthropometric, but also socioeconomic and lifestyle factors in a relatively large number of children and adolescents with obesity and normal weight control subjects in a well-harmonized European dual-centre study. The fact that no significant correlations between lifestyle and socioeconomic variables and glucose intolerance could been proven in this study reflects also that these variables are harder to classify and less reliable than laboratory and anthropometric parameters. Further multi-centre studies comparing well-harmonized and well-characterized larger childhood obesity cohorts and including healthy control subjects will be needed to confirm national or regional differences in the prevalence of glucose intolerance and to investigate possible biological, socioeconomic, and behavioural explanations for the observed differences in prevalence.

Another strength of the study presented in Paper II is that it provides longitudinal data including repeated oral glucose tolerance tests from children

and adolescents with obesity and glucose intolerance at the two clinical sites. Limitations of the study were selection bias related to different local routines for the referral of patients that resulted in different BMI-SDS distribution at the two sites, and methodological differences both regarding certain blood samples and bioimpedance assay devices. To detect a possible ongoing rise in the prevalence of type 2 diabetes in Sweden, additional longitudinal data from childhood obesity cohorts and prevalence data from young adults specifically are of major interest.

A limitation of the study presented in Paper III may be its observational design. Although significant associations could be found, no causality can be assumed. Furthermore, the study population was relatively small, especially the control group, which may have impacted the comparison of the two groups. The relevance of the associations found in this study should be considered early findings that need to be confirmed in larger paediatric populations, particularly including more healthy controls. However, they provide support of HNMT as a marker of mast cell-related inflammation, and the role the mast cell may play for obesity and metabolic complications such as glucose intolerance.

The MINT study (Paper IV) was powered to detect differences in the primary objective of the study, which was change in BMI-SDS comparing metformin XR + lifestyle changes to lifestyle changes alone. Thus, limitations of the study include that the secondary objectives may potentially be underpowered. Another limitation of the study was the inability to include a placebo arm in addition to standard care. The strengths of the MINT study include the relatively large number of participants, the detailed description and execution of the lifestyle intervention that was common for all three treatment groups, and its academic independence. Furthermore, this study is the first to take plasma concentrations of metformin into account and provide a comparable metformin dosage based on the participants` body weight.

Conclusions

Paper I

i. Glucose intolerance was common in Sri Lankan children and adolescents with obesity and showed a prevalence of 22.5% in total.
ii. FG, 2h-Ins, and higher educational status of the father independently increased the risk of having glucose intolerance in the Sri Lankan cohort.
iii. The cumulative prevalence of different obesity comorbidities increased from NGT over iso-IFG, iso-IGT, comb IFG+IGT to type 2 diabetes both in the Sri Lankan and the ULSCO cohort, indicating that these five states of glucose intolerance in the mentioned order might reflect a timeline of disease progression from NGT to overt type 2 diabetes.
iv. A positive correlation between 2h-G and 2h-Ins was found both in the Sri Lankan and the ULSCO cohort in subjects representing earlier states of glucose intolerance, whereas there was a trend to a negative correlation in subjects with type 2 diabetes, showing decreasing levels of 2h-Ins with increasing levels of 2h-G.
v. Sri Lankan subjects had higher percentage of body fat, but less abdominal fat than Swedish subjects.
vi. The findings indicate that screening for glucose intolerance is important even in asymptomatic Sri Lankan children and adolescents with obesity to avoid progression to type 2 diabetes.

Paper II

i. The prevalence of glucose intolerance was extraordinarily high with 72% among Swedish children and adolescents with obesity from the beta-JUDO/ULSCO cohort.
ii. Both Swedish children and adolescents with obesity and normal weight control subjects showed poorer metabolic health compared with Austrian children and adolescents.

iii. Ethnic origin in another country than Sweden showed a negative correlation with 2h-G in the Uppsala cohort, indicating that Swedish origin might be a risk factor for glucose intolerance.
iv. These findings corroborate the previously reported high prevalence of glucose intolerance in Swedish children with obesity and previous findings of higher prevalence of IFG in Swedish children with obesity compared with German and Austrian children with obesity.
v. No significant correlations between lifestyle and socioeconomic variables and glucose intolerance could been proven in this study as a possible explanation of the observed differences in the prevalence of glucose intolerance.
vi. Irrespective of possible explanations, the finding that prediabetes was present already at 5 years of age and type 2 diabetes at 11 years of age in Swedish children and adolescents with obesity, is alarming and calls for strong actions including both primary and secondary prevention as well as careful examination and treatment of children and adolescents already affected by obesity.

Paper III

i. Levels of HNMT as a marker associated with mast cell activity were significantly higher in children and adolescents with obesity than in normal weight controls.
ii. Independent associations could be proven between HNMT as a marker associated with mast cell activity and fasting proinsulin as a marker of beta-cell stress in children and adolescents with obesity.
iii. These findings suggest that the molecular interactions between mast cell activity, obesity, and metabolic health previously identified in adult and animal models are already active in children and adolescents with obesity, potentially setting the stage for metabolic dysfunction even before overt diabetes develops.

Paper IV

i. Metformin XR in combination with lifestyle intervention reduces obesity more than lifestyle intervention alone in children and adolescents with obesity.
ii. Treatment with both metformin XR and metformin IR in combination with lifestyle interventions is generally well tolerated and safe for children from the age of 7 years, and lowered BMI-SDS irrespective of metformin formulation.
iii. Effects on glucose and insulin metabolism were only found in the group receiving metformin IR, possibly due to differences in the pharmacodynamic profiles of the metformin formulations that were observed in the study.
iv. Adolescents likely benefit more than younger children from the addition of metformin to lifestyle treatment.
v. In summary, both metformin IR and metformin XR are suitable options for the treatment of pediatric obesity.

Sammanfattning på svenska

Övervikt och obesitas, som är en högre grad av övervikt, ökar bland barn både i Sverige och i resten av världen. Det leder inte bara till att fler barn får komplikationer till obesitas såsom typ 2 diabetes, utan också till att sjukdomen börjar utvecklas i allt yngre åldrar. I den här avhandlingen undersöks hur vanligt det är att barn med obesitas har förhöjt blodsocker, vilka riskfaktorer som är viktigast, hur andra hälsoproblem hänger ihop med förhöjda blodsockervärden och om läkemedlet metformin kan vara till hjälp.

Studierna som beskrivs i avhandlingen bygger på tre olika barnkohorter från Sverige, Österrike och Sri Lanka. Alla svenska barn kommer från en uppsalabaserad långtidsstudie om barnobesitas som heter ”Uppsala Longitudinal Study of Childhood Obesity (ULSCO)”. Deltagarna från Österrike kommer från en liknande kohort i Salzburg området som består av barn och ungdomar, både med obesitas och normalvikt. Den österrikiska kohorten slogs ihop med den svenska ULSCO kohorten inom ett europeiskt projekt som heter ”Beta-cell function in Juvenile Diabetes and Obesity (Beta-JUDO)”. I Sri Lanka deltog nästan 400 barn med obesitas från olika skolor i staden Negombo.

Alla barn tog fasteblodprover och gjorde ett glukostoleranstest där man mäter blodsocker och insulin före och efter att de druckit en sockerlösning. Resultaten visade att förhöjda blodsockervärden, också kallade för glukosintolerans, var mycket vanligt, särskilt hos barnen i Uppsala. I den gruppen hade över 70 procent någon form av glukosintolerans, vilket är betydligt högre än i de andra länderna. Samtidigt såg man att många förbättrade sina värden vid uppföljningar över en längre tid.

En tydlig riskfaktor stack ut vid uppföljningarna: barn som hade ärftlighet för typ 2 diabetes löpte betydligt större risk att själva visa tecken på glukosintolerans. Förhöjt fasteblodsocker och höga insulinnivåer två timmar efter intaget av sockerlösningen under glukostoleranstestet ökade också risken för olika former av glukosintolerans. För de svenska och österrikiska barnen spelade även HbA1c-värdet in, medan högre utbildningsnivå hos pappan ökade risken för barn från Sri Lanka.

En av studierna undersökte betydelsen av mastceller, celler som är viktiga vid allergier och inflammation. Resultaten visade att ett av de ämnen som mastceller producerar, histamin-N-methyltransferas (HNMT), var högre hos

barn med obesitas jämfört med barn som hade normalvikt. Ökade nivåer av HNMT verkade också hänga ihop med högre nivåer av proinsulin, ett ämne som kan tyda på en påfrestning på betaceller, de celler i bukspottkörteln som producerar insulin. Det antyder att det kan finnas ett samband mellan obesitas, inflammation och påverkan på regleringen av insulin och blodsocker som börjar tidigt i livet.

Andra hälsoproblem som ofta följer med obesitas, till exempel blodfettsrubbningar och högt blodtryck, blev vanligare ju mer framskridna former av glukosintolerans barnen hade. De svenska barnen, både de med obesitas och de normalviktiga, hade generellt sämre metabola värden än de österrikiska.

Avhandlingen beskriver också läkemedelsstudien ”Metformin Intervention in Children and Adolescents with Obesity (MINT)” som undersökte om metformin, ett läkemedel som vanligtvis används vid typ 2-diabetes, kan hjälpa barn med obesitas att förbättra hälsan. Barnen som deltog i studien fick antingen levnadsvanebehandling enbart eller levnadsvanebehandling i kombination med en av två olika varianter av metformin, en långtidsverkande och en snabbverkande form. De barn som fick metformin minskade sitt BMI-SDS, som är ett mått för graden av obesitas, mer än de som bara fick levnadsvanebehandling. Hos barn som fick den snabbverkande varianten av metformin sjönk dessutom både insulin- och glukosnivåer vid flera mättillfällen under glukostoleranstestet. Effekten av metformin jämfört med enbart levnadsvanebehandling var större hos äldre barn, även om yngre barn minskade sitt BMI-SDS mer än äldre barn i alla tre grupper. Biverkningarna av läkemedlet var milda och jämförbara i alla grupper.

Sammantaget visar studierna att glukosintolerans är vanligt bland barn med obesitas, att ärftlighet spelar stor roll och att glukostolerans för många barn förbättras över tid. Nya fynd tyder på att ämnen som är kopplade till inflammation orsakad av mastceller kan påverka regleringen av insulin och glukostolerans. Läkemedelsstudien MINT visade att metformin är ett lämpligt komplement till levnadsvanebehandling för barn och ungdomar med obesitas och glukosintolerans.

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