Impaired Glucose Tolerance in Childhood Obesity

Contribution of Glucagon, GLP-1 and Inflammation

HANNES MANELL
In the wake of increased obesity prevalence, impaired glucose tolerance (IGT) and type 2 diabetes (T2D) in childhood and adolescence is increasingly common. Given the negative impacts these conditions have on health over time, understanding the pathophysiology in those affected early in life is important. Both the proglucagon-derived peptides and low-grade inflammation have been implicated in the development of obesity-related complications. The aim of this thesis was to study across the glucose tolerance spectrum in children and adolescents with obesity 1) proglucagon-derived peptides glucagon, GLP-1 and glicentin, 2) dipeptidyl peptidase-4 (DPP-4) and its degradation of GLP-1 and 3) novel inflammatory markers. To this end, children and adolescents of the Uppsala Longitudinal Study of Childhood Obesity were studied.

Children and adolescents with obesity had higher fasting plasma glucagon concentrations than lean controls. In particular visceral adiposity, hyperinsulinemia, triglycerides and free fatty acids (FFAs) were associated with high plasma glucagon concentrations. In isolated islets elevated FFAs caused hypersecretion of glucagon. In children and adolescents with IGT or T2D, fasting plasma glucagon was further elevated and the GLP-1 and glicentin response to an oral glucose tolerance test (OGTT) was decreased. In T2D plasma glucagon increased during the first 15 minutes of OGTT. Plasma DPP-4 concentrations were elevated in obesity and associated with lower proportion of intact GLP-1 but not with IGT. Several pro-inflammatory markers were elevated in children and adolescents with obesity but not further elevated in IGT or T2D with the exception of low plasma Tumor necrosis factor-related weak inducer of apoptosis (TWEAK) levels, which were associated with IGT, hyperinsulinemia and hyperglucagonemia. High plasma hepatocyte growth factor (HGF) concentration was associated with increased risk of further weight gain in children and adolescents with obesity.

In conclusion, elevated glucagon concentration at fasting, a hyperglucagonemic response to OGTT and reduced GLP-1 and glicentin are characteristics of IGT and T2D development in childhood obesity reflecting altered usage of the proglucagon gene. DPP-4 concentrations are elevated in childhood obesity but not associated with IGT. Reduced circulating TWEAK was identified as a novel marker of IGT early in life. Children with obesity and high HGF are less likely to respond well to lifestyle intervention.

Keywords: Childhood obesity, impaired glucose tolerance, type 2 diabetes, glucagon, glucagon-like peptide-1, dipeptidyl peptidase-4, inflammation, free fatty acids, insulin, visceral adiposity

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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.


* Co first-authors

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## Abbreviations

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<tr>
<td>AUC</td>
<td>Area under curve</td>
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<tr>
<td>BMI SDS</td>
<td>Body mass index standard deviation score</td>
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<td>CDCP1</td>
<td>CUB-domain containing protein 1</td>
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<td>DPP-4</td>
<td>Dipeptidyl peptidase-4</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbant assay</td>
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<td>FFA</td>
<td>Free fatty acid</td>
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<td>GLP-1</td>
<td>Glucagon-like peptide 1</td>
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<td>HGF</td>
<td>Hepatocyte growth factor</td>
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<td>IFG</td>
<td>Impaired fasting glucose</td>
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<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NGT</td>
<td>Normal glucose tolerance</td>
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<td>NPX</td>
<td>Normalised protein expression</td>
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<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>PC</td>
<td>Prohormone convertase</td>
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<tr>
<td>PEA</td>
<td>Proximity extension assay</td>
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<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
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<tr>
<td>TWEAK</td>
<td>TNF-related weak inducer of apoptosis</td>
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<tr>
<td>ULSCO</td>
<td>Uppsala longitudinal study of childhood obesity</td>
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<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
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Introduction

Obesity is defined by the World Health Organization (WHO) as “abnormal or excessive fat accumulation that presents a risk to health” (1). Since the 1980s there has been a substantial increase in the prevalence of obesity and there are estimates from US data that the rates of overweight have increased throughout the 20th century (2,3). The increase is also seen among children and adolescents and estimates predict that obesity rates will continue increasing (2,4). Among the comorbidities of obesity, type 2 diabetes (T2D) is of importance as it is associated with excess mortality from a number of causes (5).

In the regulation of blood glucose, in addition to insulin, hormones of the proglucagon family are key players (6). Glucagon, secreted mainly from pancreatic alpha-cells elevates blood glucose through glycogenolysis and increased endogenous glucose production from the liver (6). Glucagon-like peptide-1 (GLP-1), produced by the enteroendocrine L-cells, although encoded by the same gene has opposite effects in that it lowers blood glucose concentrations in hyperglycemic conditions by stimulating insulin secretion (7). Obesity is associated with disturbed regulation of these two and other hormones, such as the blood glucose lowering hormone insulin. A major contributor to the disturbed hormone secretion is the low-grade inflammation occurring in obesity which has been coupled with dampening effects on e.g. insulin signaling (8). Effects of obesity-related inflammation on the proglucagon derived hormones are less defined.

Type 2 diabetes, which in the past was a disease of old age, is diagnosed increasingly also in children and adolescents (9). In children and adolescents, overweight is a more important risk factor for T2D than in adults and the vast majority of individuals diagnosed with T2D before the age of 18 are overweight or obese (10). Although much knowledge has been gained in the past decades, the causes of type 2 diabetes and its pre-stage, impaired glucose tolerance in children and adolescents are still less defined than in adults.

This thesis aims to shed light on the pathophysiology of impaired glucose tolerance (IGT) and T2D in children and adolescents by studying glucagon, GLP-1 and inflammatory markers in subjects from the Uppsala longitudinal study of childhood obesity (ULSCO) (11).
Background

Childhood obesity

Prevalence and definitions

The prevalence of overweight and obesity in youth has increased globally at least since the 1980s and has been estimated to nearly one in four children and adolescents in developed countries (2). As direct measurement of body fat, for instance with dual x-ray absorptiometry (DXA) is cumbersome, the commonly used measurement to diagnose overweight and obesity is the body mass index (BMI), calculated as weight over height squared (12). The BMI increases across childhood and adolescence as a result of normal growth, without reflecting excess adiposity. Thus, definitions of obesity in childhood are based on deviations from reference populations, expressed as percentiles or as standard deviation scores (SDS) (13). WHO cut-offs for children age 5-19 are >1 in BMI SDS for overweight and >2 for obesity (1).

The increasing trend in obesity is seen in all parts of the world with the highest relative rates in Polynesian and Micronesian island nations where more than 50% of children have overweight or obesity (14). In high-income Western countries such as the United States, the prevalence increase seems to have leveled off over the past 20 years (15,16). There is a similar leveling off or even a small decrease in the childhood obesity prevalence in Sweden with estimates that around 20% of children and adolescents are overweight or obese (17–20). In contrast, in many parts of the world such as Latin America, Africa and China the rates of childhood obesity is still increasing (14,21–23).

Complications

Obesity in childhood and adolescence tends to carry over into adulthood with estimates that 60-75% of those with obesity in childhood have obesity also in adulthood (24–27). However, it is important to note that this does not mean that most adults with obesity had obesity also as children, which likely explains the poor correlations found between childhood BMI and adult BMI (28–30). The carry over of obesity was seen also from childhood to adolescence where half of adolescents with obesity had obesity in childhood while 90% of 3-year olds with obesity remained overweight or obese as adoles-
cents (31). Together this illustrates that established obesity tends to persist regardless of whether it is established in early childhood or adolescence.

This is of importance as obesity is a risk factor for a number of diseases and BMI has a J-shaped relationship with mortality with lowest risk around the upper normal range and increasing with overweight and even further with obesity (32,33). Obesity in childhood increases cardiovascular and all-cause mortality later in life (34,35). Apart from the future risk, several comorbidities to childhood obesity are present already in childhood and/or adolescence. These include non-alcoholic fatty liver disease (NAFLD) with fibrosis in some cases (36), obstructive sleep apnea (37), asthma (38) and depressive symptoms (39). Obesity in childhood and adolescence is also associated with endocrine disorders such as T2D and reduced insulin sensitivity (9,40,41). Interestingly though, childhood obesity is inversely correlated with adult cardiovascular risk factors when adjusting for adult BMI, indicating that the increased risk might be attributable to the persistence of obesity from childhood to adulthood (42–44). In line with this, the cardiovascular risk of lean adults with obesity as children was similar to that of adults who never had obesity (45).

**Obesity-associated inflammation**

One key feature of obesity is low-grade chronic inflammation which occurs in multiple tissues of importance to metabolic homeostasis such as the liver, muscle and adipose tissue (8). Changes in the adipose tissue in obesity include increased hypoxia and the change in macrophage phenotype, resulting in higher expression of pro-inflammatory genes (46,47). The production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) from adipose tissue increases with obesity (48). Pro-inflammatory cytokines can interfere with insulin signaling in target tissues, for example through the NF-κB and JAK/STAT pathway (49,50). On the other hand, reduced insulin signaling in adipocytes can cause inflammation and thus the directions of causality in the associations between inflammation and reduced insulin signaling in obesity is not entirely clear (51). Inflammation can also impact the islets of Langerhans where exposure to cytokines Interleukin-1 beta (IL-1β), Interferon gamma (IFN-γ) and/or TNF-α can induce β-cell apoptosis (52). However, the effect seems to be time- and concentration-dependent as short term exposure to IL-1β and activation of NF-κB can stimulate insulin secretion (53). The increased inflammation can be detected in the circulation with elevated concentrations of several pro-inflammatory cytokines such as TNF-α, Interleukin 6 (IL-6) and Interleukin 1 β (IL-1β) in childhood obesity (54,55). Furthermore, adipose tissue in children with obesity has increased number of macrophages, more and larger adipocytes but not increased TNF-α expression (56). Thus, the response of increased inflammation seems to be an early event in obesity development. In adults, the inflammatory state
seems to contribute to the development of T2D as high IL-6 is a risk factor for development of the disease (57). So far treatments targeting TNF-α has not been successful in increasing insulin sensitivity (58). However, there are studies showing a modest reduction in glycaemia in T2D with anti-IL-1β treatment (59). However, a recent large study did not find beneficial effects of anti-IL-1β treatment on either long-term glycemic control or on diabetes incidence (60). Also salicylates have a glucose lowering effect, however at the cost of weight gain and a worsening lipid profile (61).

Impaired glucose tolerance and type 2 diabetes

Diabetes mellitus is the inability to maintain normal blood glucose regulation, where the blood glucose lowering hormone insulin plays a key role. It was early understood that patients with diabetes could be divided into those with absolute requirement for insulin, type 1 diabetes (T1D), and those with relative deficiency of the hormone, T2D. T2D is a heterogeneous disease with some individuals characterized by altered secretion of insulin and others by insensitivity to insulin but often a mixture of these two traits are observed (62).

Type 2 diabetes is diagnosed by elevated blood glucose concentrations, measured either at fasting (≥7mmol/l), 2-hours into an oral glucose tolerance test (OGTT; ≥11.1 mmol/l) or elevated blood glycated hemoglobin (HbA1c; ≥48 mmol/mol) or elevated non-fasting blood glucose (≥11.1 mmol/l) in the presence of classic symptoms of hyperglycemia (63).

Risk factors for developing T2D in adults include old age, obesity, unhealthy dietary pattern, low physical activity and smoking (64). There is an increase in mortality in individuals with T2D with larger relative increased risk in younger age (65). In the short term T2D can lead to emergencies such as hyperglycemic hyperosmolar state, with high mortality (66). In the long-term type 2 diabetes and hyperglycemia is associated with cardiovascular disease (67–69), renal failure (70), retinopathy (71) and foot ulcers with risk of amputation (72), all with profound negative impact on quality of life.

The development of hyperglycemia in T2D is progressive and thus intermediate hyperglycemia, either impaired fasting glucose (IFG; ≥5.6 mmol/l according to the American Diabetes Association or ≥6.1 mmol/l according to the WHO) and/or IGT (2-hour OGTT glucose ≥7.8 mmol/l) is labeled prediabetes (63,73). IFG and IGT is associated with a high risk of developing T2D and cardiovascular disease (5,74). However, the risk increase is gradual with increasing glycaemia as demonstrated by the risk increase also in high normal fasting blood glucose concentrations (75).
Prevalence in childhood and adolescence

Increased prevalence of T2D in children and adolescents started to gain attention in the 1990s (76–79) although there had been previous reports of its occurrence in high-risk populations (80,81). The prevalence varies greatly across regions and ethnic groups, highest in indigenous American populations in the USA and Canada (130 – 5100 per 100 000 children/adolescents), followed by Hispanic and African Americans (46-106 per 100 000) and lower rates in non-Hispanic whites in the USA (18 per 100 000) and European countries (0-9 per 100 000) (82). One Swedish study found an incidence of 5.6 per 100 000 person-years in 10-19 year olds, which is high among European countries (83).

When looking specifically at the prevalence of T2D among children and adolescents with obesity, higher rates of 0.1-5% were observed (84–89). More recent data from the US and UK suggest that there is an increasing trend in the prevalence of adolescent T2D (90,91). High prevalence of IGT at more than 20% among children and adolescents in obesity clinics gained attention in the early 2000s (84,92). There are several, mostly small studies of the prevalence of IGT among children and adolescents with obesity from different parts of the world. Prevalence estimates range from 2-27% with German and Italian studies at the low end and US and Turkey at the high end of the spectrum (85–88,93–109). The two conditions, IGT and IFG are overlapping to some degree but fasting glucose is known to be a poor tool to detect children with IGT (86,89,110). In Sweden the prevalence of IFG and IGT was found to be high in adolescents with obesity (111–113).

Risk factors for developing type 2 diabetes in childhood and adolescence

There are a number of characteristics of childhood- or adolescent-onset T2D as compared to adult onset T2D. The association with BMI is even stronger in adolescent onset T2D. Nearly all individuals newly diagnosed with T2D in adolescence have overweight or obesity, although in East Asians the proportion with obesity might be somewhat less (10,114,115). Type 2 diabetes in adolescence is more common in females than males with a ratio of approximately 2:1, the reason for this is not yet known (10,116). In longitudinal study of children with obesity, the progression of IGT to T2D was rapid and associated with high baseline BMI, weight gain to follow-up and African American ethnicity (117). IGT and the progression to IGT/T2D in children and adolescents is, like in adults, characterized by a reduction of β-cell function in the presence of hyperinsulinemia (117–121). The disease development is gradual, which is reflected by the finding that high fasting or 2-hour glucose within the normal range is associated with reduced β-cell response to glucose and lower insulin sensitivity in childhood obesity.
Another important risk factor is hepatic steatosis that is associated with worse insulin sensitivity and secretion and with the worsening of glucose tolerance in children and adolescents with obesity (125). Some risk factors of diabetes might have effect very early in life as offspring of diabetic mothers have an increased risk of early onset T2D, interestingly this is strongly associated with intrauterine hyperinsulinemia (126,127). Other factors of potential importance are elevated plasma free fatty acid (FFA) concentrations in childhood which is associated with hyperinsulinemic response to OGTT while in adolescence high FFA concentrations were associated with delayed insulin secretory response (128). Interestingly, a recent study placed a hyperinsulinemic response to OGTT proximal to obesity in the pathophysiological chain of events (129). Genetic risk is likely to be important in the development of adolescent IGT/T2D as the vast majority of adolescents developing the disease have a family history of diabetes (116). One recent study showed that a high T2D risk allele of the TCF7L2 gene was associated with impaired β-cell function and IGT/T2D development in adolescence (130).

Consequences and treatment of type 2 diabetes in childhood and adolescence

The risk associated with IGT early in life is illustrated by studies showing that high 2-hour glucose concentrations, along with high fasting glucose concentrations, BMI and low HDL cholesterol concentrations are significant predictors of T2D in adulthood (131,132). Furthermore, glucose intolerance in childhood was associated with increased mortality before 55 years of age (133). Together this suggests that prediabetes is a valid label of IFG and IGT although more longitudinal studies are warranted. Apart from the long-term risk, adolescents with T2D develop signs of complications to hyperglycemia before the age of 18, such as microalbuminuria (134,135) and retinopathy (136,137).

The results from the few clinical trials in adolescent T2D are somewhat disheartening. In the TODAY trial the time to failure of treatment with metformin was short with less than 50% of patients reaching glycemic target with metformin alone after three years (138). The addition of Rosiglitazone improved treatment outcome somewhat. In the RISE study, neither metformin nor metformin together with insulin could halt the progressive loss of β-cell function (139). Other treatment options such as bariatric surgery and very low calorie diet have showed more promise (140,141).

In summary, T2D in children is still rare in many parts of the world but rising numbers are observed. It is a serious illness associated with rapid progression and increased mortality and it is difficult to treat. This emphasizes
the need to better understand the pathophysiology of IGT and T2D in childhood and adolescence to better identify those at risk.

**Proglucagon-derived peptides**

The proglucagon gene is expressed by the pancreatic alpha-cells and by enteroendocrine L-cells in the gastrointestinal tract (142). Different post-translational processing in the different tissues yields different sets of peptides (142,143). Whereas pro-hormone convertase 2 (PC2) yields glucagon along with glucagon-related polypeptide and major proglucagon fragment, processing by pro-hormone convertase 1/3 (PC1/3) yields glucagon-like peptide (GLP) 1 and 2 along with glicentin and oxyntomodulin (Figure 1) (144). Pancreatic alpha-cells express mainly PC2 to produce glucagon and enteroendocrine L-cells express mainly PC1/3 to produce GLP-1 and GLP-2. Under certain circumstances such as after pancreatectomy the gut produces glucagon in physiologically relevant amounts (145). Likewise, under stress from hyperglycemia, hyperlipidemia or inflammation pancreatic alpha-cells produce GLP-1 (146,147). Due to the potential multiple cellular origins of both glucagon and GLP-1, the specific origin of these two hormones cannot be determined in plasma. Also, depending on assay antibody specificity quantification of glucagon can be confounded by glicentin and oxyntomodulin.

Figure 1. Schematic illustration of the results of post-translational processing of proglucagon by pro-protein convertase 1/3 (PC1/3) occurring predominantly in enteroendocrine glucagon expressing cells and by pro-protein convertase 2 (PC2) occurring predominantly in alpha-cells of the pancreas.

Glucagon signals through the G-protein coupled glucagon receptor, increasing intracellular cyclic-AMP (cAMP) and protein-kinase A (PKA) activity.
In many ways glucagon opposes the actions of insulin as it promotes glycogenolysis and gluconeogenesis while inhibiting glycolysis in the liver, resulting in increased glucose output from the liver and elevated blood glucose (148). Glucagon also affects hepatic lipid metabolism through inhibition of acetyl-CoA carboxylase resulting in decreased fatty acid synthesis and increased fatty acid oxidation (149). It also affects this pathway in adipose tissue along with an activating effect on hormone-sensitive lipase, increasing mobilization of fatty acid stores in adipocytes (150). Also protein and amino acid metabolism is affected by glucagon as amino acid catabolism is increased by glucagon (151). Glucagon thus works to promote mobilization of fat stores for energy and increases the use of amino acids for substrates for glucose production in the fasting situation. Other effects of glucagon include decreased appetite and increased energy expenditure (152,153). Elevated glucagon is a feature of both T1D and T2D (154,155). Glucagon is high also in obesity and further elevated in subjects with impaired glucose tolerance, accompanied by elevated endogenous glucose production (156,157).

GLP-1 is, together with glucose-dependent insulinohippolic polypeptide (GLP), conveying the incretin effect, i.e. the larger insulin response to blood glucose elevation through ingested rather than injected glucose. The stimulation of insulin secretion by GLP-1 is glucose-dependent, that is the higher glucose concentration the larger the effect of GLP-1 (158). The big effect of GLP-1 on blood glucose lowering is demonstrated by the completely normalized plasma glucose concentrations in type 2 diabetes patients with poor glycemic control upon GLP-1 infusion (159). Other important effects of GLP-1 in blood glucose regulation post-prandially is the inhibitory effect on gastric emptying and inhibition of glucagon secretion (160,161). Besides the effects of GLP-1 on blood glucose regulation it also lowers appetite and reduces food intake (162). Treatment with GLP-1 agonists lowers blood glucose and body weight and is associated with reduced cardiovascular morbidity and mortality in patients with T2D (163,164).

The two forms of GLP-1 with biological effects are the 7-36-NH2 and 7-37 forms, the latter less abundant in humans (165). The biologically intact GLP-1 is rapidly cleaved in the circulation by dipeptidyl-peptidase 4 (DPP-4) to biologically inactive form 9-36-NH2 (166). The result of this is that the majority of secreted GLP-1 is degraded and in its inactive form upon reaching the systemic circulation (167). Instead, the biological effects of GLP-1 are at least in part conveyed through effect on nerve cells locally in the gut, signaling through the brain to several target tissues including the pancreas (168). DPP-4 exists in both membrane bound and soluble form (169). Apart from its metabolism of GLP-1 it also degrades other peptides such as neuropeptide Y and peptide YY and also glucagon to lesser extent (170,171). Fur-
thermore, DPP-4 is involved in the immune system through its role in T-cell development and activation (172).

Glicentin is also secreted from L-cells together with GLP-1 and oxyntomodulin. There is no known glicentin receptor and only a few studies of glicentin (173). After gastrectomy and gastric bypass surgery glicentin concentrations increase in the circulation, similar to what happens with GLP-1 concentrations in these conditions (174,175). There are a few studies showing that glicentin might share some biological effects with other proglucagon-derived peptides, for instance modulating gastrointestinal motility and stimulation of insulin secretion (176,177). However, other groups did not find any effect on insulin secretion and whether glicentin has any meaningful physiological effects in humans remain unclear (178,179).

In summary, the proglucagon gene gives rise to a set of peptides central to blood glucose regulation and metabolism with in some cases overlapping and in some cases opposing effects.
Aims

The aim of this thesis was to investigate the involvement of glucagon, GLP-1 and inflammation in impaired glucose tolerance and type 2 diabetes in childhood obesity.

Specific aims of the thesis were:
1. To study the fasting and postprandial plasma concentrations of the proglucagon-derived hormones glucagon, intact GLP-1 and glicentin in adolescents with obesity and NGT, IGT and T2D.
2. To investigate circulating DPP-4 in children and adolescents and its association with circulating intact GLP-1, BMI, glucose tolerance and visceral, subcutaneous and liver fat compartments.
3. To investigate mechanisms for hyperglucagonemia in childhood obesity by, firstly studying the association between fasting plasma glucagon and fat compartments, plasma free fatty acids (FFAs) and triglycerides and secondly studying glucagon secretion from isolated islets of Langerhans in response to elevated FFAs.
4. To identify novel proteins related to inflammation and childhood obesity and obesity-associated phenotypes such as IGT, T2D, hyperinsulinemia and hyperglucagonemia.
Materials and methods

Study population

Study subjects included in this thesis were part of the Uppsala Longitudinal Study of Childhood Obesity (ULSCO) cohort (11). The cohort was started in 2012 at the childhood obesity clinic at Uppsala University Children’s Hospital. Children and adolescents with obesity and below 18 years of age referred to the childhood obesity clinic at Uppsala University Children’s Hospital were invited to participate in the study. Lean children and adolescents were recruited by advertisement among the hospital staff and at a local school. The age spans of the subjects included in the thesis were 10-18 years (Paper I), 7-18 years (Paper II), 5-19 years (Paper III) and 5-18 years (Paper IV). Paper III was a two-center study that also included children and adolescents from the childhood obesity clinic at Paracelsus Medical University (PMU) Hospital in Salzburg, Austria. Both centers were part of the European Commission 7th Framework project ‘Beta-cell function in juvenile type 2 diabetes and obesity’ (Beta-JUDO). At PMU, children and adolescents with overweight or obesity that visited the clinic either through self-referral or referral from a physician were invited to participate. Those included in the present studies visited the clinic between April 2012 and October 2016. BMI was calculated as weight/height² and obesity defined by BMI standard deviation score (BMI SDS) ≥2 according to the WHO 2006/2007 growth reference (180,181). Lean controls had BMI SDS of >-2 and ≤1. In paper III, six individuals with BMI SDS between 1 and 2 (i.e. with overweight but not obesity) were included as part of the obesity group. Exclusion criteria were a known diagnosis of T1D or ongoing antihyperglycemic medication. The definition of T2D was fasting plasma glucose of ≥7 mmol/l and/or 2-hour glucose during OGTT ≥11.1 mmol/l. The definition of IGT was 2-hour OGTT plasma glucose of ≥7.8 and <11.1 mmol/l and fasting plasma glucose of <7 mmol/l. The definition of NGT was 2-hour glucose <7.8 mmol/l and fasting plasma glucose of <7 mmol/l. Written informed consent was given by study subjects and legal guardians. The local ethics committee approved of the studies (ref no 2012/318).
Blood sampling

Blood was sampled from a venous catheter inserted after the application of a local anesthetic patch to the skin (EMLA, AstraZeneca, Cambridge, UK). Blood samples for biobank storage were collected in EDTA or P800 tubes (Becton Dickinson, Franklin Lakes, NJ, USA), the latter containing inhibitors of elastase, proteinase and DPP-4. Samples for biobank storage were immediately put on ice and centrifuged at 4°C at 10 000 RPM for 10 minutes after which plasma was aliquoted and stored in -80°C. The samples were stored in -80°C within 45 minutes from sampling.

Oral glucose tolerance test

Study subjects visited the clinic in the morning after an overnight fasting of at least 10 hours and are instructed to avoid any big changes to diet or level of physical activity in the three days before the visit. Fasting blood samples were obtained and thereafter subjects drank a solution of glucose (APL, Stockholm, Sweden) 1.75 g/kg body weight but maximum 75g glucose dissolved in 300 ml water. To be included in the studies subjects had to finish the glucose solution within 5 minutes. Blood samples were collected at 5, 10, 15, 30, 60, 90 and 120 minutes after finishing glucose ingestion.

Plasma sample analyses

Enzyme-linked immunosorbent assays (ELISA) were used to quantify plasma insulin, glucagon, and glicentin concentrations (Mercodia, Uppsala, Sweden). The glucagon assay uses two end-viewing antibodies to minimize cross-reactivity to other proglucagon-derived peptides. To quantify plasma total GLP-1 concentrations a luminescence ELISA that detects amidated forms of GLP-1 (including intact 7-36-amide and metabolite 9-36-amide) was used (Mercodia). To quantify plasma intact GLP-1 concentrations an electrochemiluminescence immunoassay that detects intact amidated GLP-1 (7-36-amide) and with 30% cross-reactivity with 7-37 GLP-1 was used (MSD, Rockville, MD, USA). Plasma DPP-4 concentrations were analyzed by sandwich ELISA (R&D Systems, Minneapolis, MN, USA) and plasma DPP-4 activity measured by an enzyme activity kit with a chromogenic substrate for DPP-4 (Enzo Life Sciences, Farmingdale, NY, USA). Hepatocyte growth factor (HGF) was analyzed by an electrochemiluminescence immunoassay (MSD). Plasma FFA concentrations were analyzed by ultra-performance convergence chromatography (UPC²) coupled with tandem mass-spectrometry. Nonanoic (C9:0) and heptanoic (C17:0) fatty acids were added to plasma samples as internal standards to allow for quantification. To
extract lipids, plasma samples were mixed with a isopropanol, heptane and hydrochloric acid mixture after which one ml of water was added to the sample and lipids were extracted into heptane. The heptane phase was dried and redissolved in heptane and stored in -20° before analysis.

For screening of proteins related to inflammation a 92-plex proximity extension assay (PEA) was used (Olink Biosciences, Uppsala, Sweden). The assay utilizes two antibodies for each protein carrying complementary oligonucleotide strands. When bound at the target protein these come into proximity of one another and can form a double-stranded DNA sequence. These can then be amplified by quantitative polymerase chain reaction (qPCR) and thereby provide relative protein abundance in plasma. The output is as normalized protein expression (NPX) on a log2-scale. For 24 proteins, plasma concentrations were below limit of detection in more than 25% of subjects and these were removed from further analyses. One protein, Brain-derived neurotrophic factor (BDNF) was removed due to technical issues with the antibody. Thus, 66 proteins were included in further analyses.

For insulin, glicentin, DPP-4, FFA, inflammatory proteins and HGF quantification biobank EDTA plasma was used. For glucagon and GLP-1 quantification biobank P800 plasma was used.

Glucose was measured by glucose dehydrogenase method, C-reactive protein (CRP) by turbidimetry, triglycerides, LDL cholesterol and HDL cholesterol by photometry and ALAT by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method all on Abbott Architect instrument (Abbott Diagnostics, Lake Forest, IL, USA) at the Uppsala University Hospital central laboratory from fresh plasma samples.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) was performed in close proximity to the OGTT, for most individuals on the same day. MRI scans were performed after the OGTT and after a light meal. Scans were run 16.8 cm along the craniocaudal axis with center on L2-L3 vertebrae. Water-fat imaging techniques were used. Adipose tissue volumes were determined by a fully automated segmentation method separating the visceral adipose tissue (VAT) that is surrounded by lean tissues from the subcutaneous adipose tissue (SAT) that is not (182). One operator delineated the pancreas and liver volumes in the water images using the ImageJ software (version 1.42q, http://rsbweb.nih.gov/ij/). Median fat fraction of the whole segmented liver volume was used as the estimated liver fat fraction (LFF). The mean of two separate segmentations of the pancreas was used as the estimated pancreatic fat fraction (PFF).
Human islets

Isolated human islets from three adult donors were obtained from the Uppsala University Islet Transplantation Unit. Islets were picked up individually to reduce the residual exocrine tissue content and obtain pure islets. Culture was done in CMRL 1066 medium (Thermo Fisher, Waltham, MA, USA) at 5.5 mmol/l glucose and supplemented with 10% fetal bovine serum (Thermo Fisher) and 0.5% fatty acid free bovine serum albumin (BSA, Roche Diagnostics, Basel, Switzerland). Ethical permission to use human islets isolated from healthy individuals has been obtained from the local ethics committee (Ref no 2010/006). For fatty acid treatment, palmitate, palmitoleate, oleate and stearate were dissolved in 50% ethanol to create a 100 mmol/l stock solution except for palmitoleate which was dissolved in 97% ethanol to a concentration of 200 mmol/l. Stock solutions were diluted in culture medium to a concentration of 0.5 mmol/l fatty acid. The four different 0.5 mmol/l FFA media were mixed in equal parts to create a final medium with a total FFA concentration of 0.5 mmol/l and equal concentration of the four different FFAs. Thirty human islets were cultured in the presence or absence of the 0.5 mmol/l FFA mix for 24 hours. The medium was then collected and glucagon concentration measured by ELISA (Mercodia).

Pathway analysis of inflammatory markers

Enrichment analysis was done by examining KEGG pathways that were overrepresented when comparing the proteins with differing concentrations in obesity to the ones not differing in obesity. Pathways with false discovery rate (FDR) <0.05 in the analysis of differing proteins and with a false discovery rate >0.05 in the analysis of non-differing proteins were considered uniquely enriched among differing proteins. Upstream regulator analysis was performed by Ingenuity Pathway Analysis (IPA; QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) on the 27 proteins differing in obesity. Upstream regulators with an activation z-score ≥2.0 or ≤2.0 were considered significant and reported. Molecule types “chemical – kinase inhibitor”, “chemical drug”, “chemical reagent”, “chemical toxicant”, “chemical – endogenous mammalian” and “chemical non-endogenous mammalian” were excluded from the analysis.

Calculations and statistical analysis

β-cell function (paper I) was measured by the oral disposition index as the product of 1/insulin_{fasting} and the insulinogenic index ((insulin_{30 min} - insulin_{0 min})/(glucose_{30 min} - glucose_{0 min})). Early-phase hormone response (paper I)
during OGTT was estimated by a stimulation index, calculated as the hormone concentration at 30 min into the OGTT divided by fasting concentration. Area under the curve was calculated by the trapezoid method with 0 as baseline. Percent intact GLP-1 (paper II) was calculated as intact/total GLP-1 at fasting or AUC multiplied by 100. Differences between two groups (papers II, III and IV) were tested by Student’s T-test or Mann-Whitney U-test as appropriate according to normally or non-normally distributed data. Normality of data was determined by Shapiro-Wilk test and assessment of histograms, box-plots and QQ-plots. Differences between several groups were tested by one-way ANOVA or Kruskal-Wallis test with post hoc Fisher’s least significant difference or Dunn’s test comparing all groups with the NGT group (paper I) or lowest to highest quartile of plasma glucagon (paper III). Where linear regression models were applied, data was log-transformed if residuals were non-normally distributed or there was heterogeneity of variance. In paper II, linear regression models were applied with plasma DPP-4 as dependent variable and anthropometric measurements, fat compartments and biochemical analytes as independent variable in separate models with and without age and sex as covariates included in all models. In paper III, linear regression models were applied with glucagon as dependent variable and anthropometric measurements, body fat compartments, plasma lipids, insulin and glucose as independent variable in separate models with BMI SDS, age, sex, family history of diabetes and study centre as covariates included in all models. In paper IV, in the analyses including the entire panel of proteins, multiple testing was adjusted for by the Benjamini-Hochberg FDR method with FDR=0.05 throughout. In paper IV, logistic regression was used to determine the odds ratio of weight gain after follow-up by quartile of baseline plasma HGF concentrations with age, BMI SDS at baseline and time to follow-up as covariates.
Results and discussion

Glucagon

Fasting hyperglucagonemia was present in children with obesity and NGT and gradually increased in IGT and T2D (Table 1, Paper I). There was also a significant association between fasting plasma glucagon and two-hour glucose after adjusting for age, sex and family history of diabetes (Table 3, Paper III). The response of glucagon to an oral glucose challenge was intact in obesity and IGT (Fig 1B, Paper I and Fig 5, Paper III). However, in T2D although glucagon concentrations decreased, they stayed elevated throughout the OGTT (Fig 1B, Paper I). Fasting hyperglucagonemia is found also in adults with obesity and is further elevated in T2D (183). In children with obesity, high plasma glucagon concentrations relative to the ambient glucose concentrations was found in T2D subjects (184,185). One study found higher fasting glucagon concentrations in adolescents with obesity and IGT (186). The early hyperglucagonemic response to OGTT seen in T2D adolescents (Fig 1B, Paper I) is found during OGTT also in adults with T2D. However, the same glucose excursion from intravenously administered glucose does not give this response (187). Thus, the mechanism explaining the hyperglucagonemic response is likely found in the gastrointestinal tract. One candidate would be glucose-dependent insulinoitropic polypeptide (GIP), which is known to stimulate glucagon release (188). However, preliminary data from our group show that GIP secretion is the lowest in T2D (189). Another possibility is that GLP-1 secreting L-cells in the gut shift towards production of more glucagon than GLP-1. This was shown to happen in a cell-line model upon treatment with FFAs (190).

The hyperglucagonemia in childhood obesity was found to be associated with hyperinsulinemia, hypertriglyceridemia and elevated FFAs (Fig 2, Paper III). Furthermore, hyperglucagonemia was associated with high visceral rather than subcutaneous adipose tissue (Fig 3, Paper III). This is in line with the finding that hyperglucagonemia has been associated with a high waist-hip-ratio in adults (191). To test whether elevated blood lipids is a potential stimulant of glucagon secretion, isolated human islets were treated with a mixture of FFAs. Treatment of isolated islets with FFAs induced more than a doubling of glucagon secretion at basal glucose concentrations (Fig 6, Paper III). Work from our group has shown that the glucagon response to FFAs is likely the result of beta-oxidation of the FFA and signaling through G-
protein coupled receptor (GPR) 40 (192). The reduced glucagon secretion in
the alpha-cell carnitine palmitoyl transferase-1 (CPT-1) knockout mouse
demonstrates the importance of beta-oxidation of FFAs for glucagon secre-
tion (193). High plasma glucagon concentrations has been associated with
elevated fasting plasma triglyceride concentrations and plasma glucagon
concentrations increases after lipid rich meals (194,195). However, acutely
elevated plasma FFA concentrations through the infusion of heparin lowered
glucagon secretion in man, possibly reflecting differences in acute and long-
term effects of FFAs on glucagon secretion (196).

In the MRI studies, high plasma glucagon concentrations were associated
with hepatic steatosis (Table 2, Paper III). This association was found also
in adults with non-alcoholic fatty liver disease and associated with elevated
amino acids (197). This is interesting as studies of glucagon receptor knock-
out mice identified amino acids and in particular glutamine as a causal factor
in alpha-cell proliferation (198).

The strongest correlate overall of hyperglucagonemia was hyperinsulin-
emia (Table 3, paper III). The combined hyperglucagonemia and hyperinsu-
linemia has been shown previously in adults (199). Interestingly, high fast-
ing plasma insulin concentrations were associated with a worse suppression
of glucagon in the first 10 minutes of OGTT (Fig 5, Paper III). The findings
of worse early glucagon suppression in hyperinsulinemia was shown also in
adults (200). One possible interpretation of this is that a high sensitivity of
alpha-cells to paracrine inhibition by β-cells is important for the early gluca-
gon response to OGTT. In summary, plasma concentrations of insulin and
glucagon increase in parallel in obesity and impaired glucose tolerance in
childhood and adolescence although the insulin increase is greater than the
glucagon increase.

While glucagon receptor antagonism lowers blood glucose in T2D, this
might be at the expense of increased blood lipids and hepatic steatosis (201).
This is in line with mechanistic studies in the glucagon receptor knock-out
mouse that have reduced liver beta-oxidation of lipids in favor of triglyceride
formation and are prone to develop hepatic steatosis (202). On the other
hand, glucagon receptor agonism together with GLP-1 receptor agonism
seems to be favorable in T2D patients with obesity (203). It is possible that
glucagon receptor antagonism would be more appropriate in T2D stages
more characterized by absolute insulin deficiency. In childhood and adoles-
cent obesity, characterized by hyperinsulinemia, glucagon and GLP-1 recep-
tor co-agonism might be a better option in terms of potential future treat-
ment.
GLP-1, Glicentin and DPP-4

Plasma concentrations of intact GLP-1 (iGLP-1) and glicentin during OGTT were highest in lean controls and decreased with lowering glucose tolerance in children and adolescents with obesity (Fig 2C&D, Paper I). In line with this, also plasma total GLP-1 (tGLP-1) concentrations were lower during OGTT in children and adolescents with obesity (Table 1, Paper II). As expected, iGLP-1 concentrations were low compared to glicentin or tGLP-1 concentrations but the pattern across the glucose tolerance spectrum was similar. When viewed together with the changes in glucagon, there was a shift from intestinally to pancreatically processed proglucagon in the plasma of adolescents with obesity and more so in IGT and T2D (Fig 3D, Paper I). A reduced incretin effect has been found in T2D in adolescents with obesity (185,204). This was not coupled to changes in GLP-1 secretion, although in both studies there were hardly any stimulation of the GLP-1 release upon oral glucose intake. Important to note is that the reduced incretin effect observed in obesity with NGT was largely due to an exaggerated insulin response to iv glucose (204). In adults, GLP-1 concentrations during OGTT is modestly reduced in T2D and obesity (205).

The circulating DPP-4 concentrations were slightly higher in obesity than in lean controls (Fig 1A, Paper II). One previous study of childhood obesity found that plasma DPP-4 concentrations decreases with weight loss after a one-year lifestyle intervention (206). The children with obesity had a lower proportion of circulating GLP-1 in its intact form in the fasting state (Table 1, Paper II). In one adult study, although both tGLP-1 and iGLP-1 was decreased in obesity, the AUCOGTT of iGLP was 55% but tGLP-1 was 85% of lean controls (207). Subjects with obesity also had a higher DPP-4 activity in plasma (207). The high DPP-4 concentrations were associated with male gender and a higher waist/hip ratio (Table 2, Paper II). There are reports of higher release of DPP-4 from visceral than subcutaneous adipocytes, potentially explaining this observation (208). While high DPP-4 concentrations were associated with a lower proportion of intact GLP-1 (Fig 2, Paper II) it was not associated with glucose intolerance (Table 3, Paper II). In adults with T2D, plasma DPP-4 was reported to be increased and correlated with glycemic control measured by HbA1c (209,210). The apparent discrepancy could be explained by the fact that while our study included some IGT individuals, none were T2D and HbA1c was normal (Table 1, Paper II). On the other hand, a longitudinal study in adults found baseline DPP-4 activity to be predictive of IGT and T2D (211). Nevertheless, the majority of GLP-1 is metabolized locally in the gut and the local DPP-4 activity might be of greater importance than plasma DPP-4 activity for glucose tolerance (167).

Given the apparent reduction in incretin hormone secretion in children and adolescents and the beneficial effect in adult T2D and obesity with GLP-1 receptor agonist treatments, these are appealing as future treatment in
childhood obesity. There are promising results in terms of reduced BMI with GLP-1 receptor agonists in adolescents with obesity (212).

**Inflammatory markers**

Of the 66 proteins included in the screening of inflammatory markers the following were higher in obesity, in order of largest to smallest fold increase: Fibroblast growth factor-21 (FGF-21), IL-6, Oncostatin M (OSM), NAD-dependent deacetylase sirtuin 2 (SIRT2), AXIN1, STAM-binding protein (STAMBP), Tumor necrosis factor ligand superfamily member 14 (TNFSF14), CUB domain-containing protein 1 (CDCP1), Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), Interleukin-18 receptor 1 (IL-18R1), HGF, C-C motif chemokine 3 (CCL3), Interleukin-18 (IL-18), TNF-related activation-induced cytokine (TRANCE), Cluster of differentiation 40 (CD40), Vascular endothelial growth factor A (VEGF-A), C-C motif chemokine 19 (CCL19), Interleukin-10 receptor subunit beta (IL-10RB), Fibroblast growth factor-23 (FGF-23), Macrophage colony-stimulating factor 1 (CSF-1), Caspase-8 (CASP-8) and TNF-related apoptosis-inducing ligand (TRAIL) (Fig 1, Paper IV). The following proteins were lower in obesity, in order of largest to smallest decrease: Fibroblast growth factor-19 (FGF-19), TNF-related weak inducer of apoptosis (TWEAK), Delta- and Notch-like epidermal growth factor-related receptor (DNER) and Tumor necrosis factor-beta (TNFB) (Fig 1, Paper IV).

Pathway analysis revealed that the top uniquely enriched pathways among the proteins differing in obesity were the PI3K-Akt, Ras and Rap-1 signaling pathways (Table 3, Paper IV). A common effect shared by these pathways is increased cell survival and proliferation and they have been implicated in the observed elevated cancer risk in obesity (213). The PI3K-Akt signaling pathway is also of importance for insulin signaling and secretion (214). Interestingly, individuals with loss-of-function variants in the Phosphatase and tensin homolog (PTEN) gene, a negative regulator of PI3K-Akt signaling have increased cancer risk along with increased insulin sensitivity and adiposity (215). Thus, an upregulation of the PI3K-Akt pathway could contribute to maintaining obesity. Upstream regulator analysis revealed a pattern of pro-inflammatory cytokines as predicted to be upregulated and anti-inflammatory cytokine IL-10 as predicted to be inhibited (Table 4, Paper IV).

The observed associations between the inflammatory proteins and IGT, hyperinsulinemia and hyperglucagonemia are presented in Figure 2. The only protein associated with glucose intolerance was TWEAK, which was lower in children and adolescents with IGT/T2D (Fig 4A, Paper IV). Interestingly, lower TWEAK was also associated with hyperinsulinemia and hyperglucagonemia (Fig 4B&C, Paper IV). This protein is a member of the
TNF-family and also known by the name TNF superfamily member 12 (TNFSF12). In agreement with our findings, low plasma TWEAK concentrations were shown in adults to be predictive of incident T2D and associated with fasting hyperinsulinemia (216). Interestingly, while soluble TWEAK (sTWEAK) is lower in obesity, membrane bound TWEAK (mTWEAK) in subcutaneous adipose tissue is higher (217). In the adipose tissue, primarily anti-inflammatory M2 macrophages seem to express TWEAK while adipocytes express its receptor Fn14 (217). Accordingly, pretreatment of adipocytes with TWEAK reduced TNF-α induced gene expression, indicating a role of TWEAK in moderating TNF-α signaling (217). Whether a low sTWEAK is predictive of glucose intolerance in children and adolescents warrants further longitudinal study.

![Figure 2](image)

Figure 2. Summary of observed associations between inflammatory markers and impaired glucose tolerance, hyperglucagonemia and hyperinsulinemia in children and adolescents with obesity. Green text indicates a positive association and red text indicates a negative association. * Association with glucagon not independent of age. † Association with insulin not independent by age.

In children and adolescents with high insulin, FGF-21, IL-18R1, HGF and CDCP1 were elevated. The IL-18R1 is a subunit of the IL-18 receptor complex, known to circulate and with the potential to decrease IL-18 signaling (218). It’s known that genetic loss of IL-18 signaling causes hyperphagia and obesity in mice (219). On the other hand we found IL-18 to be increased in obesity, similar to previous findings in adults and children (220). A re-
duced effect of IL-18 in obesity, which was demonstrated in isolated leukocytes, might explain this apparent paradox (221).

The involvement of CDCP1 in obesity and hyperinsulinemia has not been shown previously. CDCP1 is a membrane-bound protein that functions as a substrate for src-kinases. Increased CDCP1 activity is associated with tumor cell proliferation and migration and thereby increased metastasis (222). The cleavage of a 65-kDa extracellular fragment of CDCP1 increases its activity and among known intracellular pathways activated is the PI3K-Akt signaling pathway (223). This is of interest given the central role of PI3K-Akt in both insulin signaling and secretion.

In a longitudinal assessment, children with high plasma HGF concentrations at baseline increased in average 0.1 BMI SDS units and those with low plasma HGF concentrations decreased on average 0.36 BMI SDS units (Fig 6A, Paper IV). The risk of weight gain to follow-up was higher with high baseline HGF independent of baseline BMI, age or time to follow-up (Fig 6F, Paper IV). A correlation between plasma HGF concentrations and obesity in adolescents has been shown previously in cross-sectional study (224). Increased HGF seems to promote insulin signaling and also β-cell proliferation in situations of increased insulin demand such as pregnancy or high-calorie diet (225–227). Given the anabolic and obesity-promoting effect of insulin (228), a combined increased insulin sensitivity and production would be one possible mechanism through which HGF could cause weight gain.

Strengths and limitations

The strengths of the studies in this thesis include the careful characterization of phenotype with MRI measurements and blood samples not only in adolescence but also childhood and combined by in vitro islet work. By studying younger subjects there is likely less confounding by comorbidities or concomitant medication and allows for characterization of the early stages of disease development in obesity and glucose intolerance. The recruitment to the ULSCO cohort of lean controls in the same age span as the children and adolescents with obesity is a strength of the studies. However, among those asked to participate as controls only a few accepted. Also, some controls were recruited by announcement at the hospital leading to a substantial part of controls being offspring to hospital staff. Thus there is a risk that the lean controls are selected from a different socioeconomic background than the average child at the obesity clinic. One limitation of particular importance is the cross-sectional nature of most studies of the thesis that stops us from being able to study the timing of the various events. As the work of the ULSCO cohort proceeds and more patients are followed, the observations of this thesis should be studied longitudinally.
Summary and conclusions

In summary, IGT and T2D in children and adolescents with obesity were associated with hyperglucagonemia particularly at fasting and this is associated with fasting hyperinsulinemia. In subjects that develop T2D, increased glucagon concentrations at early time points during OGTT are common, similar to what is seen in adults. At the same time, GLP-1 and glicentin response to OGTT was blunted. The hyperglucagonemia in childhood obesity is particularly associated with elevated visceral adiposity and higher circulating lipids. DPP-4 was elevated in childhood obesity and associated with lower proportion of GLP-1 in its intact form. However, as this was not associated with IGT it is likely not of importance for obesity-related impairment of glucose metabolism early in life. Potential drivers of the parallel increase in insulin and glucagon was identified in elevated free fatty acids and lowered fasting plasma TWEAK, which was also associated with IGT. A novel obesity- and hyperinsulinemia-associated protein was discovered in CDCP1. The obesity state in children and adolescents is associated with elevation of multiple pro-inflammatory cytokines, particularly related to the PI3K-Akt pathway. However, these were mostly not associated with IGT and T2D development. Thus, inflammation seems closely related to the obesity state but independent of IGT or T2D development. Finally, HGF was found to be associated with further weight gain in children and adolescents with obesity during lifestyle intervention. This positions HGF as a potential marker to identify individuals with obesity that are particularly hard to treat and that might benefit from other forms of interventions earlier.

In conclusion, IGT and T2D development in childhood and adolescent obesity is characterized by increased glucagon but reduced GLP-1 and glicentin, indicating that these are early events in obesity-related impairment of glucose metabolism. These results point to altered pro-glucagon processing and GLP-1 agonism as a potential future treatment of promise to halt IGT and T2D development in children and adolescents with obesity.
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