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LONG-ACTING INSULIN ANALOGS IN TYPE 1 DIABETES - EFFECTS ON METABOLIC CONTROL, ENDOGENOUS INSULIN PRODUCTION AND THE GH-IGF-AXIS

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Long-acting insulin analogs in type 1 diabetes - effects on metabolic control, endogenous insulin production and the GH-IGF-axis

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To my family

ABSTRACT

The treatment goal in type 1 diabetes is to achieve near-normal glycemia. Despite of the advancements of subcutaneous insulin therapy and glucose monitoring, metabolic control is not fully normalized and secondary endocrine disturbances in the growth-hormone (GH) - Insulin-like Growth Factor (IGF)-axis are important for the deterioration of metabolic control, particularly in children at puberty. The long-acting insulin analogs, glargine and detemir, have prolonged effect duration compared to intermediate-acting NPH insulin. Sustained nightly insulin actions could be particularly important in pubertal children with type 1 diabetes by opposing the low IGF-I production and increase feedback inhibition of elevated GH. Even the successive decline in endogenous insulin production could be affected. The purpose of this thesis was to evaluate if glargine or detemir, compared to NPH, could improve metabolic control, prolong endogenous insulin production and reverse the abnormalities in the GH-IGF-axis in children and adolescents with type 1 diabetes.

In **Paper I** we studied the effects of changing insulin therapy from NPH to glargine for up to 12 weeks on the GH-IGF-axis and metabolic control in 12 pubertal subjects with type 1 diabetes. A fifty percent increase in IGF-I levels, decreased overnight IGFBP-1 secretion and unchanged overnight GH secretion were associated with a 1 % unit (10 mmol/mol) lower (P= 0.008) 12-week HbA1c. These findings indicate that glargine reverses some of the abnormalities in the GH-IGF-axis and improves metabolic control. Suppression of IGFBP-1 suggests that hepatic insulin sensitivity is improved.

In **Paper II** we retrospectively compared the first 49 children and adolescents that we treated with glargine from diagnosis of type 1 diabetes with 49 patients treated with NPH, for up to one year. We found 0.8 % unit (8 mmol/mol) lower (P < 0.01) 12-month HbA1c and lower insulin requirements in the glargine treated subjects without affecting weight gain. These findings support a long-term improvement of HbA1c in children and adolescents treated from diagnosis with glargine. We hypothesized that improved metabolic control and lower insulin requirements could result from normalization of the GH-IGF-axis and/or improved endogenous insulin production.

In **Paper III** we randomized children and adolescents stratified for puberty to treatment with glargine or detemir vs. NPH from diagnosis of type 1 diabetes. We found 9 mmol/mol (0.9 % unit) lower (P = 0.008) 12-month HbA1c and lower fasting-glucose with glargine or detemir in pubertal children, with no difference in prepubertal children. Meal-stimulated C-peptide AUC or glucose variability by CGM did not differ. These findings demonstrate that long-term improvement of metabolic control is obtained with glargine or detemir treatment but not associated with improved preservation of beta cell function.

In **Paper IV** we reported changes in the GH-IGF-axis in the subjects studied in paper III. We found lower 12-month IGFBP-1 with glargine or detemir in pubertal subjects. IGF-I SDS was subnormal from diagnosis throughout the 12 months study, particularly low in the pubertal individuals, and did not differ among the treatment groups. Lower IGFBP-1 suggests that hepatic insulin action is improved, which may have contributed to the improved 12-month HbA1c. However, the improved hepatic insulin action with long-acting insulin analogs was not sufficient to normalize IGF-I.

In summary, this thesis supports that long-acting insulin analogs, glargine or detemir, are used from diagnosis of type 1 diabetes in pubertal children with insulin injection therapy to improve metabolic control. Although endogenous insulin secretion is not better preserved and IGF-I remains subnormal, hepatic insulin sensitivity may be improved as indicated by lower IGFBP-1. Given the link between abnormalities in the GH-IGF-axis associated with subcutaneous insulin therapy and the development of diabetic complications new treatment strategies are needed until beta-cell function can be fully preserved.

SAMMANFATTNING PÅ SVENSKA

Typ 1 diabetes orsakas av autoimmun nedbrytning av de insulinproducerande cellerna i bukspottkörteln. De nordiska länderna har högst förekomst i världen av typ 1 diabetes hos barn och ungdomar. Målet med insulin-injektionsbehandlingen är att hålla blodsockret på en så normal nivå som möjligt, vilket kan följas genom kontroll av långtidsblodsockret, HbA1c. Förutom snabbverkande insulin vid måltider ges ett basinsulin med längre verkan. De senaste femton åren har två basinsuliner, glargine och detemir, s.k. analoger med längre verkan, tagits fram som alternativ till medellångverkande NPH insulin. Innan detta avhandlingsarbete påbörjades saknades studier av barn och ungdomar som klart visade att långverkande insulin analoger gav bättre HbA1c över ett år. Bättre insulinverkan på natten kan vara extra viktig under puberteten, för att höja nivåerna av hormon med insulin-liknande effekt (Insulin-like Growth Factor I; IGF-I) och motverka en kompensatorisk höjning av hormon med insulin-motverkande effekt (tillväxthormon; GH). I denna avhandling har vi undersökt om långverkande basinsulin har fördelar framför basinsulin med medellång verkan, vad gäller HbA1c, kroppens egen insulin produktion, som finns kvar under några år från diabetes insjuknandet och nivåerna av GH-IGF-axelns hormoner.

I **studie I** undersökte vi effekterna på GH-IGF-axeln och HbA1c efter byte av basinsulin-behandling från NPH till glargine under 12 veckor hos 12 pubertala ungdomar med typ 1 diabetes. Vi fann ett väsentligt lägre HbA1c, markant ökning av IGF-I, sänkt nattligt IGFBP-1 samt oförändrad nattlig nivå av GH. Dessa fynd talar för att glargine delvis kan återställa störningar i GH-IGF-axeln samt förbättra långtidsblodsockret.

I **studie II** samlade vi in data från 49 barn och ungdomar, som vi behandlade från diagnos av typ 1 diabetes med glargine, efter att det blivit tillgängligt, och jämförde med 49 barn och ungdomar, som tidigare behandlats med NPH med samma omhändertagande. Vi visade att glargine-behandlade patienter hade väsentligt bättre HbA1c och samtidigt lägre insulindos efter 12 månader. Detta talar för att behandling med glargine från diabetesdebut ger bättre långtidsblodsocker. De första två studierna var grunden för vår hypotes att en återställning av GH-IGF-axeln och/eller förbättrad kroppsegen insulinproduktion är viktig för det förbättrade långtidsblodsockret.

Studie III var en randomiserad kontrollerad studie där vi jämförde basinsulinbehandling med glargine eller detemir med NPH till barn och ungdomar från diagnos av typ 1 diabetes. Vi visade en sänkning av 12-månaders HbA1c och ett lägre faste-blodsocker hos pubertala ungdomar behandlade med glargine eller detemir, men ingen skillnad hos prepubertala barn. Det fanns ingen skillnad i kroppsegen insulinproduktion eller blodsockervariabilitet.

I **studie IV** undersökte vi förändringar i GH-IGF-axeln hos de ungdomar som studerats i studie III. Vi fann lägre 12-månaders IGFBP-1 med glargine eller detemir. IGF-I var subnormalt för åldern under hela studiens första år och det var inga skillnader mellan behandlingsgrupperna. Lägre IGFBP-1 talar för att bättre insulinkänslighet på levernivå kan ha bidragit till att förbättra HbA1c.

Sammanfattningsvis talar denna avhandling för att behandling med långverkande basinsuliner, glargine och detemir, bör användas från diagnos av typ 1 diabetes hos ungdomar i pubertet, för att förbättra den metabola kontrollen. Den kroppsegna insulinproduktionen förbättras inte genom denna behandling men en viss återställning av GH-IGF-axeln sker, talande för att insulinkänsligheten på levernivå är förbättrad. I avvaktan på en behandling, som återställer den egna insulinproduktionen, skulle det behövas en behandling som normaliserar GH-IGF-axeln och minskar risken för komplikationer.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following original articles and manuscripts. The papers will be referred to in the text by their Roman numerals.

I. Ekström K, **Salemyr J**, Zachrisson I, Carlsson-Skwirut C, Örtqvist E, Bang P. Normalization of the IGF-IGFBP Axis by Sustained Nightly Insulinization in Type 1 Diabetes.
Diabetes Care. 2007 Jun;30(6):1357-63

II. **Salemyr J**, Bang P, Örtqvist E.
Lower HbA1c after 1 year, in children with type 1 diabetes treated with insulin glargine vs. NPH insulin from diagnosis: a retrospective study.
Pediatric Diabetes. 2011 Aug;12(5):501-5

III. **Salemyr J*,** Örtqvist E*, Ekström K, Carlsson-Skwirut C, Pulkkinen M-A. Brorsson A-L, Bang P (*contributed equally).
Lower HbA1c with long-acting insulin analogs versus NPH insulin from diagnosis of type 1 diabetes in pubertal children - a Randomized Controlled Trial.
Manuscript

IV. **Salemyr J**, Ekström K, Carlsson-Skwirut C, Brismar K, Örtqvist E, Bang P. Lower IGFBP-1 is a marker of improved hepatic insulin sensitivity in pubertal children treated from diagnosis of type 1 diabetes with long-acting insulin analogs versus NPH insulin.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ALS	Acid-Label Subunit
AUC	Area Under the Curve
BFP	Body fat percentage
BMI	Body Mass Index
CIPII	Continuous Intra Peritoneal Insulin Infusion
CSII	Continuous Subcutaneous Insulin Infusion
CGM	Continuous Glucose Monitoring
DCCT	Diabetes Control and Complications Trial
GAD	Glutamic Acid Decarboxylase
GH	Growth Hormone
GHBP	Growth Hormone Binding Protein
GHR	Growth Hormone Receptor
HbA1c	Glycosylated hemoglobin A1c
HGP	Hepatic Glucose Production
IGF-I	Insulin-like Growth Factor-I
IGFBP3-PA	Insulin-like Growth Factor Binding Protein-3 Proteolysis
IGF-1R	Insulin-like Growth Factor type 1 Receptor
IR	Insulin Receptor
iv.	Intra venous
MAGE	Mean Amplitude of Glycemic Excursion
MIT	Multiple Injection Therapy
MMTT	Mixed Meal Tolerance Test
NPH	Neutral Protamin Hagedorn insulin
PI	Pre-Insulin (before iv.insulin)
PS	Pre-Subcutaneous (before start of subcutaneous insulin)
RCT	Randomized Controlled (clinical) Trial
rhIGF-I	Recombinant human IGF-I
RIA	Radioimmunoassay
SDS	Standard Deviation Score
SMBG	Self Monitored Blood Glucose

THESIS AT A GLANCE

Paper	AIM	METHOD/DESIGN	RESULTS	CONCLUSIONS
I	To study effects of changing from NPH to glargine on the GH-IGF-axis and its association with metabolic control in adolescents with type 1 diabetes.	12 pubertal subjects with type 1 diabetes were studied with blood sampling every 30 min for 20 hours, CGM and microdialysis once on NPH and once 6 weeks after starting glargine. Fasting samples for IGF-I and HbA1c were obtained at 1, 2, 4, 8 and 12 weeks.	HbA1c decreased from 8.3±0.6 to 7.3±0.3%, (P<0.01) at 12 weeks. IGF-I increased by 50 % and the change was inversely correlated with HbA1c. IGFBP-1 decreased while GH was unchanged.	Treatment with glargine normalizes IGF-I. The increase in IGF-I correlates with decreased HbA1c.The decrease in IGFBP-1, suggests that hepatic insulin action is improved and the increase in IGF-I indicate that GH receptor function is normalized.
II	To compare metabolic control during the first year in children with type 1 diabetes treated from diagnosis with glargine vs. NPH.	Retrospective descriptive study of the 49 first patients treated with glargine from diagnosis, compared with 49 consecutive children treated with NPH. HbA1c, reported insulin requirements and change in weight were studied at 3, 6, and 12 months diabetes duration.	12-month HbA1c was lower with glargine vs. NPH (6.3% vs. 7.1%, P<0.01). 12-month insulin dose was lower with glargine (0.6 IU / kg vs. 0.85 IU / kg, P<0.001).	Glargine treatment from diagnosis improves long-term metabolic control. We hypothesized that improved metabolic control and lower insulin requirements could result from normalization of the GH-IGF-axis and/or improved endogenous insulin production.
III	To study if treatment with long-acting insulin analogs, glargine or detemir, in children and adolescents from diagnosis of type 1 diabetes results in better 12-month metabolic control and if this could at least partly be explained by preserved endogenous insulin production.	Prospective 12-month study of 120 children and adolescents, stratified for puberty and randomized at diagnosis to NPH, glargine or detemir (1:1:1) in an MIT regimen with insulin aspart. Patients were studied at diagnosis and at 2 weeks, 3, 6, 9 and 12 months with anthropometric data, bioimpedance, blood samples for HbA1c and fasting-glucose. 3 days CGM for study of glucose variability at 3 occasions. Endogenous insulin production was determined by MMTT (mixed meal tolerance test) at 4 different time-points (2 weeks, 3, 6 and 12 months).	The 12-month HbA1c was lower in children treated with analogs vs. NPH (52.7 ±1.0 vs. 57.9±2.2 mmol/mol, P=0.019). The difference was due to lower HbA1c in children in puberty at inclusion (60.2±3.2 vs. 51.0±1.7 mmol/mol, P= 0.008). At 12 months, f-glucose was lower in pubertal children on analogs vs. NPH (P=0.017).Glucose variability assessed by CGM and endogenous insulin production measured as stimulated C-peptide did not differ. No differences were found between glargine and detemir except for higher insulin dose with detemir.	This is the first randomized controlled trial from diagnosis of type 1 diabetes in children and adolescents to demonstrate lower HbA1c and lower fasting-glucose with glargine or detemir, compared to NPH in pubertal children. These findings could not be explained by improved preservation of beta cell function.
IV	To study if treatment with long-acting insulin analogs from the onset of type 1 diabetes in adolescents results in changes in the GH-IGF-axis incl. increased IGF-I and decreased IGFBP-1	In the same study settings as in paper III. Blood samples for IGF-I and IGFBP-1 were taken before iv.insulin, before randomization to glargine, detemir or NPH and at 2 weeks and 3, 6, and 12 months.	The 12-month IGFBP-1 was lower in pubertal children treated with analogs, glargine or detemir vs. NPH (69.4±6.9 vs. 98.7±13.9 µg/l, P= 0.04). IGF-I SDS was subnormal from diagnosis throughout the 12 months, particularly low in the pubertal individuals, and did not differ among the treatment groups.	Lower IGFBP-1 suggests that hepatic insulin action was improved which may contribute to the improved 12-month HbA1c in children with type 1 diabetes treated with glargine or detemir. However, the improved hepatic insulin action with long-acting insulin analogs was not sufficient to increase IGF-I.

1 INTRODUCTION

In my clinical work I meet children with type 1 diabetes and their families almost every day. I see that they struggle to find a way to cope with the disease and that their daily lives are deeply affected. They live always with the fear of acute and long-term complications. Most of all they have to follow the child's blood sugar levels and repeatedly adjust the insulin dose. It has been said that they must act like a beta-cell, taking blood sugar levels into consideration as often as possible, and use this information to make decisions about insulin dosing. Many of my patients are teenagers who, in addition to all the other issues related to adolescence also struggle with a disease that, owing to hormonal changes, becomes even harder to manage during these years. In order to confront these challenges in a fruitful way, both children and adolescents need all the help they can get from various sources. For one thing, they need insulin therapy with an action profile that mimics the physiological release of insulin.

When I started working in the area of diabetes, insulin analogs, with a target to be short acting and to accompany a meal, had been in use for some years and they were very useful and appreciated by both children and parents. However, no basal insulin could meet the standard of physiological insulinization, especially the increased need for insulin at dawn during puberty (Dunger 1992). In 2002 we started using the long-acting basal insulin analog, glargine, which has a 24 hours action profile (Wang, Carabino et al. 2003). After some months of glargine usage clinical opinion indicated that this treatment was much appreciated and produced a better glucose profile than older treatments. Our research group then decided to investigate whether the clinical observations of improved insulinization could be replicated in research studies. For me, a very clinically oriented medical doctor, with a heart for the patients, this was an ideal entrée into research.

Type 1 diabetes most often emerges early in life and as a chronic disease it cannot be forgotten for a single day. Its development is influenced by both genetic and environmental factors. Incidence is high in Sweden and has almost doubled during the last decades. There are several theories regarding this increase. The goal in treating type 1 diabetes is to maintain blood glucose levels that are as normal as possible. Long-term deteriorated metabolic control leads to both micro- and macro-vascular complications. It has become well known since the large Diabetes Control and Complications Trial (DCCT) was conducted that it is possible to delay these complications with better blood sugar control achieved through intensive insulin treatment (DCCT 1993).

To achieve good metabolic control, insulin treatment must mimic the physiological release of insulin. Insulin regimens that employ the intermediate-acting, Neutral Protamin Hagedorn insulin (NPH) fail to do this (Lepore, Pampanelli et al. 2000), so instead multiple injection therapy (MIT) with long-acting analogs, glargine or detemir, (Heise and Pieber 2007) or insulin pump treatment (CSII) is preferred (Bolli, Andreoli et al. 2011). The Growth Hormone (GH) Insulin-like Growth Factor (IGF)-axis acts near related to insulin in controlling both growth and metabolism. During puberty GH levels increase, leading to insulin resistance and in teenagers with type 1 diabetes a deteriorated metabolic control. Treating adolescents by creating an insulin profile that mimics as closely as possible, the physiological one is therefore of great importance. This thesis work has focused on the long-acting insulin analogs, glargine and detemir, and their effect on metabolic control, endogenous insulin production and the GH-IGF-axis, examining all these aspects in different studies of children and adolescents with type 1 diabetes.

2 BACKGROUND - TYPE 1 DIABETES

2.1 INCIDENCE

The incidence of type 1 diabetes is not equal throughout the world, being highest in northern Europe and lowest in Asia and South America (Patterson, Guariguata et al. 2014). Finland (57.6 cases per 100,000 people under 15 years old) and Sweden (43.1 cases per 100,000 people under 15 years old) have very high incidences. There has been a steady increase in incidence during the last fifty years, the steepest rise occurring in children under the age of five (Gale 2002; Patterson, Dahlquist et al. 2009). Onkamo et al. reported the world wide increase to be 3.0% per year during the period from 1960 to 1996 (Onkamo, Vaananen et al. 1999), while the increase in Europe for the same time was slightly higher (EURODIAB ACE study group 2000). It was later reported the same increase from 1989 to 2008 in all registered European countries (Patterson, Gyurus et al. 2012), although several reports from the Nordic countries have shown that the increase in incidence seems to have leveled off during recent years (Berhan, Waernbaum et al. 2011; Harjutsalo, Sund et al. 2013; Skrivarhaug, Stene et al. 2014).

2.2 ETIOLOGY

Type 1 diabetes is caused by autoimmune destruction (Bottazzo, Cudworth et al. 1978) of the insulin-producing beta-cells in the pancreas, influenced by both genetic and environmental factors. The lifetime risk of developing type 1 diabetes for the general population is approximately 0.4%, and increases if a first-degree relative has the disease. Siblings have 3-6% risk of developing type 1 diabetes (Mehers and Gillespie 2008) and the risk for diabetes is lower if the mother has diabetes (2.6%) than if the father (5.7%) has the disease (Tuomilehto, Lounamaa et al. 1992).

Multiple autoantibodies, have been identified as leading to the autoimmune beta cell destruction; glutamic acid decarboxylase antibodies (GADA) (Baekkeskov, Aanstoot et al. 1990), protein tyrosine phosphatase antibodies (IA-2A) (Hawkes, Wasmeier et al. 1996) and antibodies to insulin (IAA) (Palmer, Asplin et al. 1983). In 2007 another autoantibody was identified to be involved in risk of developing type 1 diabetes, antibodies to the Zink transporter complex (ZnT8A) (Wenzlau, Juhl et al. 2007).The autoimmune process begins early in life and autoantibodies can be detected before one year of age, in children, genetically at risk. The incidence of activation of autoimmunity is high between 9 months and 2 years of age (Ziegler and Bonifacio 2012). There are high risk HLA types with which the risk of developing autoantibodies are three times higher than it is for individuals with moderate risk genotypes (Kukko, Virtanen et al. 2004). Different environmental triggers influence the onset of type 1 diabetes in genetically predisposed individuals and they are becoming more and more important in the disease's rate of development. In recent years more children with less genetic susceptibility have been developing diabetes (Gillespie, Bain et al. 2004; Fourlanos, Varney et al. 2008; Resic-Lindehammer, Larsson et al. 2008; Carlsson, Kockum et al. 2012).

Addressing the observation that the environment seems to interact with the development of type 1 diabetes earlier in life are some hypotheses about why this happens. One is the hygiene

hypothesis, first proposed in 1989 (Strachan 1989), which suggests that, with a decreasing number of infections, the immune response is upregulated increasing the risk of allergic and autoimmune disorders (Cooke 2009; Kondrashova, Seiskari et al. 2013). Another theory about the increased incidence and lower ages for diagnosis is the accelerator hypothesis, which states that rapid growth and weight gain causes greater stress on the beta cells and precipitate earlier onset of the disease (Kibirige, Metcalf et al. 2003; Wilkin 2012).

Apart from these theories it has long been thought that the disease process leading to overt type 1 diabetes is triggered by an infectious agent. Perhaps the enterovirus acts as an antigen and causes inflammation in the gut, thus driving the autoimmune process (Knip and Simell 2012). Other viruses discussed as possibly associated with type 1 diabetes are rotavirus and Ljunganvirus (Nilsson, Vaziri-Sani et al. 2013). Another environmental factor that early on was thought to be involved was protection by breastfeeding, but influences seems small (Samuelsson, Johansson et al. 1993). Likewise, the time at which cow milk is introduced into a child's diet and the amount of cow milk consumed, have been studied as potentially influential factors, but data are conflicting (Dahlquist, Blom et al. 1990). Although a large intervention study introducing a cow milk free supplementation after breastfeeding stops for children at risk of diabetes has failed to decrease the appearance of diabetes associated antibodies (Knip, Akerblom et al. 2014) Finally, a meta-analysis based on five studies has shown that supplementation with vitamin D early in life may be associated with lesser risk of diabetes (Zipitis and Akobeng 2008).

2.3 PHYSIOLOGY

2.3.1 Insulin and its role in glucose metabolism

Insulin is produced in pancreatic beta cells as proinsulin (Steiner 1969). This molecule is then cleaved into insulin and C-peptide; these two molecules are stored and later secreted into portal circulation in equimolar concentrations. While insulin is extracted by the liver to a large extent, almost all C-peptide reaches the peripheral circulation and can thus be used as a marker of endogenous insulin production (Faber and Binder 1986).

Insulin is involved in carbohydrate, protein and lipid metabolism. Regarding glucose, insulin enhances transport over the cell membrane, increases the rate of glycolysis, stimulates glycogen synthesis and decreases glycogen breakdown. In lipid metabolism, insulin decreases lipolysis in adipose tissue and stimulates synthesis of fatty acids and glycerol as well as the uptake of triglycerides from the blood into adipose tissue and muscle. In the liver and muscle tissues insulin decreases the rate of fatty acid oxidation. Insulin also stimulates uptake and the synthesis of protein and is overall therefore an anabolic hormone (Dimitriadis, Mitrou et al. 2011).

Glucose is moved by several glucose transporters (GLUTs) (Zhao and Keating 2007). GLUT-2, which is expressed in liver and beta cell, is insulin independent. Glucose entering the cell starts a chain reaction, involving closing of ATP-sensitive potassium channels that in turn opens calcium channels. The entry of calcium stimulates insulin release into portal circulation (Hussain 2008). GLUT-4 is the insulin dependent glucose transporter, present in skeletal

muscle and adipose tissue (Birnbaum 1989) and therefore plays a key role in glucose metabolism and insulin resistance (Govers 2014).

2.3.2 Insulin in relation to the GH-IGF-axis

Insulin interacts with the GH-IGF-axis in two ways in the liver. First it increases sensitivity to GH by increasing the expression of the GH receptor (GHR), its downstream signaling and thereby the production of IGF-I (Leung, Doyle et al. 2000).Second it down regulates IGFBP-1 and thus increases the bioavailability of IGF-I (Brismar, Fernqvist-Forbes et al. 1994).

IGF-I is a polypeptide structurally homologous to insulin that has insulin-like metabolic and growth-promoting actions (Daughaday, Phillips et al. 1976; Hall, Bang et al. 1995; Mauras and Haymond 2005). IGF-I acts with GH to induce growth via the proliferation and expansion of the growth plate (Kaplan and Cohen 2007). In glucose metabolism it acts against GH by producing insulin-like effects and increasing glucose uptake in muscles via the IGF type 1 receptor (IGF-1R) (Dohm, Elton et al. 1990; Crowne, Samra et al. 1998). IGF-I effects in adipose tissue and liver are minimal as the IGF-1R is not expressed in significant quantities in humans (Bolinder, Lindblad et al. 1987). IGF-I also down regulates GH by negative feedback (Yakar, Setser et al. 2004; Clemmons 2006). Complete lack of IGF-I expression in a human subject increased insulin needs and lowered insulin sensitivity (Woods, Camacho-Hubner et al. 1996). IGF-I and insulin cross-reacts on their respective receptors but these effects are less important under most conditions, than are the hypoglycemic effects on their respective receptors. It has been suggested that hybrid receptors, composed of one-half insulin receptor (IR) and one-half IGF-1R are important for mediating IGF-I effects in muscle (Fernandez, Kim et al. 2001). Most of the circulating, metabolically active IGF-I is produced in the liver (Yakar, Liu et al. 1999), but IGF-I is also produced locally in most tissues including skeletal muscle (Loughna, Mason et al. 1992), adipose tissue (Nam and Marcus 2000), and the growth plate (Yakar, Liu et al. 1999), where its contributions to local IGF-I actions are important. Unlike insulin, IGF-I is not delivered on demand, but maintains a more stable diurnal level caused by its bond to a group of IGF binding proteins (IGFBP)-1-6 (Juul 2003), regulating its bioavailability. IGFBP-3 and -5 bind to IGF-I or to IGF-II and then associates with the acid labile subunit (ALS) to form large ternary complexes with long half-lives. Binary complexes of IGF-I bound to BP-1, 2, 4 and 6 and have shorter half-lives in circulation (Holly and Perks 2006; Clemmons 2012).

Proteolysis of IGFBP-3 is an important mechanism for increasing the bioavailability of IGF-I for peripheral tissues (Bang 1995). This action has been shown to increase in states characterized by increased insulin resistance, such as pregnancy (Giudice, Farrell et al. 1990; Yan, Payet et al. 2009) and type 2 diabetes (Bang, Brismar et al. 1994). It seems to contribute to adaption to insulin deficiency, but not to disturbances in GH secretion that lead to low IGF-I secretion (Lassarre, Duron et al. 2001). The protease activity is considerable in healthy children at a young age, but decreases with age, reflecting higher levels of IGF-I (Renes, van Doorn et al. 2014).

IGFBP-1 is mainly produced in the liver and is particularly interesting in the context of type 1 diabetes because its production is inversely related to the insulin levels in the portal vein (Brismar, Fernqvist-Forbes et al. 1994; Lee, Giudice et al. 1997). It has been established that

IGFBP-1 can serve as a marker of portal insulin action or sensitivity (Hilding, Brismar et al. 1995; Kotronen, Lewitt et al. 2008). Low levels of IGFBP-1 in circulation lead to increased levels of IGF-I and may increase the bioactivity of IGF-I in the tissues, although experimental proof is lacking (Lewitt, Denyer et al. 1991).

GH is produced in the pituitary, and secretion is regulated by several factors: glucose, fatty acids, and hormones like GH releasing hormone, somatostatin, leptin and ghrelin, in addition to the negative feed-back of IGF-I. GH induces insulin resistance (LeRoith and Yakar 2007) through several mechanisms, doing so directly by stimulating lipolysis (Williams, Amin et al. 2003; Vijayakumar, Novosyadlyy et al. 2010), thereby increasing hepatic glucose production (HGP) (Salgin, Marcovecchio et al. 2009) and doing so indirectly by interacting with the IR/IGF-IR signaling (Dominici, Argentino et al. 2005).

GH hyper secretion, which occurs during puberty to promote growth, leads to insulin resistance (Moller, Jorgensen et al. 1991; Moran, Jacobs et al. 2002). Maximal insulin resistance is seen in mid and late puberty, which is Tanner stadium 3 and 4 (Smith, Dunger et al. 1989; Caprio, Cline et al. 1994). IGF-I levels are increased in relation to the increased GH. Estrogen allows a concomitant rise in GH and IGF-I by relaxing the negative feed-back axis of IGF-I (Veldhuis and Bowers 2003). The increase of insulin becomes physiologically meaningful by promoting anabolism and linear growth. IGF-I balances the diabetogenic effects of GH. Because of the increased levels of insulin IGFBP-1 is decreased during puberty in healthy adolescents (Juul, Main et al. 1994).

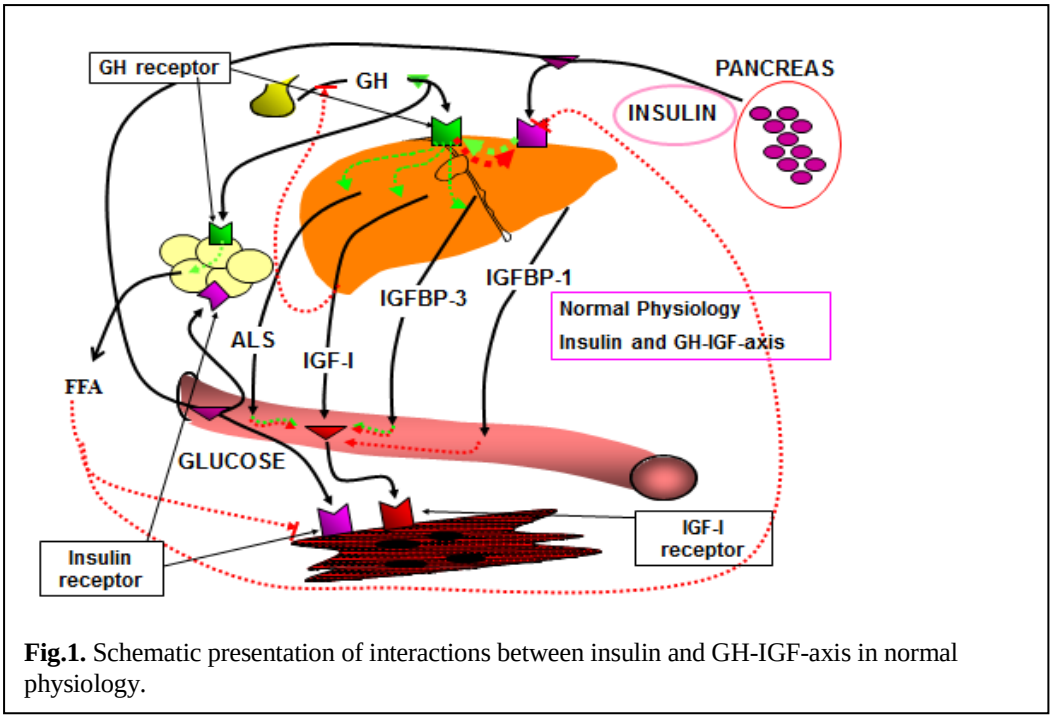

Fig.1. Schematic presentation of interactions between insulin and GH-IGF-axis in normal physiology.

2.4 PATHOPHYSIOLOGY

2.4.1 Insulin deficiency and endogenous insulin production

The biochemical abnormality in type 1 diabetes is insulin deficiency, but the insulin secretion from the beta cell must be reduced to almost 10 % before hyperglycemia is induced (Pipeleers and Ling 1992; Gorus, Keymeulen et al. 2013). After diagnosis and the start of insulin treatment most patients do recover some endogenous insulin production, measured as increased C-peptide. The clinical remission with recovery of endogenous insulin production peaks at three to six months (Agner, Damm et al. 1987). Differences in insulin sensitivity also affect the remission period, and increased insulin sensitivity is shown to precede increased beta-cell activity in newly diagnosed type 1 diabetes (Linn, Ebener et al. 1995). Several factors, among them season, gender, age, glycosylated hemoglobin (HbA1c) at diagnosis and the number of autoantibodies have been shown to affect both the level of C-peptide at diagnosis and its decline (Ludvigsson and Hellstrom 1997; Ortqvist, Falorni et al. 1997; Ludvigsson, Carlsson et al. 2013; Samuelsson, Lindblad et al. 2013). Age is inversely related to a reduction in C-peptide (Ludvigsson, Carlsson et al. 2013) or to the duration of clinical remission (Ortqvist, Falorni et al. 1997). C-peptide declined roughly 50% in the first year after diagnosis among children, while the first year decline among adults was 20% (Greenbaum, Beam et al. 2012). Intensive insulin treatment beginning at diagnosis has been shown to improve C-peptide levels and metabolic control for one year or more in both adults (Linn, Ortac et al. 1996) and adolescents (Shah, Malone et al. 1989). Several interventions to induce beta-cell rest have been conducted. Somatostatin (Bjork, Berne et al. 1998) and diazoxide (Ortqvist, Bjork et al. 2004) have both been shown to induce prolonged endogenous insulin production via temporary beta-cell rest but neither has come into clinical use owing to unwanted side-effects.

Different kinds of immunomodulation to prevent beta cell destruction have been tested. Cyclosporine was reported to induce beta-cell rest in the 1990's (Skyler and Rabinovitch 1992), but was never used because of severe adverse reactions. In later years clinical trials of immunotherapy directed toward lymphocytes using anti-CD3 (teplizumab or otelixizumab) and anti-CD20 (rituximab) resulted in transient insulin secretion (Coppieters, Harrison et al. 2013). More antigen-specific agents such as GAD65 alum have been tested in children and the first study indicates that beta-cell capacity could be preserved (Ludvigsson, Faresjo et al. 2008) in children recently diagnosed with type 1 diabetes, although this could not be repeated in a second larger trial (Ludvigsson, Krisky et al. 2012). Anti-cytokine therapies blocking IL-1, IL-6 and tumor necrosis factor have also been tried (Nepom, Ehlers et al. 2013). Anti-oxidants, including nicotinamide and vitamin E (Pozzilli, Visalli et al. 1997) have been investigated and more recently the use of vitamin D was proposed (Ludvigsson 2012). All immunomodulation efforts and future perspectives in this area were recently summarized in a review (Lord and Greenbaum 2015).

A follow-up of the DCCT study has shown that a stimulated C-peptide level of > 0.2 nmol/l at study inclusion was important both for delaying vascular complications and for reducing the risk of hypoglycemic episodes (Steffes, Sibley et al. 2003). A recent, more thorough analysis of the same data has revealed that there is a near-linear regression with no threshold in the decline of C-peptide, in addition very small amounts of remaining C-peptide seems to be of

great importance (Lachin, McGee et al. 2014). In the "medalist study" some subjects who had been living with diabetes for over 50 years were still C-peptide positive (Keenan, Sun et al. 2010).

2.4.2 Insulin deficiency in relation to the GH-IGF-axis

Type 1 diabetes causes deleterious changes in the GH-IGF-axis (Dunger and Cheetham 1996). Subjects with type 1 diabetes treated with subcutaneous insulin injections have a portal insulin deficiency, which affects hepatic GHR function (Baxter, Brown et al. 1980), as a result of a decreased number of GHRs (Menon, Arslanian et al. 1992) and post receptor defects in GH action (Maes, Underwood et al. 1986; Hanaire-Broutin, Sallerin-Caute et al. 1996). This impaired GH action leads to decreased levels of both circulating total and free IGF-I (Taylor, Dunger et al. 1988; Frystyk, Bek et al. 2003; Hedman, Frystyk et al. 2004). The low levels of IGF-I create a lack of negative feedback and consequently GH secretion increases, leading to greater insulin resistance (Dunger, Cheetham et al. 1995; Frystyk 2004). Insulin administration at diagnosis of type 1 diabetes increases GH binding protein (GHBP, a marker of hepatic GHR numbers. This further underlines the importance of insulin in regulating the GHR. However, GHBP levels remain subnormal (Arslanian, Menon et al. 1993). The portal insulin deficiency also causes increased IGFBP-1 levels in both children and adolescents (Hall, Johansson et al. 1989; Radetti, Paganini et al. 1997) and adults (Suikkari, Koivisto et al. 1988; Ekman, Nystrom et al. 2000). IGFPB-3 and ALS are decreased although some IGFBP-3 immunoassays may detect an increase (Diamandi, Mistry et al. 2000), since IGFBP-3 proteolysis is increased in adolescents with type 1 diabetes (Zachrisson, Brismar et al. 2000). IGF-I levels are low in both prepubertal (Salardi, Cacciari et al. 1986; Strasser-Vogel, Blum et al. 1995) and pubertal (Zachrisson, Brismar et al. 1997) children with type 1 diabetes. Glycemic control has been shown to affect IGF-I levels in diabetic subjects during puberty, but not before (Rogers, Sherman et al. 1991). In one study age-adjusted values for IGF-I correlated inversely with HbA1c (Strasser-Vogel, Blum et al. 1995).

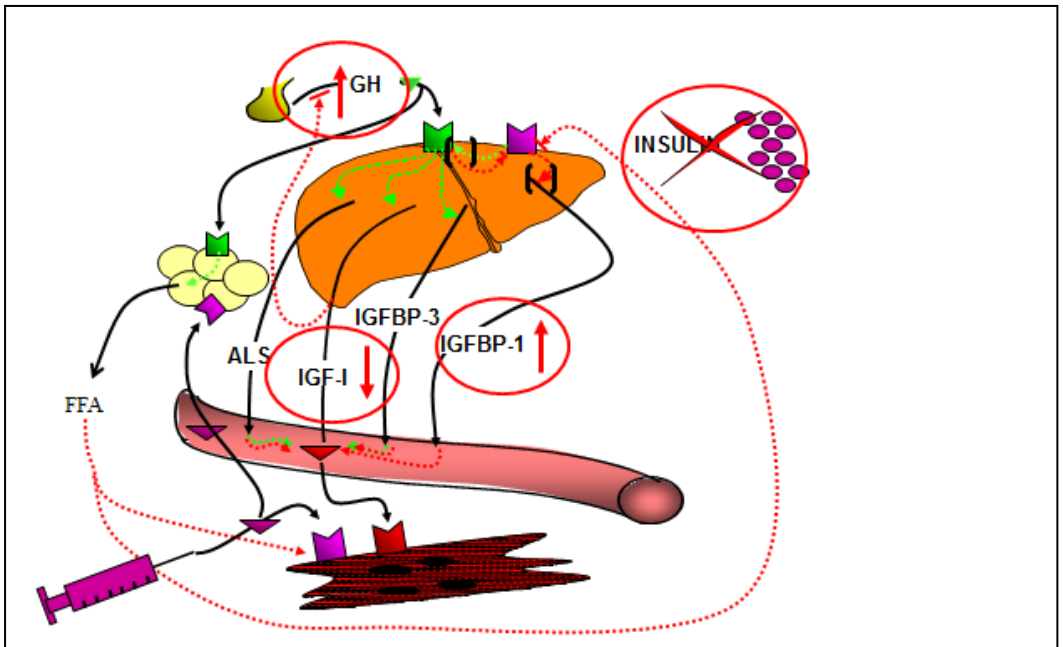

Fig.2. Schematic presentation of changes in GH-IGF-axis in type 1 diabetes when endogenous insulin secretion is replaced by subcutaneous insulin injections.

Bereket et al., who studied children and adolescents before and after the initiation of insulin therapy, found that IGF-I was very low (75 % less) at diagnosis, but increased one week after the initiation of insulin treatment. IGFBP-1 levels were elevated up to sevenfold, and decreased within 24 hours after the first insulin dose (Bereket, Lang et al. 1995). The decrease in IGFBP-1 led to increased free IGF-I (Bereket, Lang et al. 1996) preceding the increase of circulating IGF-I, suggesting that IGFBP-1 plays a role in the acute regulation of glucose metabolism and interacts with the glucose rise at dawn (Cotterill, Daly et al. 1995; Kobayashi, Amemiya et al. 1997). Before insulin initiation Bereket et al. also found that IGFBP-3 proteolysis was increased but reversed by insulin treatment, thus suggesting that insulin regulates this proteolysis activity and may help to counteract the catabolic state induced by severe insulin deficiency (Bereket, Lang et al. 1995).

Intra-peritoneal delivery of insulin can restore the decreased IGF-I levels (Hanaire-Broutin, Sallerin-Caute et al. 1996; Hedman, Frystyk et al. 2014), but only intra-portal delivery of insulin can induce a complete restoration of the changes in the GH-IGF-axis (Shishko, Dreval et al. 1994).

During puberty GH levels are even more increased in subjects with type 1 diabetes than in healthy subjects (Amiel, Sherwin et al. 1986; Dunger and Acerini 1998). This is shown to be more prominent in girls and does not affect only nightly GH secretion (Halldin, Tylleskar et al. 1998). Circulating IGF-I is lower (Zachrisson, Brismar et al. 1997), and IGFBP-1 is further increased (Zachrisson, Dahlquist et al. 2000), leading to increased insulin resistance and often to deteriorated metabolic control (Acerini, Williams et al. 2001). Increased IGFBP-3 proteolytic activity (IGFBP3-PA) is also present and may play a compensatory role in attenuating the decreased free IGF-I and thus to some extent increasing insulin sensitivity and restoring glycemic control (Zachrisson, Brismar et al. 2000).

Type 1 diabetes		
Components in GH-IGF-axis	**Prepuberty**	**Puberty**
GH	↗	↗↗
IGF-I	↘↘	↘↘↘
IGFBP-1	↗↗	↗↗
IGFBP3-PA	↗	↗↗

Table 1: Changes in serum concentration of components in the GH-IGF-axis in type 1 diabetes, before and during puberty, compared to healthy subjects.

2.5 METABOLIC CONTROL AND LONG-TERM COMPLICATIONS

In healthy humans, glucose levels are kept in a narrow physiological interval of approximately 3.5-7 mmol/l by insulin, which is by instant feedback secreted into the portal vein in response to the plasma glucose level. In diabetes the goal of treatment is to keep the blood glucose levels as normal as possible. Hyperglycemia causes increased oxidative stress (Chung, Ho et al. 2003) and inflammation and is thereby toxic for the endothelial cells (Lorenzi, Toledo et al. 1987). The long-term result of this damage is both micro and macro vascular complications. The most vulnerable vessels are those in the kidney (de Boer 2014) and those in the retina (Aiello 2014).

The DCCT, in which conventional insulin therapy, using one or two daily injections, was compared to intensive insulin treatment, using three or more daily injections or an insulin pump (CSII) showed the importance of maintaining good metabolic control in order to prevent and delay long-term micro vascular complications (DCCT 1993). The follow up study of the DCCT, dubbed the Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that there is a "metabolic memory" (Nathan 2014), meaning that good metabolic control in early years after diagnosis is remembered despite later deterioration. This can be explained by the glycosylation of mitochondrial proteins (Ceriello 2009). Furthermore intensive insulin treatment in the DCCT for subjects with remaining C-peptide was shown to increase endogenous insulin production (DCCT 1998).

In a Swedish follow-up examining a group of adults with type 1 diabetes who had been diagnosed in childhood and who had been living with diabetes for at least 15 years, the diabetes duration and long-term HbA1c were both risk factors for developing retinopathy and nephropathy (Nordwall, Arnqvist et al. 2009). In a recent long-term follow up study, no one with a long-time HbA1c below 7,6% (DCCT) (60 mmol/mol) had developed severe retinopathy or nephropathy (Nordwall, Abrahamsson et al. 2015). Another Swedish population based study showed that despite intensive insulin therapy about half the patients developed microvascular complications after 12 years. Inadequate HbA1c during the first years accelerated the complications, although young age at diagnosis delayed the incidence (Svensson, Eriksson et al. 2004). A recent follow up using two Swedish diabetes registers show that metabolic control during the first year after diagnosis in children influences the risk of developing retinopathy and microalbuminuria in early adult years (Samuelsson, Steineck et al. 2014).

2.6 MEASUREMENTS OF METABOLIC CONTROL

HbA1c has been considered the main measurement of long-term metabolic control. In recent years, however, it has been suggested that glucose variability also contributes to the development of long-term complications. Data are conflicting and the clinical correlates detected so far are limited (Cavalot 2013), although both experimental (Ceriello, Esposito et al. 2008) and clinical studies (Monnier, Colette et al. 2010) have shown that both long-term stable hyperglycemia and intermittent hyperglycemia over the short and the long-term induce oxidative stress that causes vascular damage (Saisho 2014). In a Swedish study of adults with type 1 diabetes glycemic variability was shown to possibly predict the development of long-term neuropathy (Bragd, Adamson et al. 2008).

2.6.1 **HbA1c**

The glycosylation of hemoglobin is a non-enzymatic, irreversible reaction affecting hemoglobin and other proteins exposed to glucose. Since the erythrocyte normally has a lifespan of 120 days, the degree of glycosylation reflects in practice the average glucose concentration over the last two to three months. In conditions with increased turnover of erythrocytes, such as thalassemia and hemolytic anemia, false low values may be obtained in the measurement of HbA1c (Landin-Olsson, Jeppsson et al. 2010).

There are several standards in measuring HbA1c. In Sweden the Mono-S method was used until 2010, but was approximately 1% unit lower than the National Glycohemoglobin Standardization Program (NGSP)/DCCT standard, used worldwide. To coordinate all measures it was agreed in October 2010 to use a new worldwide standard – the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Hanas and John 2010). The IFCC values are reported in mmol/mol.

Mono-S HbA1c (%)	DCCT HbA1c (%)	IFCC HbA1c (mmol/mol)
4.0	5.0	31
4.5	5.5	36
5.0	6.0	42
5.5	6.4	47
6.0	6.9	52
6.5	7.4	57
7.0	7.9	63
8.0	8.8	73
9.0	9.8	83
10.0	10.7	94
12.0	12.7	115

Table 2: Comparison between Mono-S, DCCT and IFCC standard for HbA1c.

2.6.2 **Glucose variability**

The first and basal way to measure glucose is through self-monitoring of blood glucose (SMBG). A relation between the numbers of SMBG events per day and HbA1c reduction has been described (Schutt, Kern et al. 2006). But SMBG provides only intermittent single blood glucose levels, most often only during the day, without giving a 24 hour view. Continuous glucose monitoring (CGM) can for this purpose be very helpful. Blinded CGM, where no measures are shown in real time, can be used in research and "real time" CGM, where the glucose values can be followed continuously, is becoming a routine method. Use of "real-time" CGM has been shown to improve HbA1c in both children and adults (Battelino, Phillip et al. 2011).

Several factors are important regarding glucose variability; among them are time, glucose level and amplitude. The most direct way to monitor glucose variability is to use the mean value of glucose derived either from SMBG or from CGM. The standard deviation (SD) of the mean is often used as a measurement of glucose variability, although it does not evaluate whether the observed deviations represent hypoglycemia or hyperglycemia (Kovatchev, Cox et al. 2002). Mean blood glucose levels have been shown to correlate to HbA1c (Sacks 2007). Besides the simple mean and SD of mean, several mathematical methods may be used to estimate glucose variability. One is the Mean Amplitude of Glycemic Excursions (MAGE), first described in 1970 (Service, Molnar et al. 1970) from continuous glucose venous

sampling. It constitutes of all glucose variations above one standard deviation. There are two possible advantages with MAGE, first it measures major glycemic swings but excludes minor ones and second it is not dependent on the glucose mean (Monnier, Colette et al. 2008). Several studies have shown that HbA1c is positively associated with MAGE and it is still used as a reliable index of glucose variability (Ferenci, Korner et al. 2015). Other variability indexes are the M-value (Schlichtkrull, Munck et al. 1965) and day-to-day glycemic variations of excursions (MODD) (Molnar, Taylor et al. 1972). Low blood glucose and high glucose index are also often reported. Which glucose variability index is superior and should be used is still debated (Service 2013).

2.7 TREATMENT OF TYPE 1 DIABETES

2.7.1 Historical aspects

Insulin was discovered in 1921 by Frederic Banting, Charles Best, and their co-workers from a pancreas extract. It was first tested on a severely ill teenager, Leonard Thompson, in 1922 (Herrington 1995). The first injection was complicated by a severe allergic reaction, but after further purification of the insulin the treatment became a success and several dying children could be treated. The researchers were rewarded the Nobel Prize in 1923 for this life saving development.

After this landmark, insulin preparations were manufactured and insulin therapy spread. From the beginning insulin was extracted from bovine pancreas. A major problem was the short duration of the effects of water soluble insulin preparations. The first insulin, with prolonged duration, was protamine zinc insulin, developed in the 1930s. In the 1940s NPH insulin was developed in Denmark, but all insulin was still extracted from animals. It was not until the 1980s, that human insulin was produced, following advances in molecular biology (Levinson 2003). During the last 20 years hybrid DNA process has made it possible to refine products and modified insulin analogs have been developed.

2.7.2 Different insulin regimens

In healthy humans, insulin is secreted in a pulsatile manner to meet the increased need after meals and maintaining a basal level of about 50 % of total insulin secretion (Polonsky, Given et al. 1988).The goal in treating type 1 diabetes is to mimic the physiological release of insulin, which is characterized by a stable basal level that peaks in response to meals (Owens, Zinman et al. 2001). By administrating insulin subcutaneously it is difficult to mimic the physiological release because of a variable insulin uptake from the subcutaneous fat and often great day-to day variability (Lepore, Pampanelli et al. 2000). Before 1970 most patients with type 1 diabetes were treated with insulin twice daily. Since then multiple injection therapy (MIT) with NPH insulin as basal component and short-acting insulin before meals became more common and resulted in an improved metabolic control in many patients (Eschwege, Guyot-Argenton et al. 1976). However, the imitation of physiological insulin release was still poor and to address this problem, insulin analogs were developed using a DNA-recombinant technique. First developed were two short-acting insulin analogs, lispro and aspart, with faster absorption than regular human insulin and hence improved postprandial blood glucose levels (Bolli, Di Marchi et al. 1999; Heise 2007). But there remained a need for a basal insulin that

was more stable and reproducible than NPH (Heise and Pieber 2007). For this purpose the two long-acting insulin analogs, glargine and detemir, with a time profile of nearly 24 hours were developed. Today the most widely used insulin regimen among children and adolescents with type 1 diabetes in Sweden is MIT, also called the basal-bolus regimen, which entails multiple injections per day of meal boluses, of a rapid-acting analog together with one or two injections of basal insulin. Another way to more closely mimic the physiological release is through subcutaneous continuously insulin infusion (CSII), insulin pump, which is used by approximately 50 % of children and adolescents in Sweden (Swediabkids year report 2014).

Although the regimens for subcutaneous insulin delivery aim to match the body's insulin need it is impossible to mimic physiological insulin secretion perfectly, since insulin is delivered to the portal system in humans rather than into peripheral circulation. Subcutaneously injected insulin reaches the portal region only to a limited extent (Rachmiel, Perlman et al. 2005).

Insulin type	Appearance	Onset	Peak	Duration
Fast-acting insulins	Clear	0.5–1	2–5	6-8
Rapid-acting analogs (aspart, glulisin, lispro)	Clear	5–10 min	0.5–2	3–4
Intermediate-acting insulin e.g. NPH	Cloudy	1–3	5–8	12–18
Long-acting analogs (glargine, detemir)	Clear	1.5–4	None	20–24
Ultra-long-acting analog (degludec)	Clear		None	> 40

Table 3: Characteristics of different insulins (time in hours unless given). Modified from Rachmiel et al. 2005.

2.7.3 **Basal insulins - pharmacological aspects**

My thesis work has focused on the effects of long-acting insulin analogs in comparison to intermediate-acting NPH insulin. The first long-acting insulin analog to enter the market was insulin glargine (Lantus®) in 2000 (Bolli and Owens 2000). In 2004 another long-acting insulin analog, insulin detemir (Levemir®) was introduced in Europe, in 2005 it was approved for use in children (EMA assessment report 2011). Both glargine and detemir have lower peaks and longer durations than NPH does (Rachmiel, Perlman et al. 2005). Recently, the ultra-long-acting insulin, degludec (Tresiba®) was introduced and in February 2015 it was approved for use in children. It is almost identical to human insulin, but with small amino acid changes and a link to a fatty acid (Rendell 2013). The formation of multi hexamer chains in subcutaneous fat yields a duration of > 40 hours in both adults and children (Biester, Blaesig et al. 2014). Degludec had not yet been introduced when the studies in my thesis were planned and therefore was not included in my comparison, but may be of interest in future research projects.

2.7.3.1 *NPH insulin - intermediate-acting insulin*

NPH insulin is a crystal preparation with reduced solubility at physiological pH, resulting in slower absorption from the subcutaneous tissue (Owens, Zinman et al. 2001). This absorption is highly variable. Because of the peak between five and eight hours after injection, which is attenuated with increased dosing, there is a risk of night-time hypoglycemia (Rachmiel, Perlman et al. 2005). These pharmacological properties contributes to unsatisfactory control of fasting-glucose (Lepore, Pampanelli et al. 2000), especially among adolescents, who show insulin resistance during late night, because of increased levels of GH (Edge, Matthews et al. 1990).

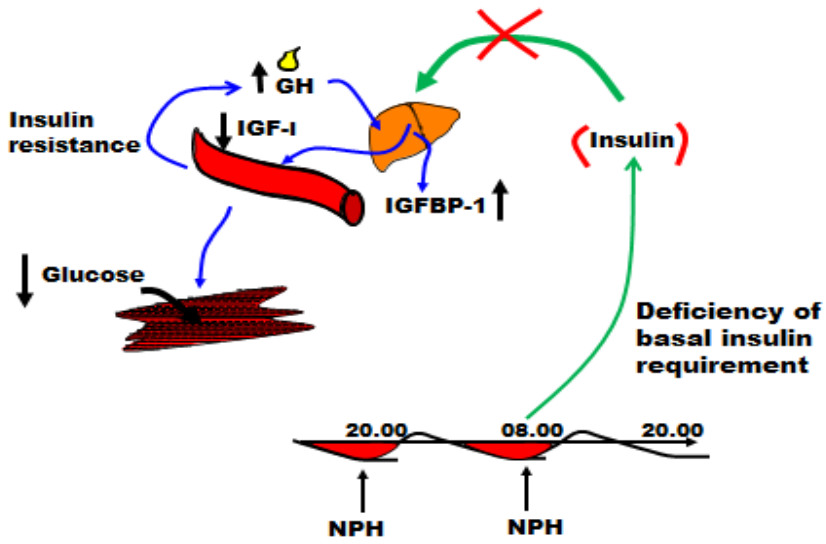

Fig.3. Schematic presentation of the changes in the GH-IGF-axis when NPH is used for basal insulin supplementation in puberty.

The late night under insulinization that accompanies NPH treatment results in increased levels of IGFBP-1and in decreased free IGF-I and may contribute to greater insulin resistance (Yagasaki, Kobayashi et al. 2010).

2.7.3.2 *Insulin glargine – long-acting insulin analog*

Glargine is produced by recombinant DNA technology. The structure is different from human insulin in three amino acids (Wang, Carabino et al. 2003). This modification results in a delayed onset and a longer duration. Glargine has normal solubility in the preparation owing to its slightly acidic pH while it forms crystals and precipitates at the neutral subcutaneous pH (Bolli and Owens 2000; Rachmiel, Perlman et al. 2005). This leads to a more stable diurnal release of insulin into circulation without pronounced peaks and produces an action time of approximately 24 hours (Lepore, Pampanelli et al. 2000).

Glargine binds to both the IR and the IGF-1R, because of this, concerns have arisen about whether glargine could have mitogenic actions (Kurtzhals, Schaffer et al. 2000). This opinion is not supported in studies on cell lines, using in vivo concentrations of glargine (Chisalita and

Arnqvist 2004). Furthermore glargine is converted into two metabolites in the subcutaneous fat, M1 and M2. These metabolites have similar glucose lowering effects but less growth promoting actions (Sommerfeld, Muller et al. 2010).

2.7.3.3 *Insulin detemir – long-acting insulin analog*

Detemir, like glargine, has been developed using recombinant DNA technology and is a soluble preparation. It differs from human insulin by one amino acid and an acetylated fatty acid. Its duration is prolonged, primarily because of slow absorption into the blood that results from self-association and albumin binding (Kurtzhals 2007). After a single dose of 0.4 U/kg the duration of action was shown to be 22-24 hours (Lepore, Pampanelli et al. 2000). One clamp study in adults with type 1 diabetes did, however, show that detemir had a duration of only 17.5 hours at a dose of 0.35 U/kg in contrast to glargine's 24 hours of action (Porcellati, Rossetti et al. 2007). In a review of several clamp studies comparing glargine's and detemir's duration Heise et al. considered the shorter duration of detemir in Porcellati's study an outlier (Heise and Pieber 2007). Detemir has been associated with significantly less within-subject variability than both NPH and glargine (Heise, Nosek et al. 2004). In a clamp study of healthy adults detemir, compared to NPH in equipotent doses, had a lesser effect on suppressing lipolysis in peripheral tissue, but a higher effect on glucose metabolism in the liver than NPH (Hordern, Wright et al. 2005), which might explain why detemir is associated with less weight gain than NPH (Home, Bartley et al. 2004; Russell-Jones, Simpson et al. 2004). Detemir is also shown to have a molar ratio of 5:1 compared to NPH in healthy individuals (Hordern, Wright et al. 2005) and also a higher molar ratio compared to glargine (Porcellati, Bolli et al. 2011). This molar difference is, however, compensated for in one unit of detemir in the manufactured preparation, Levemir® (Owens and Bolli 2008).

2.7.4 **Studies comparing long-acting insulin analogs**

A recent systematic review and network meta-analysis of studies comparing long-acting analogs to NPH used to treat adults with type 1 diabetes concluded that long-acting insulin analogs are superior to intermediate-acting insulin analogs, the difference in HbA1c is although small (Tricco, Ashoor et al. 2014). In a meta-analysis of randomized controlled (clinical) trials (RCTs) comparing both glargine and detemir to NPH , the long-acting analogs showed a small, but significantly better effect on HbA1c and a reduced risk of severe and nocturnal hypoglycemia (Monami, Marchionni et al. 2009). It included mostly studies of adults, but three studies had examined children and adolescents. The effects of the long-acting analogs, on children and adolescents, are not entirely clear and depend on study design and the length of study period. Table 4 shows selected RCTs comparing glargine or detemir with NPH.

2.7.4.1 *Studies comparing glargine and NPH*

Several RCTs have compared glargine and NPH for treating type 1 diabetes in adults (Ratner, Hirsch et al. 2000; Porcellati, Rossetti et al. 2004; Fulcher, Gilbert et al. 2005; Home, Rosskamp et al. 2005; Chatterjee, Jarvis-Kay et al. 2007). Three of them show lower HbA1c and lower fasting-glucose with glargine (Porcellati, Rossetti et al. 2004; Fulcher, Gilbert et al. 2005; Chatterjee, Jarvis-Kay et al. 2007). Only one was performed over a longer period of one

year (Porcellati, Rossetti et al. 2004) and also reports a lower incidence of over-all hypoglycemia with glargine. All studies were performed on subjects who had been diagnosed with diabetes several years earlier. None of them show any difference between glargine and NPH in terms of insulin-dose or weight gain.

Four RCTs have compared glargine and NPH in children and adolescents (Schober, Schoenle et al. 2002; Murphy, Keane et al. 2003; Chase, Arslanian et al. 2008; Hassan, Rodriguez et al. 2008). Only one of them reported lower HbA1c in children recently diagnosed with type 1 diabetes and studied for not more than three months (Hassan, Rodriguez et al. 2008). Schober et al. failed to show lower HbA1c, but found decreased fasting-glucose after 26 weeks. In a crossover study performed for 2 × 16 weeks Murphy et al. found a lower incidence of nocturnal hypoglycemia and lower fasting-glucose. Chase et al. found no overall differences in HbA1c, but did report an improvement with glargine versus NPH in a subgroup of adolescents with high HbA1c. In non-randomized, mostly retrospective studies of children and adolescents with ongoing type 1 diabetes the data on HbA1c are diverse (Chase, Dixon et al. 2003; Hathout, Fujishige et al. 2003; Tan, Wilson et al. 2004; Alemzadeh, Berhe et al. 2005; Colino, Lopez-Capape et al. 2005). Some studies show lower HbA1c, but several find improved fasting-glucose and less nocturnal hypoglycemia. Furthermore insulin regimens and observational times vary between studies. Therefore reliable interpretations are difficult to make. One study included subjects at the time of diagnosis of type 1 diabetes and found lower HbA1c with glargine (Adhikari, Adams-Huet et al. 2009).

2.7.4.2 *Studies comparing detemir and NPH*

Several randomized studies have compared detemir and NPH in adults over periods ranging from 16 to 52 weeks (Vague, Selam et al. 2003; Home, Bartley et al. 2004; Russell-Jones, Simpson et al. 2004; Standl, Lang et al. 2004; De Leeuw, Vague et al. 2005). Only Home et al. showed improved metabolic control in a 16 weeks RCT. Although the glucose variability and the prevalence of hypoglycemia were lower in most studies, only two showed lower fasting-glucose (Home, Bartley et al. 2004; Russell-Jones, Simpson et al. 2004). All the reported studies showed less weight gain with detemir.

For children and adolescents comparisons of detemir and NPH have failed to demonstrate improved HbA1c (Robertson, Schoenle et al. 2007; Thalange, Bereket et al. 2013). Both studies showed less nocturnal hypoglycemia and less BMI-gain with detemir and one study showed lower fasting-glucose (Robertson, Schoenle et al. 2007). No difference in insulin dose was seen. Two observational studies have reported better HbA1c in children on detemir than among those on NPH over a limited time. One examined the cohort of Turkish children (Kurtoglu, Atabek et al. 2009) in a large multinational observational study of both type 1 and type 2 diabetes comparing detemir with NPH over 14 weeks. The other, a multicenter study from Japan (Jinno, Urakami et al. 2012), showed lower HbA1c at 3 and 6 months, but no difference after 12 months.

2.7.4.3 *Studies comparing glargine and detemir*

Two RCTs of adults with type 1 diabetes compared the two long-acting analogs and found no differences in HbA1c after 26 weeks (Pieber, Treichel et al. 2007) or 52 weeks (Heller, Koenen et al. 2009). The 26-week parallel comparison showed lower fasting-glucose with

glargine, but less risk of nocturnal hypoglycemia and higher insulin doses with detemir. There were no differences regarding within-subject variation or weight gain (Pieber, Treichel et al. 2007). For children, a retrospective observational study showed no differences between glargine and detemir, except for higher doses with detemir (Abali, Turan et al. 2014).

2.7.4.4 *Studies comparing CSII and NPH or long-acting analogs*

Data on the advantages of CSII treatment over long-acting analogs and NPH are conflicting, probably because they compare different treatment strategies. A fairly recent Cochrane Review, comparing CSII to MIT with either NPH or analogs, performed in all age groups, stated that there is some evidence for the effectiveness of CSII (Misso, Egberts et al. 2010). A meta-analysis in children showed a small positive effect with CSII versus MIT (Pankowska, Blazik et al. 2009) and only one of the included studies reported improved HbA1c with CSII versus glargine (Doyle, Weinzimer et al. 2004). A more recent study in children showed no beneficial effects with CSII versus glargine on HbA1c (Starkman, Frydman et al. 2011) and an RCT comparing CSII and MIT with NPH in children, from diagnosis likewise showed no difference (Skogsberg, Fors et al. 2008). However two retrospective studies showed a greater reduction in HbA1c when changing treatment from NPH to CSII than when moving from NPH to glargine (Alemzadeh, Ellis et al. 2004; Schiaffini, Ciampalini et al. 2005). Over the long-term (two years) one retrospective study of children comparing CSII to glargine reported equal metabolic control (Garcia-Garcia, Galera et al. 2007) and another report from our group, comparing CSII and MIT with either NPH or long-acting analog showed lower HbA1c at 12 months with CSII, but after two years the difference had diminished (Brorsson, Viklund et al. 2014).

2.7.4.5 *Studies comparing long-acting analogs and degludec*

A meta-analysis of available trials showed a statistically significant reduction in the basal insulin dose in the degludec group compared to groups treated with long-acting analogs. There was also a significant reduction of nocturnal hypoglycemia in the degludec treated group but no differences between the groups in terms of HbA1c, fasting-glucose or adverse events (Dzygalo, Golicki et al. 2014). One RCT examining children over 26 weeks showed a comparable effect on HbA1c with degludec versus detemir, while fasting-glucose and insulin dose were lower with degludec (Thalange, Deeb et al. 2015).

Table 4 (On page 17)

RCTs comparing long-acting insulin analogs (ANA), glargine (GL) or detemir (DT) vs. NPH, in adults or children and adolescents with type 1 diabetes. HbA1c is given as DCCT standard (%),*mean or ANA/NPH, nr = not registered, ns = non-significant. For hypoglycemia, BMI/body weight (BW), and insulin dose, differences at end-point between GL/DT and NPH are given.

Reference Name and year	Age* years	Duration* years	Study design weeks	Regimen	Numbers included ANA/NPH	HbA1c* Baseline	HbA1c Endpoint ANA/NPH	P =	f-glucose* Baseline mmol/l	f-glucose Endpoint ANA/NPH	P =	Hypo-glycemia P	CGM use	BMI or BW P	Insulin dose
RCTs comparing glargine and NPH in adults															
Chatterjee 2007	42.9	18.2	16x2 cross over	MIT, GL x1 vs. NPHx2	60/60	8.5	8.1/8.3	0.04	11.5	8.4/11.4	<0.01	ns	yes	ns	ns
Porcellati 2004	35.0	14.0	52	MIT, GL x1 vs. NPHx4	61/60	7.1	6.7/7.1	<0.05	nr	6.7/7.5	0.05	<0.05	no	ns	ns
Fulcher 2005	40.5	17.5	30	MIT, GL x1 vs. NPHx1	62/63	9.2/9.7	8.3/9.1	0.009	11.2/11.4	-3.5/ -2.3	0.03	Severe hypo,0.05	no	ns	ns
Home 2005	39.0	16.0	28	MIT, GL x1 vs. NPH x1- 2	292/293	7.9	-0.2/-0.1	ns	9.3	-0.8/ -0.8	ns	ns	no	nr	ns
Ratner 2000	38.5	17.4	28	MIT, GL x1 vs. NPHx1-2	264/270	7.7	7.5/7.5	ns	11.8	-1.7/ -0.3	0.01	Severe hypo,0.03	no	nr	ns
RCTs comparing detemir and NPH in adults															
Vague 2003	40.0	17.2	26	MIT, DT x2 vs. NPHx2	301/146	8.1/8.2	7.6/7.6	ns	11.6	9.2/9.9	ns	Noct.hypo <0.005	no	<0.001	ns
Russell-Jones 2004	40.5	17.0	26	MIT ,DTx1 vs. NPHx1	491/256	8.4	8.3/8.4	ns	11.9/11.6	10.3/11.4	0.001	Noct.hypo 0.003	yes	0.024	ns
Standl 2004	41.0	16.0	52	MIT, DT x2 vs. NPH x2	154/134	7.7	7.9/7.8	ns	10.9	10.1/9.8	ns	ns	no	0.002	ns
Home 2004	40.0	17.0	16	MIT, DTx2 vs. NPHx2	276/132	8.6	7.75/7.9	0.027	11.6/12.2	9.3/11.2	<0.001	0.002	yes	Lower DT	ns
De Leeuw 2005	40.5	17.1	52	MIT, DT x2 vs. NPH x2	216/99	8.2/8.0	7.5/7.6	ns	11.9/11.5	10.7/10.8	ns	Noct.hypo, 0.016	no	<0.001	ns
RCTs comparing glargine and NPH in children and adolescents															
Hassan 2008	11.0	0.3	12	Not MIT, GL x2 vs. NPHx2	23/19	6.8/6.9	6.7/7.6	0.029	6.6/7.2	5.7/9.6	0.008	ns	no	ns	ns
Schober 2002	11.6	4.8	26	MIT, GL x1 vs. NPH x1-2	174/175	8.5/8.8	8.8/9.1	ns	10.8/10.6	9.5/9.9	0.02	ns	no	nr	ns
Chase 2008	13.3	5.3	24	MIT, GL x1 vs. NPH x2	84/84	7.8/8.0	-0.25/0.05	ns	10.4/11.3	-0.2/0.05	ns	ns	no	nr	ns
Murphy 2003	14.8	7.3	16x2 crossover	MIT, GL x1 vs. NPH x1	25 crossover	9.3	8.7/9.1	ns	9.8	8.0/9.2	<0.001	Noct.hypo <0.05	no	ns	Lower w. GL
RCTs comparing detemir and. NPH in children and adolescents															
Robertson 2007	11.8	5.0	26	MIT, DTx1-2 vs. NPH x1- 2	232/115	8.8/8.7	8.0/7.9	ns	11.2/11.1	8.4/9.6	0.022	Noct.hypo 0.011	no	<0.001	ns
Thalange 2013	9.9	3.7	52	MDI, DTx1-2 vs. NPH x1-2	177/170	8.4	8.7/8.6	ns	8.5	0.02/-0.6	ns	Noct.hypo 0.001	no	<0.001	ns

3 HYPOTHESIS AND AIMS

Hypothesis

Treatment with long-acting insulin analogs, glargine or detemir, in children and adolescents with type 1 diabetes results in better metabolic control, increased endogenous insulin production, and normalization of the GH-IGF-axis.

Specific aims

Paper I
To study the effect of changing from NPH to glargine, on the GH-IGF-axis and its association with metabolic control in adolescents with type 1 diabetes.

Paper II
To retrospectively study if treatment with glargine compared to NPH from the onset of type 1 diabetes in children and adolescents results in lower HbA1c during the first year of disease.

Paper III
To prospectively study if randomization to treatment with glargine or detemir compared to NPH, from the onset of type 1 diabetes in children and adolescents, results in lower HbA1c, decreased glucose variability and better preserved endogenous insulin production during the first year of disease.

Paper IV
To study if lower HbA1c in paper III is related to normalization of the GH-IGF-axis, including increased IGF-I and decreased IGFBP-1, a marker of hepatic insulin action.

4 SUBJECTS AND METHODS

Paper	Group		n	Age yrs (range)	Sex F/M	Diabetes duration yrs (range)	HbA1c % (mmol/mol)	Study duration
I	Pubertal NPH->Glargine		12	12.7 (11.1-15.0)	8/4	3.1 (1.0-6.0)	8.3±0.6 (76.1±6.3)	12 weeks
II	NPH		49	12.0 (9.0–16.0)	18/31	From diagnosis	9.6 ± 2.2* (89.7±23.0)	12 months
	Glargine		49	12.5 (8.0–17.0)	21/28		11.0 ± 2.3* (104.3±24.1)	
III+IV	NPH	prepubertal	12	9.2 (7-11)	3/9	From diagnosis	108.7±6.3	12 months
		pubertal	16	13.8 (10-17)	6/10		100.3±4.5	
	Glargine	prepubertal	18	9.2 (7-11)	6/12		93.5±3.8	
		pubertal	12	13.5 (12-15)	4/8		111.5±4.7	
	Detemir	prepubertal	17	9.1 (7-11)	5/12		95.6±4.0	
		pubertal	17	13.7 (10-17)	7/10		107.6±6.7	

Table 5: Subjects characteristics at baseline. Data are given as mean±SE (* ±SD) and range for age and diabetes duration. HbA1c is given in Mono-S standard, % and (IFCC, mmol/mol) for paper I and II, in IFCC, mmol/mol for paper III + IV.

4.1 SUBJECT SELECTION

4.1.1 Paper I

The abnormalities in the GH-IGF-axis in subjects with type 1 diabetes, which includes increased GH secretion during day and night (Halldin, Tylleskar et al. 1998) and subnormal IGF-I concentrations are more pronounced in puberty (Acerini, Williams et al. 2001). The underlying mechanism responsible for these abnormalities is the lack of hepatic insulin actions. Therefore, we hypothesized that improved nightly insulinization achieved with long-acting analog glargine could be of importance, particularly in puberty. Because of this we studied 12 adolescents in pubertal Tanner stages 2 or 3. All subjects and their parents were informed and signed informed consent.

4.1.2 Paper II

The clinical use of glargine in the pediatric population was established very fast in Sweden. Glargine was soon used from the diagnosis of type 1 diabetes. At clinical follow up, the effects on HbA1c appeared to be superior compared to treatment with NPH, in accordance with results from paper I. Therefore a systematic retrospective study was initiated. Treatment effects with glargine were compared to treatment with NPH, both given in a MIT regimen. A power calculation showed that 50 patients in each treatment arm were needed to show a difference in HbA1c of 1 %-unit. We included the first 49 patients that were treated with

glargine from diagnosis and compared with 49 consecutive patients treated close in time with NPH. Both groups received the same care and management from the diabetes team. The age of the patients included was from 7-17 years. Patients younger than 7 years were not treated with glargine at the time, and we wanted to allow the one year follow up to take place at the pediatric department before the age of 18 years. The glargine group was diagnosed from August 2002 until September 2003. At that time-point the initial patient management program changed and no more patients could therefore be included. The NPH group was collected between March 2000 and December 2001. All patients remained on the same initial treatment during the first year from diagnosis.

4.1.3 **Paper III+IV**

The effects of different insulin regimens on reversing the abnormalities of the GH-IGF-axis are assumed to be more pronounced in individuals lacking endogenous insulin production. However, the effects of initial treatment from diagnosis has been shown to be of great importance for metabolic control later on (Ludvigsson and Bolli 2001). Following the results of paper II, we planned a prospective randomized controlled trial from diagnosis of type 1 diabetes to investigate effects on metabolic control and underlying mechanisms. At that time a second long-acting insulin analog, detemir had been approved and it was, besides glargine included in the investigation. A power calculation showed that 40 patients in each treatment arm were needed to show a difference in HbA1c of 1 %-unit compared with either of the long-acting insulin arms. Since metabolic control, endogenous insulin production and abnormalities in the GH-IGF-axis are affected by puberty, the subjects were stratified for pubertal status and gender.

120 patients were enrolled from September 2005 until March 2010. Of the 120 patients included, 40, 41 and 39 subjects were assigned to NPH, glargine or detemir. Newly diagnosed children with type 1 diabetes were enrolled in the study before they received their first subcutaneous insulin injection. While receiving intra venous (iv.) insulin infusion (up to 48 hours), they and their families were informed about the study. Patients were enrolled if they were novel to insulin therapy at admission, age $\geq$7 years and $\leq$17 years and they and their legal guardians had signed the informed consent form. The exclusion criteria were: Moderate to severe diabetes ketoacidosis (pH <7.2 and/or standard bicarbonate < 10 mmol/l), suspected non-type 1 diabetes, known celiac disease, hypothyroidism (if not well controlled), suspected syndrome, any eating disorder, neuropsychiatric diagnosis or cancer.

A total of 27 subjects were excluded. Celiac disease is, like type 1 diabetes an autoimmune disease and the coexistence is approximately 8 %, varying from 3-16% in different studies (Cohn, Sofia et al. 2014). All subjects were screened for celiac disease with IgA transglutaminase antibodies at diagnosis, but the test result often came after start of subcutaneous insulin. Thus we had to inform and include the patients before the test result arrived. A total of 8 (7%) subjects had positive IgA transglutaminase antibodies indicating preexisting undiagnosed celiac disease and these patients had to be excluded. 17 subjects were excluded on their own request, during the study treatment phase, mainly because of fear for the test procedures that involved iv.catheters and CGM. This reason was, as expected, a little more prevalent among the younger children. 2 subjects were excluded because of protocol violence, both because they were non-compliant to the insulin regimen resulting in a

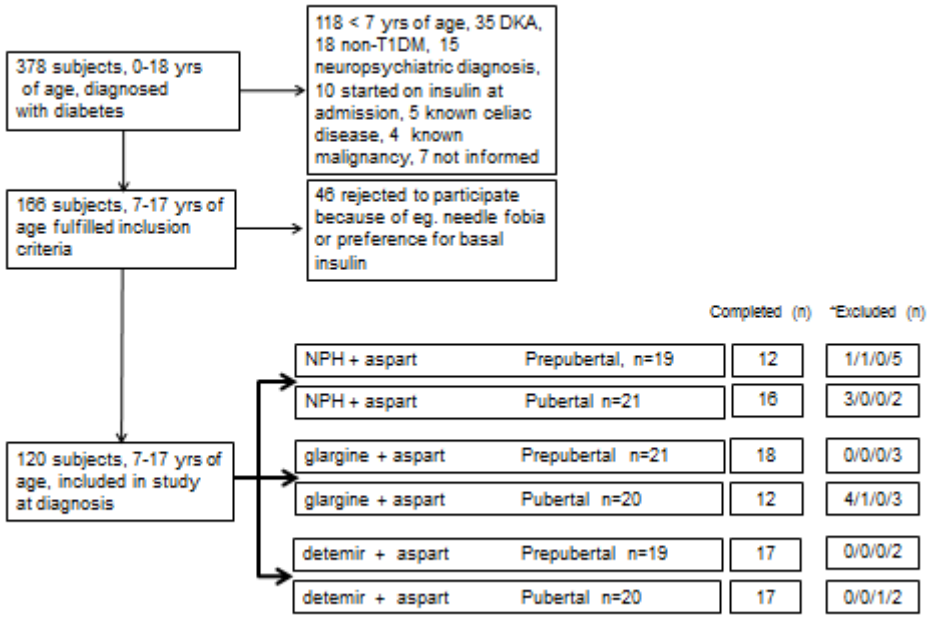

Fig.4. Included and excluded and reasons for exclusion in paper III+IV. * The reason for exclusion is given by number of subjects: Diagnosed with celiac disease /found non-compliant /diagnosed with neuropsychiatric disorder /patient´s or family´s request

deteriorated metabolic control. Finally one subject was excluded due to newly diagnosed Asperger´s syndrome during the study. A flow-chart of included and excluded subjects and reasons for exclusion is presented in Fig.4.

4.2 RESEARCH DESIGN

4.2.1 Research design in Paper I

The main purpose of paper I was to explore and identify potential reversal of abnormalities in the GH-IGF-axis induced by glargine in pubertal adolescents. By the time of planning for the study glargine was recently approved. Each subject was her/his own control. A randomized cross-over study could be planned later on, if we found support for the involvement of the GH-IGF-axis in the improvement of metabolic control.

At inclusion all 12 subjects were treated with NPH in a MIT regimen. Each subject was admitted for a 26 hours period at two different occasions First, still treated with NPH and then after 6 weeks on glargine. Subjects were also studied with fasting blood samples in the morning after 1, 2, 4, 8, and 12 weeks. Subjects were advised to adjust insulin doses, both basal and meal insulin, in order to optimize their glycemic control.

At start of each admission two iv.catheters were applied and blood samples were obtained at start and then every 30 minutes from 21:00 until 17:00 the next day. Hormone analysis taken

between 00:00 and 07:00 were considered as overnight values. The first dose of glargine was given at 18.00 at the end of the first admission. The research design is presented in Fig.5.

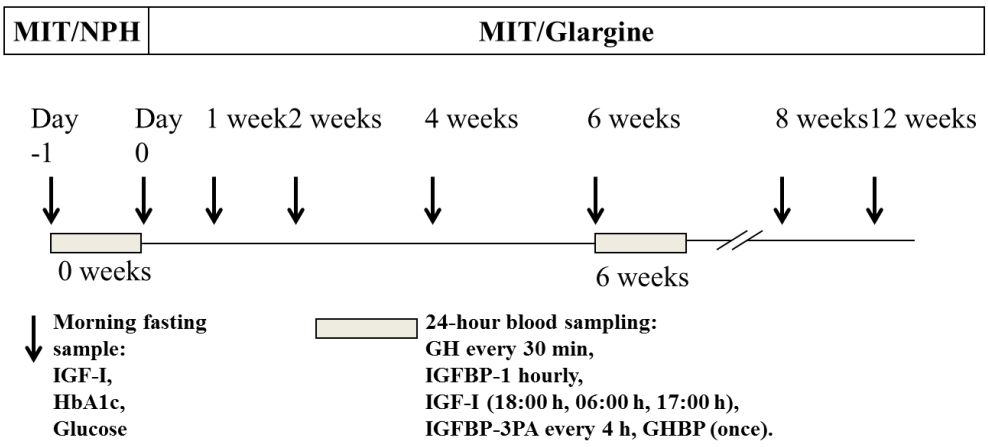

Fig.5. A schematic presentation of the research design in Paper I

4.2.2 **Research design in Paper II**

This was a retrospective observational study from diagnosis of type 1 diabetes comparing the use of glargine versus NPH, both as a part of a MIT insulin regimen. From our clinical patient database we collected measurements regarding HbA1c, insulin doses and weight, at diagnosis, after 3-5 months, 6-8 months, and 12-15 months of diabetes duration. Gain in weight was analyzed as difference in body weight (BW) from 1 month after diagnosis until final visit of the study. Clinical visits were not always at exact expected time and therefore the time-points are given as an interval (e.g. 3-5 months).

A retrospective, observational study has several limitations and a high risk of confounders. We wanted to take advantage of clinical data in this retrospective design before a costly and time-consuming RCT was conducted.

4.2.3 **Research design in Paper III+IV**

This was a randomized controlled clinical trial from diagnosis of type 1 diabetes in children and adolescents. We wanted to test the hypothesis that long-acting insulin analogs, because of their prolonged effect duration and thereby improved nightly insulinization (Heise and Pieber 2007), improve metabolic control, prolong endogenous insulin production and reverse the abnormalities of the GH-IGF-axis. The hypothesis was generated based from the results in papers I and II. Since a second long-acting analog, detemir had been approved, it was included in the comparison. Randomization was therefore done to three different basal insulins, intermediate- acting NPH or either of the two long-acting analogs, glargine or detemir.

Patients were admitted at diagnosis to the diabetes unit at Astrid Lindgren´s children hospital. They were initially treated with iv.insulin infusion for up to 48 hours according to our standard treatment protocol. Randomization to NPH, glargine or detemir took place before the first subcutaneous insulin injection. The randomization process was stratified for gender

and puberty. It was designed to give equal numbers of subjects in each treatment group. All subjects followed the same educational program and were hospitalized for approximately one week, and thereafter had close contact and/or daily visits to the hospital for another week. Doses at start of subcutaneous insulin injection therapy were calculated based on insulin requirements during iv.insulin infusion according to a predefined scheme. Insulin doses were optimized according to the Swedish National Guidelines.

Blood samples were collected before iv.insulin treatment was started (PI= preinsulin), before subcutaneous insulin (PS= presubcutaneous) was started, and with fasting in the morning at each study visit at 2 weeks, and at 3, 6, 9 and 12 months. A clinical evaluation including pubertal staging and auxology was obtained at each visit.

The mixed meal tolerance test (MMTT) was performed at 2 weeks and at 3, 6 and 12 months to evaluate endogenous insulin production. To evaluate glucose variability, subjects used a blinded CGM for 3 days at 3, 6 and 12 months. The 24-hour and overnight (00:00-07:00) individual means and SD were calculated for the first full 24 hours available. A glucose variability index, MAGE, was calculated from the 24-hour CGM glucose data. Time spent at a low (≤ 3.9 mmol/l) or at a high (≥ 10.0 mmol/l) glucose was also calculated. Patients were advised to report hypoglycemia in diaries that were collected at each visit. The research design is presented in Fig.6.

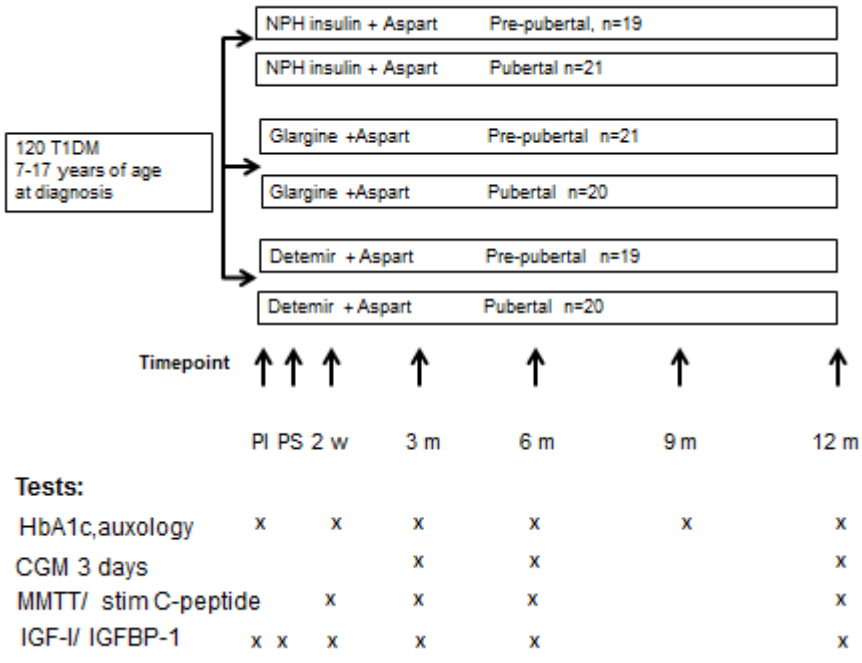

Fig.6. Schematic presentation of the research design in Paper III+IV.

4.3 METHODS

4.3.1 Glycemic control

4.3.1.1 *HbA1c*

Since the DCCT study (DCCT 1995) showed a correlation between intensive insulin treatment, improvement in HbA1c and reduction in prevalence of long-term complications, HbA1c is a parameter of metabolic control that is used as primary endpoint in most clinical comparisons of different insulin regimens in type 1 diabetes.

In paper I the change in HbA1c from start of the study until the 12 weeks endpoint was compared. The HbA1c after 6 weeks was also interpreted, although 6 weeks is a short time to monitor a difference in HbA1c. In papers II and III the difference in HbA1c between the treatment groups after 12 months was the primary end point. HbA1c was also measured with 3 months interval to find out the time of nadir.

In Sweden HbA1c was measured by the Mono-S standard until December 2010, while the most used standard for HbA1c is the DCCT standard, which is approximately 1 % unit lower than Mono-S standard. For reasons of uniformity the standard was in 2011 changed to IFCC standard. In paper III conversions were made using the formula: [(10.45 x HbA1c Mono S standard %) – 10.62] = HbA1c IFCC mmol/mol, which is the conversion formula stated to be used by the Swedish standardization organ, Equalis. This formula differs just slightly from the IFCC master equation, but since our central laboratory used the Equalis standard it was essential for us to also do so.

In paper I, all HbA1c samples were collected on filter paper, analyzed at the central laboratory and reported as Mono-S standard. In paper II HbA1c was analyzed either with DCA 2000 at the outpatient clinic or on filter paper, analyzed on the central laboratory, expressed as the Mono-S standard. In paper III all samples were collected on filter paper. Values were then reported as IFCC values after conversion.

4.3.1.2 *Fasting-glucose*

Second to HbA1c, fasting-glucose is the most common marker of glycemic control in clinical trials of diabetes. When comparing basal insulins with different duration, it is of great importance to compare the fasting-glucose. This measurement is also of great importance because of the glucose rise in the dawn phenomena (King, Clark et al. 2012). We studied adolescents and the fasting-glucose is of importance in regard to the deterioration in glucose control seen in pubertal individuals during late night caused by increased GH secretion (Dunger 1992). The sample was obtained after an overnight fast as the zero time-point of the MMTT. Given that the subjects had to travel to the hospital, it is possible that the glucose value may have been raised by the stress as compared to what we would have obtained at wake-up.

4.3.2 Continuous glucose monitoring (CGM)

In paper III, a blinded CGM system (CGM Medtronic gold®) was applied for three days at 3, 6 and 12 months. CGM consists of a disposable transcutaneous glucose sensor along with an

electronic transmitter/receiver unit (Wolpert 2010). Because the measurement is blinded to the patient, the aim was only to register the glucose variability, not to use it for dose adjustments. In general the accuracy of CGM values to blood glucose values has been shown to be high, except for very low glucose values (Bay, Kristensen et al. 2013). Some children considered it painful to have an iv.catheter or the probe for the CGM inserted. Therefore a considerable number rejected this examination. In other cases, we experienced technique problems with the CGM. Because of this we have a lower number of observations with these parameters.

From the CGM curves we derived different measures of glucose variability. The 24-hour and overnight (00:00-07:00) individual means and SD were calculated for the first full 24 hours available. A glucose variability index, MAGE, was calculated from the same 24 hours. Time spent at a low (≤ 3.9 mmol/L) or at a high (≥ 10.0 mmol/L) glucose was also calculated. The index MAGE was described already in 1970 (Service, Molnar et al. 1970) and it is still used as a reliable tool for assessing glucose variability (White, Chase et al. 2009; Ferenci, Korner et al. 2015). MAGE doesn´t take the actual glucose level into consideration, but instead only the glycemic swings, that exceed one SD. To compensate for this lack of information we also registered the time spent in low or high glucose.

4.3.3 Endogenous insulin production

To evaluate remaining endogenous insulin production in paper III we performed an MMTT. MMTT stimulated C-peptide area under the curve (AUC) is regarded as the golden standard (Greenbaum, Mandrup-Poulsen et al. 2008) for evaluation of preserved beta cell capacity. The MMTT (Ludvigsson, Faresjo et al. 2008) was performed at 2 weeks and at 3, 6 and 12 months. In brief, a mixed meal drink (Sustacal - Boost®; 6ml/kg) was given after an overnight fast (from 24:00), omitting basal and meal insulin in the morning. Blood samples for blood glucose and C-peptide were obtained at 0, 30, 60, 90 and 120 minutes. C-peptide AUC was calculated using the trapezoidal rule. The AUC mean equals the AUC divided by the interval of time, as with 120 minutes for a 2-hour MMTT, for example.

4.3.4 Anthropometric measurements and body composition

It is established that the body mass index (BMI) is an index of relative fatness. The measure has been validated against body fat measured by DXA in children and adolescents (Lindsay, Hanson et al. 2001). In papers I-III the height and weight of all subjects were registered at each clinical visit. From this data we calculated the BMI and this value was in paper III correlated to the Swedish standard of individuals of the same age and pubertal status to form the BMI standard deviation scores (SDS) (Wikland, Luo et al. 2002). In paper III the change in BMI from 2 weeks until 12 months was also registered, to consider the change in BMI during the study. By doing this this we could compare the influence of the different treatments and didn´t have to adjust for group differences at baseline. The reason to compare the 12-month value to the value of 2 weeks instead of baseline was that all individuals at diagnosis of type 1 diabetes are in varying state of metabolic derangement and hydration and often gain several kilos over the first week after rehydration and initiation of insulin therapy. By comparing to the 2-week BMI we abolished the effect of this acute weight loss. In the retrospective paper II, only weight was registered at every time-point. Therefore no BMI

could be calculated and registered variable of body composition was weight gain from 1 month after diagnosis until end of study at 12-15 months. The rational for comparison to 1 month weight instead of baseline was the same as in paper III.

Body fat determinations by bioimpedance have been validated versus DXA, including the instrument that we have used (InBody720 2002) and is often easier to perform and does not have irradiation effects. In paper III bioimpedance measurements (8-point tactile electrode) were performed by InBody 720 (Fysiotest Europa AB, Sweden) to assess the body fat percentage (BFP) at each visit.

In papers I and III the pubertal status was validated by a clinical examination. In paper I all individuals were in early or mid-puberty, since pubertal status ≥ 2 was inclusion criteria. In paper III+IV pubertal status at baseline was also important for the randomization. The pubertal development during the 12-month study was then followed and registered.

4.3.5 **Insulin doses**

Both basal and meal insulin doses per kg were recorded in papers I-III. They may be considered a measure of insulin sensitivity. Insulin doses were reported by the subjects at each visit and this information tends to not be completely accurate. Subjects may omit dosing and still report dosing, which is a strong limitation when comparing insulin doses of different insulin regimens, although there is no reason to suspect the misreporting to be different between the insulin treatments.

4.3.6 **GH-IGF-axis**

4.3.6.1 *IGF-I*

The levels of IGF-I in serum reflects the liver production of IGF-I and are regulated by portal insulin through potentiation of the GHR, in numbers and by post receptor effects (Maes, Underwood et al. 1986; Hanaire-Broutin, Sallerin-Caute et al. 1996; Hedman, Frystyk et al. 2004). We assumed that improved insulinization using long-acting insulin analogs should have hepatic effects and increased hepatic GH sensitivity leading to increased IGF-I production and serum concentration of IGF-I. Therefore, we used serum IGF-I level as an indirect marker of hepatic insulin actions in paper I and IV. IGF-I is not directly regulated by insulin via GHR signaling: GH status as well IGFBP-3, ALS and other IGFBPs contributes by regulating production and serum clearance, respectively. Therefore IGFBP-I, which is directly insulin regulated, may be more sensitive, as a marker of hepatic insulin actions, than IGF-I.

In healthy subjects, IGF-I increases in Tanner stage 2-3, reaches a peak in Tanner 3-4 and then decreases in late puberty in parallel to the changes in GH secretion (Juul, Bang et al. 1994). It is therefore important to relate the absolute IGF-I values to pubertal stage and age to make the comparison valid. In addition, linear regression analysis is not valid throughout the pubertal age range, if IGF-I is not expressed in SD scores. In paper IV, we calculated the sex, age and pubertal stage corrected IGF-I SDS by using a formula described previously (Juul, Bang et al. 1994).

4.3.6.2 *IGFBP-I*

Circulating IGFBP-1 is produced almost entirely in the liver and is down-regulated by exposure to portal insulin (Brismar, Fernqvist-Forbes et al. 1994). In contrast to endogenous insulin, injected insulin reaches the systemic circulation and hepatic insulin levels become sub-physiological. IGFBP-1 was shown to be a marker of hepatic insulin sensitivity in healthy subjects (Kotronen, Lewitt et al. 2008) and has also been used as a marker of hepatic insulin actions in several clinical studies of type 1 diabetes (Hall, Johansson et al. 1989; Lepore, Pampanelli et al. 2000). IGFBP-1 was measured in papers I and IV to evaluate portal insulin action/ sensitivity. IGFBP-1 is acutely down-regulated by increased hepatic insulin levels and has therefore a diurnal variation. Morning levels are high due to overnight fasting and are suppressed during the day by insulin release at meals. It is therefore important to consider the time-point when IGFBP-1 is measured. In paper I blood samples for IGFBP-1 were taken every 30 minutes between 21.00 and 17.00 during the two occasions that subjects were admitted. The mean values for overnight secretion and total sampling time was evaluated. In paper IV only fasting values were obtained.

4.3.6.3 *Overnight curves*

To determine levels of hormones that have a diurnal secretory pattern like GH and IGFBP-1, it is necessary to do 24-h or overnight curves. In paper I; GH, IGFBP-1 and IGFBP3-PA were analyzed hourly from 21:00 to 17:00 at time-point 0 and at the 6-week admission.

4.4 BIOCHEMISTRY AND HORMONAL ANALYSIS

HbA1c- was analyzed on filter paper using high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA, USA) or by DCA 2000 (Bayer, Elkhart, USA). The HbA1c assay was monitored against the External Quality Assurance in Laboratory Medicine in Sweden reference standard.

Glucose- was analyzed in whole blood with a bedside glucose dehydrogenase method (HemoCue®, Ängelholm, Sweden).

C-peptide- concentrations in serum samples were determined using a time-resolved fluoroimmunoassay (AutoDELFIA™ C-peptide kit, Wallac, Turku, Finland).

IGF-I- in paper I and IV total serum IGF-I concentrations were determined using an in-house des(1-3) IGF-I radioimmunoassay (RIA) after serum extraction by the acid-ethanol method followed by cryoprecipitation (Bang, Eriksson et al. 1991).

IGFBP-1- in paper I the serum IGFBP-1 concentrations were determined by RIA as previously described (Pihl, Carlsson-Skwirut et al. 2006), also in paper IV a RIA method was used (Povoa, Roovete et al. 1984).

Serum IGFBP-3 proteolysis (IGFBP-3-PA) - was determined by in vitro degradation of 125I-IGFBP-3 as previously described (Pihl, Carlsson-Skwirut et al. 2006).

GH- was analyzed in serum by a commercial dissociation-enhanced lanthanide fluorescence immunoassay from Perkin Elmer (Turku, Finland).

GHBP- serum GHBP was analyzed with a commercial ELISA (enzyme-linked immunoassay) kit (DSL-10-48100; Diagnostic Systems Laboratories).

All analyses are described in detail in each paper.

4.5 ETHICS

In papers I and III+IV all subjects, and their parents, were informed and then gave their informed consent. All studies were approved by the Regional Ethical Committee, Stockholm. Papers I and III+IV were also approved by the Medical Products Agency, Sweden. For paper III+IV the Karolinska Trial Alliance provided monitoring.

4.6 STATISTICAL ANALYSIS

All statistical analysis were performed with Sigma Stat 2.0 (Paper I), SAS (Paper II) and Sigma Plot 11.0 and SPSS (Papers III and IV). All baseline characteristics are given as descriptive characteristics. All follow-up data were tested for normality and expressed as mean ± SD (Paper II) or mean ± SE (Papers I, III and IV) if normally distributed, otherwise as median (25^{th}-75^{th} percentiles). Follow-up data were analyzed using Student´s t-test or Rank-Sum test (Paper I-IV), one-way Anova (Papers I, III and IV), one way repeated measures Anova (Paper II) and two ways repeated measures Anova (Papers II, III and IV) when appropriate. For all pairwise multiple comparison procedures, the Holm-Sidak, or Student-Newman-Keuls methods were used. Correlations were analyzed by linear regression (Papers I and IV) and by multiple linear regressions (Paper IV). $P < 0.05$ was considered significant.

5 RESULTS

5.1 GLYCEMIC CONTROL

5.1.1 HbA1c

In papers I-III HbA1c was lower with long-acting insulin analogs (glargine or detemir) compared to NPH at study end point 12 weeks (paper I) or 12 months (papers II and III).

Paper	NPH	Analog (glargine or glargine/detemir)	Comparison
I	8.3 ± 0.6 % (76.1 ± 6.3 mmol/mol)	7.3 ± 0.3 % (65.7 ± 3.2 mmol/mol)	0.008
II	7.1 ± 1.3 % (63.6 ± 13.6 mmol/mol)	6.3 ± 1.6 % (55.2 ± 16.7mmol/mol)	< 0.01
III (all ages)	6.6 ± 0.2 % (57.9 ± 2.2 mmol/mol)	6.1 ± 0.1 % (52.7 ± 1.0 mmol/mol)	0.019
III (pubertals)	6.8 ± 0.3 % (60.2 ± 3.2 mmol/mol)	5.9 ± 0.2 % (51.0 ± 1.7 mmol/mol)	0.008

Table 6: Comparison of HbA1c at end point for papers I-III. Values are given as Mono-S, % and (IFCC, mmol/mol) and reported as mean± SE for papers I+III and as mean ± SD for paper II.

In **paper I**, treatment with glargine decreased mean ± SE HbA1c from 8.3 ± 0.6% to a nadir of 7.3 ± 0.3 % at 6 weeks (P< 0.002). Improvement in HbA1c was significant after 2 weeks (7.5 ± 0.4 %, P < 0.008) and was sustained also after12 weeks (7.3± 0.3 %, P< 0.008).

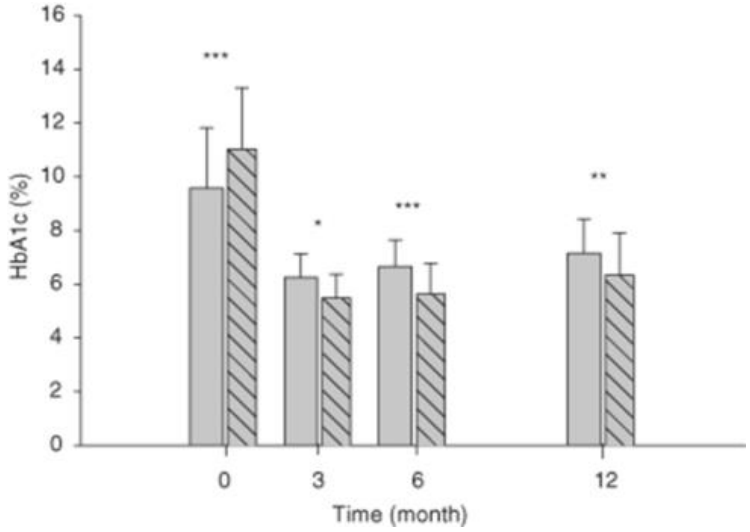

Fig.7. HbA1c at diagnosis and follow-up in paper II: at 3-5 months, 6-8 months, and 12-15 months in patients treated from diagnosis with NPH (open bars) or glargine (hatched bars) in an MIT regimen. Results presented as mean ± SD. *p < 0.05, **p < 0.01, and ***p < 0.001 by two-way repeated measures Anova.

In **paper II**, HbA1c in the glargine group was lower at all follow up time-points compared to NPH (Fig.7).

In **paper III**, mean ± SE HbA1c at 12 months was lower with analogs compared to NPH (52.7 ± 1.0 vs. 57.9 ± 2.2 mmol/mol, P = 0.019). This difference was entirely due to lower HbA1c in pubertal children (51.0 ± 1.7 vs. 60.2 ± 3.2 mmol/mol, P = 0.008) (Fig.8). The 12-month HbA1c differed among the three basal insulin treatment groups in pubertal children (P = 0.026), but not in prepubertal (P = 0.34).In pubertal subjects, HbA1c in NPH-treated (60.2 ± 3.2 mmol/mol) was borderline higher than glargine (52.3 ± 3.1 mmol/mol, P = 0.057) and significantly higher than detemir (49.8 ± 1.9 mmol/mol, P = 0.01). There were no differences between analogs and NPH at any other time-point. HbA1c decreased rapidly after start (P < 0.001) of insulin therapy and reached a nadir at 3 months in all treatment groups (Fig. 2C and 2D in paper III).

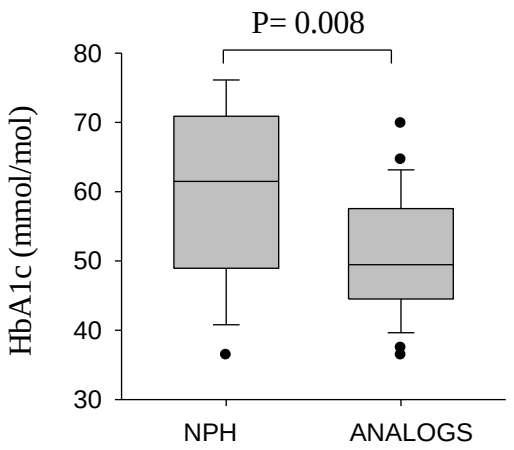

Fig.8. Hba1c at 12 months in pubertal subjects treated from diagnosis with NPH or analogs (glargine or detemir).

5.1.2 Fasting-glucose

Fasting-glucose values were only assessed in **paper III**. At 12 months mean ± SE fasting-glucose was significantly lower in pubertal subjects on analogs vs. NPH (8.2 ± 0.5 vs. 10.5 ± 0.9 mmol/l). A difference among the three pubertal groups at 12 months almost reached statistical significance. No differences were found at other times.

5.1.3 Glucose variability

Different measurements of glucose variability, calculated from CGM data were presented in **paper III**. The 12-month individual 24-hour mean and SD of mean of glucose values, mean overnight glucose, % of time spent in hyperglycemia or hypoglycemia and MAGE did not differ between NPH and analogs (table 2 of paper III) or among treatment groups.

5.2 ENDOGENOUS INSULIN PRODUCTION

In **paper III** we reported that the median (25^{th} - 75^{th} percentiles) 12-month stimulated C-peptide AUC did not differ between NPH versus analogs (0.16 (0.12 - 0.33) vs. 0.18 (0.08-0.52) nmol/lx2h, in prepubertal or 0.42 (0.18 - 0.84) vs. 0.51 (0.22 - 0.71) nmol/lx2h, in pubertal subjects). 12-month fasting C-peptide did not differ between NPH and analogs (0.35 (0.18 - 0.47) vs. 0.30 (0.11 - 0.77) nmol/l, in prepubertal or (0.41 (0.21 - 0.98) vs. 0.64 (0.31 - 1.00) nmol/l pubertal subjects. Stimulated C-peptide AUC peaked at 3 months and then declined gradually until 12 months (P< 0.001) (Fig.9). The change in stimulated C-peptide AUC between 3 and 12 months was not different between NPH and analogs (Table 2 in paper III).

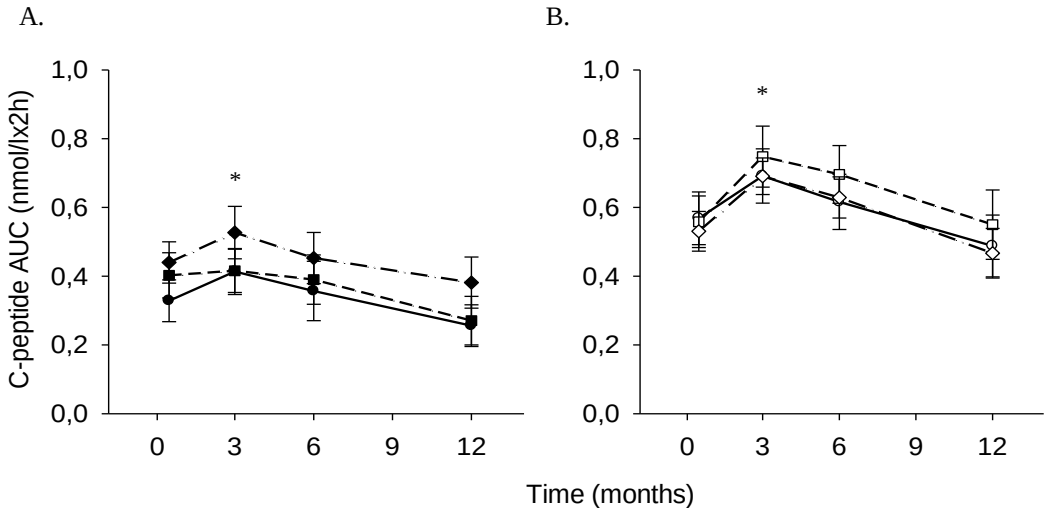

Fig.9. C-peptide AUC in A. prepubertal and B. pubertal subjects from diagnosis treated with NPH (solid line), glargine (dashed lines) and detemir (dashed and dotted lines). * P < 0.001 from all other time-points.

In **paper IV** we reported that C-peptide AUC predicted HbA1c at 6 and 12 months (r = 0.32, P = 0.002 and r = 0.30, P = 0.003, respectively). In a multivariate analysis, insulin group and C-peptide AUC at 12-months predicted 39 % of the variation in HbA1c with a contribution from both group (P = 0.018) and C-peptide AUC (P = 0.003). At 6 months only C-peptide AUC predicted HbA1c (P = 0.002).

5.3 ANTHROPOMETRIC MEASUREMENTS AND BODY COMPOSITION

In **paper I** mean ± SE body weight increased from 46.8 ± 2.5 kg to 48.3 ± 2.5 kg (P = 0.002), height from 157.8 ± 2.0 cm to 158.7 ± 2.0 cm (P = 0.002) and BMI from 18.7 ± 0.8 kg/m² to 19.1 ± 0.9 kg/m² (P = 0.03) from 0 to 6 weeks.

In **paper II** mean ± SD weight gain did not differ between groups; 5.69 ± 4.4kg in the NPH-group vs.5.69 ± 4.1 kg with glargine.

In **paper III**, no differences in 12-month BMI SDS, change in BMI SDS from 2 weeks to 12 months, 12-month BFP, or change in BFP from 2 weeks to 12 months were observed between NPH and analogs or among the treatment groups. Values are reported in table 2, paper III.

5.4 INSULIN DOSES

In **paper I**, the mean ± SE total daily insulin dose at first evaluation was 1.21 ± 0.12 IU/ kg, and after 6 weeks, 1.05± 0.11 IU /kg or 89 ± 6% of the initial dose (P = ns). However, the change of total insulin dose and in IGF-I was positively correlated (r = 0.61, P = 0.046).

In **paper II**, the mean ± SD total insulin dose (basal and meal) was similar at nadir (lowest point), 0.47 ± 0.16 U/kg with glargine vs. 0.51 ± 0.20 U/kg with NPH. At 12-15 months the dose was lower with glargine vs. NPH (0.64 ± 0.23 vs. 0.86 ± 0.31 U/kg; P < 0.001).

In **paper III**, the lowest basal and meal insulin doses were seen at 3 months, with no difference among the treatment groups. At 12 months, mean basal doses had increased approximately 30 % in NPH and glargine, and almost 50 % in detemir. The detemir dose was 0.47 units/kg, 67 % higher compared to glargine (0.28 U/kg, $P < 0.001$) and 27 % higher than NPH (0.37 U/kg, $P < 0.022$) for all subjects. The 12-month basal insulin requirements were approximately 30 % higher in pubertal children compared to prepubertal. The 12-month meal doses did not differ among the groups.

5.5 GH-IGF-AXIS

5.5.1 IGF-I

In **paper I**, the mean ± SE IGF-I level increased, from 231 ± 19 at time-point zero to 309 ± 17 µg/l after 1 week ($P < 0.001$). At the 6 weeks admission, IGF-I was 274 ± 25 µg/l ($P = 0.022$). It peaked at 12 months, 347 ± 25 µg/l ($P < 0.001$), 54 ± 9% over baseline. HbA1c at time-point zero was positively correlated with the increase of IGF-I at all time-points ($r = 0.93$, $P < 0.001$ at 6 weeks). The change in HbA1c over time mirrored that of IGF-I (Fig 1 in paper I).

The individual IGF-I SDS of all subjects in paper I is shown in Fig.10. The mean ± SE IGF-SDS was subnormal on NPH at time-point zero (-1.8 ± 0.4). Although, at 12 weeks mean IGF-I SDS had increased to -0.55 ± 0.3 SDS ($P < 0.001$).

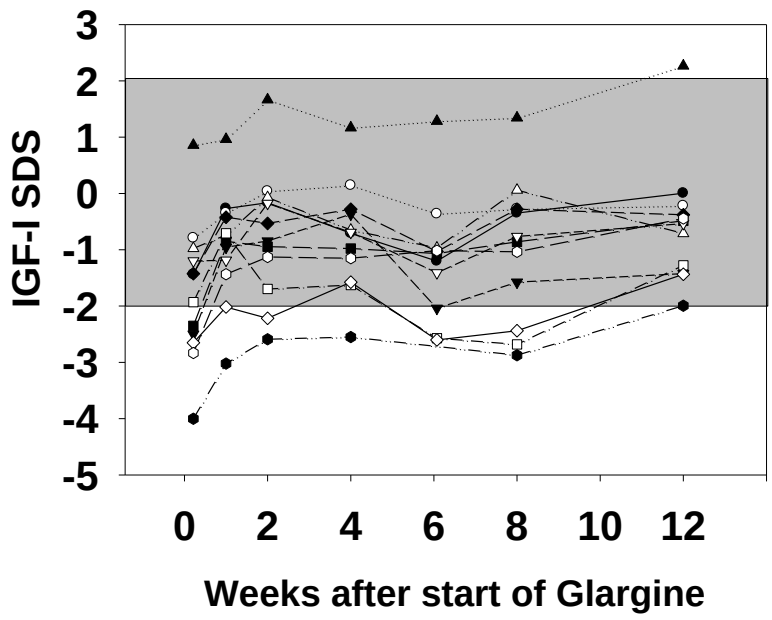

Fig.10. Changes in individual IGF-I SDS during 12 weeks of glargine treatment in adolescents with type 1 diabetes, earlier treated with NPH.

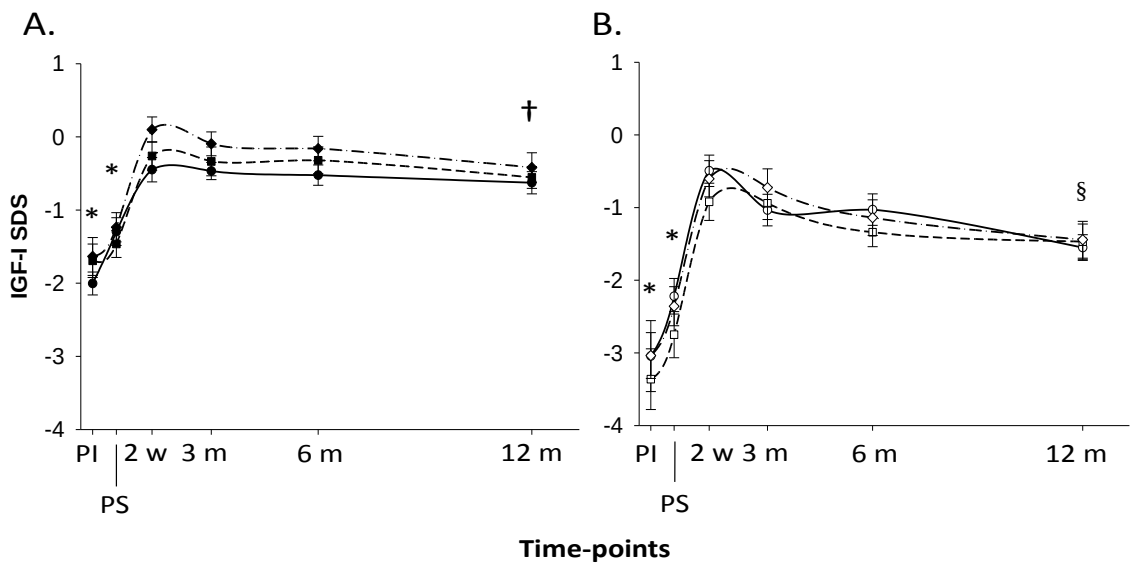

Fig.11. IGF-I SDS over time in A. prepubertal and B. pubertal subjects treated with NPH (solid line), glargine (dashed line) and detemir (dashed-dotted line) from diagnosis of type 1 diabetes.
* Difference for all subjects at that time-point compared to all other time-points, P< 0.001† Difference for all subjects at that time-point compared to 2 weeks, P= 0.006. § Difference for all subjects at that time-point compared to 2 weeks, P < 0.001.

In **paper IV** the 12-month mean IGF-I SDS did not differ among the three insulin groups or between NPH and analogs in prepubertal or pubertal subjects. Neither were there any differences at 3 and 6 months, respectively (Table 2, paper IV). IGF-I SDS changed over time, with the lowest values at PI and PS in both prepubertal and pubertal subjects. IGF-I SDS increased towards normal levels at 2 weeks in all treatment groups. In pubertal subjects, IGF-I SDS then declined to markedly subnormal levels at 12 months while the prepubertal IGF-I SDS levels were less subnormal (Fig.11).

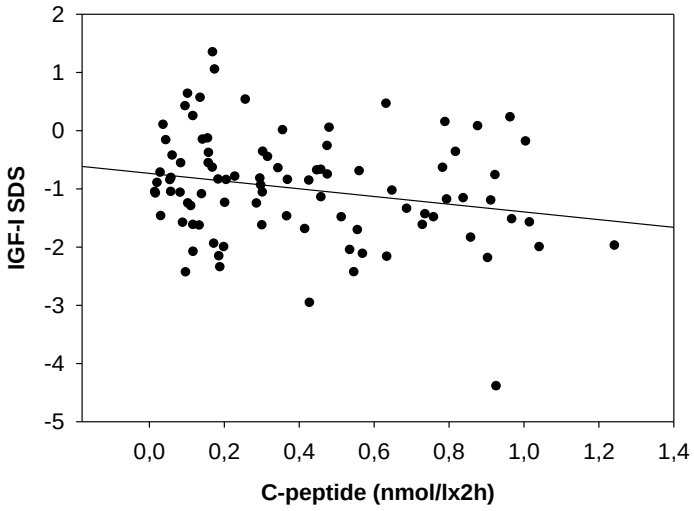

Fig.12. IGF-I SDS at 12 months was inversely correlated with C-peptide AUC at 12 months in all subjects treated with NPH, glargine or detemir from diagnosis of type 1 diabetes.

In **paper IV** univariate analysis demonstrated that 12-month IGF-I SDS was inversely dependent on C-peptide AUC (r = -0.23, P = 0.029) (Fig.12 on p. 33). The 6-month IGF-I SDS did not correlate with C-peptide AUC. However, 12-month IGF-I SDS was strongly inversely dependent on age (r = -0.47, P < 0.001) and tanner stage (r = -0.56, P < 0.001) and in multivariate analysis the dependency on C-peptide was lost.

To summarize, we found an increase in IGF-I SDS in paper I, when changing from NPH to glargine. This difference in IGF-I SDS could not be seen when comparing NPH to glargine or detemir from diagnosis in paper IV; however, there was an increase in IGF-I SDS in all treatment groups, with a maximum level at 2 weeks. In both paper I and IV all individuals and especially those in puberty had subnormal IGF-I SDS levels.

5.5.2 **IGFBP-1**

In **paper I**, individual IGFBP-1 patterns increased at late night and early morning. On glargine the morning values were in some individuals lower than on NPH (Fig. 2A in Paper I).The mean ± SE overnight IGFBP-1concentration decreased from 127 ± 21 µg/l to 90 ± 12 µg/l at 6 weeks (P = 0.035) (Fig.13), but was not significantly lower with glargine when evaluated over the total 20 hours admission period (P = 0.065).

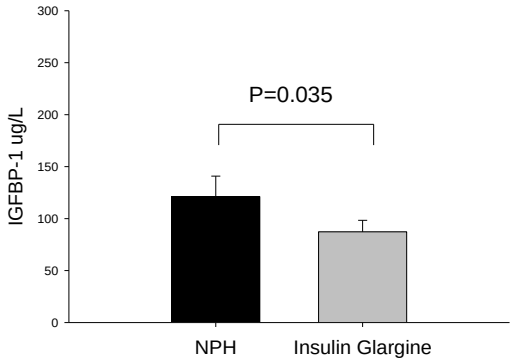

Fig.13. IGFBP-1 mean overnight concentration (00.00-10.00) on NPH (baseline) and after 6 weeks with glargine in adolescents with typ1 diabetes.

In **paper IV**, the 12-month mean ± SE IGFBP-1 was lower in pubertal subjects on analogs than on NPH (69.4 ± 6.9 vs. 98.7 ± 13.9 µg/l, P = 0.04) (Table 2, paper IV). No difference in IGFBP-1 was found among pubertal subjects on NPH (98.7 ± 14 µg/l), glargine (69.5 ± 9 µg/l) or detemir (69.2 ± 12 µg/l), P = 0.12 at 12 months with one way Anova. IGFBP-1 was also lower in pubertal subjects on analogs than on NPH at 3 and 6 months (Table 2, paper IV). In the prepubertal subjects, no difference was found between NPH and analogs at any time-point (Table 2, paper IV).

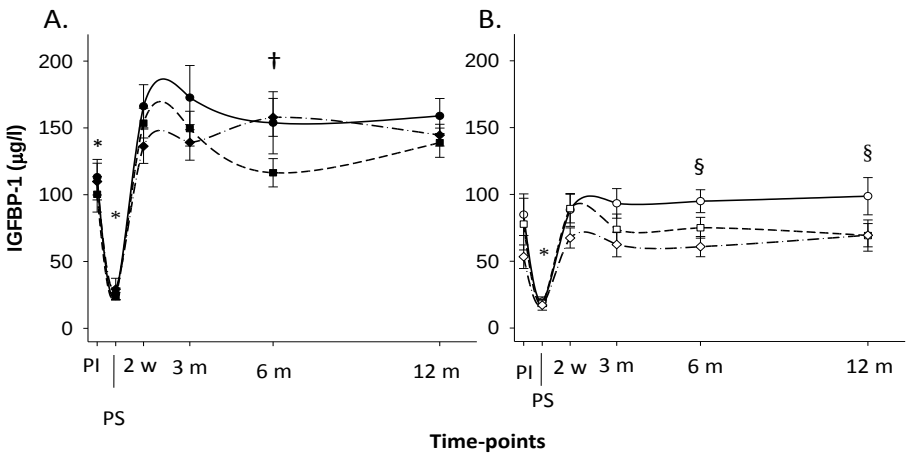

Fig.14. IGFBP-1 over time in A. prepubertal and B. pubertal subjects treated from diagnosis with NPH (solid line), glargine (dashed line) and detemir (dashed-dotted line).
* Difference for all subjects at that time-point compared to all other time-points, P< 0.001.
†Difference between subjects treated with glargine and detemir at that time-point by repeated measures Anova, P= 0.046. § Difference between subjects treated with NPH and detemir at that time-point by repeated measures Anova, P= 0.03.

IGFBP-1 was at its lowest level at PS in both prepubertal and pubertal subjects (Fig.14). With repeated measures Anova at 6 and 12 months in pubertal subjects both glargine and detemir were lower than NPH, but significantly only with detemir (Fig.14).In prepubertal subjects IGFBP-1 was lower in glargine compared to detemir by repeated measures Anova at 6 months. Prepubertal values were higher than pubertal at all time-points.

Univariate analysis demonstrated that 6- and 12-month (Fig.15) IGFBP-1 were inversely dependent on C-peptide AUC (r = -0.47, P < 0.001 and r = -0.28, P = 0.007). The 6- and 12-month IGFBP-1 was also strongly dependent on age (r = -0.55, P < 0.001 and r = -0.56, P <0.001, respectively) and tanner stage (r = -0.57, P < 0.001 and r = -0.58, P < 0.001, respectively). In multivariate analysis these parameters predicted 63 % of the variation in 6 months IGFBP-1 with C-peptide as the strongest predictor (P= 0.002), while C-peptide did not contribute to IGFBP-1 prediction at 12 months.

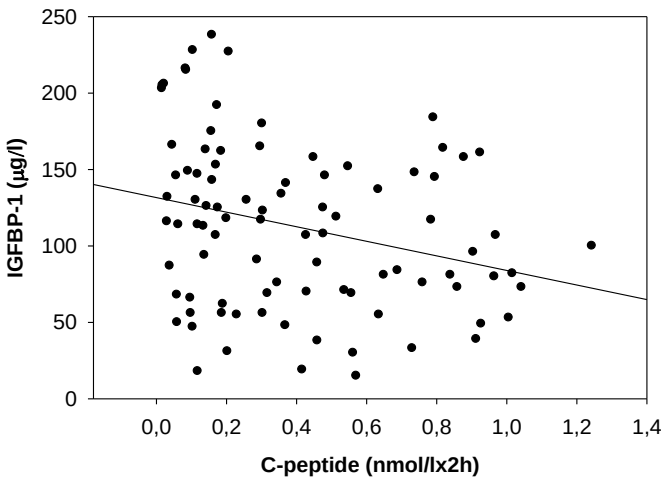

Fig.15. 12-month IGFBP-1 correlated inversely with 12-month C-peptide AUC in all subjects treated with NPH, glargine or detemir from diagnosis (r = -0.28, P = 0.007).

To summarize; lower IGFBP-1 by treatment with analogs, glargine or detemir, were shown in pubertal subjects in both papers I and IV.

5.5.3 GH, GHBP and IGFBP3-PA

GH, GHBP and IGFBP3-PA were investigated during the two admissions at 0 and 6 weeks in **paper I**. The individual GH patterns did not differ between treatment with NPH (0 weeks) or glargine (6 weeks) (Fig.2B in paper I). The mean ± SE GHBP did not did not differ between time-point zero and 6 weeks (523± 95 vs. 488± 80 pmol/l). The mean IGFBP3-PA was lower with glargine ($P < 0.001$). The mean values of IGFBP3-PA during day time were higher than values during night with both NPH and glargine.

6 GENERAL DISCUSSION

Long-acting insulin analogs, glargine and detemir, have a prolonged and more reproducible insulin profile compared with the intermediate-acting NPH (Heise and Pieber 2007). The overall hypothesis for this thesis is that treatment with long-acting insulin analogs, glargine or detemir, in children and adolescents with type 1 diabetes results in better metabolic control, increased endogenous insulin production, and normalization of the GH-IGF-axis. The four papers all address these questions in different study settings.

6.1 METABOLIC CONTROL

In paper I we found a 1 % unit (10 mmol/mol) lower HbA1c in 12 adolescents with type 1 diabetes, 12 weeks after changing from NPH to glargine. The same has been shown in other observational, non-randomized studies, both in adults (Manini, Forlani et al. 2007) and in children and adolescents (Chase, Dixon et al. 2003; Hathout, Fujishige et al. 2003; Jackson, Ternand et al. 2003; Alemzadeh, Berhe et al. 2005; Colino, Lopez-Capape et al. 2005; Urakami, Naito et al. 2014). One non-randomized study, over 12 months failed to show a difference in HbA1c (Paivarinta, Tapanainen et al. 2008), while one early randomized study showed equal HbA1c, but an improvement in fasting-glucose (Raskin, Klaff et al. 2000).

Paper I explored the potential effects of long-acting analogs on the GH-IGF-system. The study was not designed to make final conclusions regarding the effects on HbA1c, since it lacks a control group. When changing from one treatment to another in a study environment, subjects are encouraged to perform better and the tight follow up and increased supervision can therefore lead to better compliance and thereby an improved HbA1c (placebo effect). This confounder is also discussed by Jackson et al (Jackson, Ternand et al. 2003) in their study of children and adolescents with type 1 diabetes. A subgroup with poor metabolic control had greater improvement in HbA1c and they discussed whether the reason was that this group benefitted more from increased supervision. We also observed a BMI gain during the study, although, glargine is not reported to induce weight gain (Monami, Marchionni et al. 2009). Poor baseline metabolic control with a mean HbA1c of 8% (73 mmol/mol) in our subjects may be more likely to cause a BMI increase when glycemia is improved.

In paper II we showed lower HbA1c after one year in children with type 1 diabetes treated from diagnosis with glargine or NPH, in a retrospective study. Studying metabolic control from diagnosis, in patients with a significant remaining endogenous insulin production, is different from studies performed on subjects with more than one year of duration of diabetes. Few studies on type 1 diabetes are performed from diagnosis. Adhikari et al. also showed lower HbA1c 1 year from diagnosis, but they retrospectively compared glargine, once daily in a MIT regimen versus conventional therapy with NPH twice daily (Adhikari, Adams-Huet et al. 2009). Intensive insulin treatment including MIT or CSII (DCCT 1993) and also an increased number of boluses (Danne, Battelino et al. 2008) are shown to improve metabolic control. Therefore the improvement reported by Adhikari et al. may not be entirely due to the treatment with glargine, but could have a significant contribution from the intensified treatment by a MIT regimen. In another study, starting close to diagnosis, Hassan et al. showed better HbA1c with glargine compared to NPH (Hassan, Rodriguez et al. 2008). Here identical regimens were used. They randomized to either glargine or NPH, both basal insulins

given twice daily at the same time as the two daily injections of rapid-acting analog. Thus, none of these regimens can be expected to be optimal or realistic in an up-to-date clinical setting. Glargine is likely to require a MIT regimen to be advantageous, since it does not provide meal coverage for the next meal. In contrast, NPH has a significant effect peak 4-6 hours after injection which may cover lunch after a pre-breakfast injection and a snack in the evening after the pre-dinner injection. Therefore, the advantage of glargine reported by Hassan et al is somewhat surprising. With the prolonged action of glargine, compared to NPH, the insulin action profile will be more physiological with glargine given in a MIT regimen.(Hirsch 2005; Porcellati, Bolli et al. 2011).

In paper III, we compared long-acting analogs, glargine or detemir versus NPH in a RCT from diagnosis of type 1 diabetes. The primary end point was HbA1c after 12 months. We found lower HbA1c with the analogs compared to NPH. By stratifying patients for pubertal status, we were able to show that the advantage of glargine and detemir was only found in the pubertal subjects. This is the first study to show lower HbA1c in children in a RCT and this is the first study to assess the effects of long-acting insulin analogs from diagnosis over 12 months. Other RCTs in adults have also showed lower HbA1c with glargine (Porcellati, Rossetti et al. 2004; Fulcher, Gilbert et al. 2005; Chatterjee, Jarvis-Kay et al. 2007) or detemir (Home, Bartley et al. 2004), but in children and adolescents all RCTs over more than 3 months have failed to show an advantage of long-acting insulin analogs on HbA1c (Schober, Schoenle et al. 2002; Murphy, Keane et al. 2003; Robertson, Schoenle et al. 2007; Chase, Arslanian et al. 2008; Thalange, Bereket et al. 2011; Thalange, Bereket et al. 2013; Rostami, Setoodeh et al. 2014). Chase et al (Chase, Arslanian et al. 2008) showed lower HbA1c after 6 months, with glargine compared to NPH, in a subgroup of adolescents that had the highest HbA1c. It is possible that this subgroup consisted of a majority of pubertal patients, in whom suboptimal control and a greater degree of insulin resistance are common and that they benefited more from improved insulinization by glargine. Another possible reason for the improvement is that those with poor metabolic control received more benefit from the regulated study conditions and increased guidance, than those with good metabolic control, who may have already had more regulated control of their diabetes previous to the study.

In paper III we stratified for puberty and the improvement in HbA1c was only seen in the pubertal subjects treated with glargine or detemir. It is well known that insulin resistance during puberty is further augmented in adolescents with type 1 diabetes, mainly as a result of insufficient portal insulin exposure and consequently low IGF-I and hyper-secretion of GH (Dunger 1992; Acerini, Williams et al. 2001). The increase in insulin requirements, particularly during the early morning, should be favored by the pharmacokinetic profiles of glargine or detemir with sustained insulin effects. In contrast to our study, few other studies have stratified for puberty. Contrary to our findings, a Japanese survey saw less significant improvement in HbA1c with glargine in the older age group compared to those below 13 years of age (Urakami, Naito et al. 2014). Thalange et al. also subdivided their study population according to age, but the HbA1c didn´t differ in any age group in the comparison of detemir and NPH (Thalange, Bereket et al. 2013).

The use of CSII, like long-acting analogs, is a more physiological way to deliver insulin (Bolli, Andreoli et al. 2011) that has been compared to NPH or long-acting analogs with MIT in two meta-analysis in children. A small beneficial effect on HbA1c with CSII has been

found in both (Churchill, Ruppe et al. 2009; Pankowska, Blazik et al. 2009). None of the included studies assessed patients from diagnosis. A Swedish study compared CSII to NPH with MIT from diagnosis of type 1 diabetes in children and adolescents. They could not show improved HbA1c with CSII (Skogsberg, Fors et al. 2008). They studied a similar age group to our study, although they did not stratify for puberty, which may have prevented them from finding a small benefit of CSII in the pubertal group. The recently introduced ultra-long-acting insulin analog, degludec (Biester, Blaesig et al. 2014) can be expected to have a positive effect on metabolic control. However a meta-analysis has showed no beneficial effect on metabolic control compared to glargine and detemir (Dzygalo, Golicki et al. 2014). Additionally an RCT, comparing degludec to detemir in children over 26 weeks, also showed comparable HbA1c (Thalange, Deeb et al. 2015).

Fasting-glucose is an important measure of glycemic control complementing HbA1c, and it may more specifically reflect the impact of the nighttime insulin effects of basal insulin in patients with type 1 diabetes. Fasting-glucose was measured in paper III and like HbA1c it was lower in pubertal subjects treated with long-acting analogs. Several RCTs in adults showed both better HbA1c and lower fasting-glucose (Home, Bartley et al. 2004; Porcellati, Rossetti et al. 2004; Fulcher, Gilbert et al. 2005; Chatterjee, Jarvis-Kay et al. 2007). In children, the only study to show concomitant improvements in HbA1c and fasting-glucose was the study by Hassan et al.(Hassan, Rodriguez et al. 2008), where they investigated children close to diagnosis, but their study design had limitations as already discussed above. There are several studies, both in adults and children that failed to show better HbA1c. However, several of these studies reported lower fasting-glucose with glargine or detemir (Ratner, Hirsch et al. 2000; Schober, Schoenle et al. 2002; Murphy, Keane et al. 2003; Russell-Jones, Simpson et al. 2004; Robertson, Schoenle et al. 2007) compared to NPH. One reason for finding improved fasting-glucose, but not better overall improved glycemia leading to better HbA1c could be that the long-acting analogs have larger impact on nighttime insulin delivery.

A unique part of our study was to provide repeated three day CGM measurements during the study. However, the CGM measurements did not support that overnight glycemia or glucose variability calculated as SD of mean glucose or MAGE were improved. Moreover, the 24-hour data did not support the improvement in HbA1c that we found in the pubertal subjects. One possible explanation for this is that loss of data from children who rejected carrying the CGM affected our chance to detect differences. Our results accorded with a study in adults treated with glargine compared to NPH over 36 weeks, reporting lower HbA1c and lower fasting-glucose, but no difference in glucose by CGM (Chatterjee, Jarvis-Kay et al. 2007). In this study, the number of subjects that had the CGM was as in our study low. A subgroup of pubertal children with type 1 diabetes included in a RCT comparing glargine and NPH (Chase, Arslanian et al. 2008) were investigated with CGM and, in contrast to our study, White et al. showed lower glucose variability and no difference in HbA1c (White, Chase et al. 2009). Similar to our study they assessed MAGE and SD of glucose, among other glucose variability indexes. In contrast to our study population, these adolescents were C-peptide negative and had fairly poor metabolic control, which makes a comparison to our findings difficult. In conclusion, not only HbA1c, but also glucose variability is believed to be

important to delay vascular complications (Monnier, Colette et al. 2010) and should therefore also be taken into consideration when evaluating the metabolic control of a patient.

We found no differences in time spent with high (> 10 mmol/l) or low (< 3.5 mmol/l) blood glucose over 24 hours. Neither were there any differences in overnight (00 - 07) mean glucose. In contrast, the meta-analysis by Monami reported significantly less nocturnal hypoglycemia with long-acting analogs (Monami, Marchionni et al. 2009). A 16-week crossover study with NPH versus glargine in adolescents, performing overnight glucose profiles, reported lower overnight mean with NPH, meanwhile lower fasting-glucose and less nocturnal hypoglycemia with glargine (Murphy, Keane et al. 2003). Most likely these apparently contrasting results are explained by the insulin profile of NPH, with a prominent peak around midnight, causing hypoglycemia and later failure to provide adequate insulinization at dawn, resulting in higher fasting-glucose with NPH.

6.2 ENDOGENOUS INSULIN PRODUCTION AND INSULIN DOSES

Intensive insulin treatment from diagnosis of type 1 diabetes induces beta cell rest and may thereby prolong and enhance endogenous insulin production (DCCT 1998). The capacity of an individual to secrete endogenous insulin can be measured by quantification of the secreted amount of C-peptide after a mixed meal, a MMTT. Furthermore, preserved endogenous insulin can be supposed to decrease the need for exogenous insulin. However, to use the need of exogenous insulin to assess endogenous insulin production has several limitations, even if the subject has adequate metabolic control.

In paper I we assessed pubertal individuals with diabetes duration of one to six years, but did not measure endogenous insulin production. Preserved beta cells capacity is higher during the first year from diagnosis and can be found as long as up to five year from diagnosis in some individuals (Steele, Hagopian et al. 2004; Greenbaum, Anderson et al. 2009). Given this it is possible that some of the individuals in paper I had remaining endogenous insulin. It is, however, unlikely that major changes in C-peptide should occur during the 12 weeks of that study or that such changes would impact on HbA1c or the GH-IGF-axis, particularly given the C-peptide results from paper III discussed below.

In paper II we retrospectively compared children with newly diagnosed type 1 diabetes treated with NPH or glargine, through obtaining clinical data from patient's electronic records. Fasting C-peptide was not routinely measured and therefore could not be investigated. The two groups were comparable in all aspects except from basal insulin treatment. We found that the glargine treated group, not only had lower HbA1c, but also reported lower insulin doses at 12 months from diagnosis. Using exogenous insulin requirements as a proxy for endogenous insulin production, we hypothesized that the lower insulin need could be due to better preserved endogenous insulin production and that this was one factor that explained improved HbA1c in the glargine treated group. To investigate this further we performed a MMTT at 4 different time-points in paper III. We found no evidence of higher C-peptide AUC at 12 months, or a slower decrease from baseline in children on analogs versus NPH. These findings did not support our hypothesis that improved metabolic control was related to better preserved beta cell capacity. On the other hand, in paper IV we report that 6- and 12-month C-peptide AUC predicted HbA1c in a univariate analysis, and in

multivariate analysis we found that both insulin treatment group and C-peptide AUC were important for 12-month HbA1c. In contrast, only C-peptide AUC predicted 6-month HbA1c, which may suggest a role of endogenous insulin production on metabolic control at least on an individual level. Endogenous insulin is often well-preserved during the first year with type 1 diabetes in children (Greenbaum, Anderson et al. 2009). Therefore it is possible that an effect of the analogs on C-peptide on a group level would become apparent later on. In this aspect, the follow up time of only one year, is a limitation. Furthermore, newly diagnosed children may benefit from their endogenous insulin secretion, and may therefore not be so dependent on exogenous insulin. However, this did not prevent us from demonstrating an effect of analogs on HbA1c in the pubertal children.

We found the maximum stimulated C-peptide levels to occur after 3 months and then noticed a small decline until 12 months. At 12 months most individuals had levels of C-peptide > 0.2 nmol/l, which is the commonly used cut-off for C-peptide positivity These findings accorded with previous studies, showing that most children and adolescents have the capacity to secrete C-peptide 1 year from diagnosis (Greenbaum, Anderson et al. 2009). In paper III, C-peptide levels were higher in pubertal subjects versus prepubertal. This might be explained by a faster decline in C-peptide levels in younger children (Greenbaum, Beam et al. 2012), but is in opposition to our finding that the decline in C-peptide from 3 until 12 months did not differ between prepubertal and pubertal subjects.

Beta cell capacity was evaluated by a 2 hours MMTT, at fasting conditions in the morning (Ludvigsson, Faresjo et al. 2008). All individuals took their basal insulin in the evening prior to the test, but they did not take basal insulin (or meal insulin) the morning of the test. Whether a more sustained insulin effect of the analogs during the night may have affected the stimulated insulin/C-peptide release during the MMTT is not known. An increased release is possible if endogenous insulin release is spared by a sustained effect of analogs. However, it may also be speculated that a decreased release could be observed, if exogenous insulin covers part of the insulin need in patients with euglycemia during the test. Against findings that the nightly action of the basal insulin would affect the c-peptide concentration, Besser et al. found that by giving insulin during a MMTT, the C-peptide is only slightly reduced (Besser, Jones et al. 2012).

Long-acting insulin analogs given in a MIT regimen with rapid-acting meal insulin is thought to provide a more physiological insulin profile. This may also be achieved by the use of CSII (Bolli, Andreoli et al. 2011). Our research group has recently found that treatment with CSII versus NPH in a MIT regimen from diagnosis of type 1 diabetes did not increase endogenous insulin secretion in children and adolescents (Ekström, Skogsberg et al. 2013). In an older study, comparing CSII to conventional therapy from diagnosis, de Beaufort et al. reported better HbA1c after 2 years, but no difference in glucagon stimulated C-peptide or urinary C-peptide. They argued that the reason there was not better preservation of C-peptide with CSII was the young age of the patients, < 15 years, according to the inverse relation between loss of C-peptide and age (de Beaufort, Houtzagers et al. 1989). Furthermore, in a more recent study, which compared treatment with CSII to glargine in adolescents with newly diagnosed diabetes at 12 months, Thrailkill et al. found improved HbA1c (Thrailkill, Moreau et al. 2011), but failed to show significantly higher stimulated C-peptide levels with CSII. A

difficulty comparing C-peptide levels among studies are the heterogeneity of C-peptide assays used.

The exogenous insulin dose needed to lower glucose to a desired level is dependent on endogenous insulin secreting capacity, insulin sensitivity and how and where the insulin is administrated (Wiegand, Raile et al. 2008). In paper III we evaluated the basal insulin dose and found no difference between glargine and NPH treated subjects after 12 months. This is in opposition to our finding in paper II of lower doses with glargine treatment after one year. In paper III the doses of detemir at 12 months were 67 % higher than those of glargine ($P < 0.001$) and 27% higher than those of NPH ($P < 0.022$). One reason for the higher detemir doses could be the bi-daily delivery, although NPH was also given bi-daily. This dose relation between glargine and detemir is according to what Porcellati et al. found in a clamp study, directly comparing glargine and detemir, where one unit of detemir was 30% less active than 1 unit of glargine, estimated by the need of iv glucose (Porcellati, Rossetti et al. 2007). In vitro data indicates that detemir has a lower affinity to the IR and therefore has a 4-fold lower metabolic effect than human insulin (Kurtzhals, Schaffer et al. 2000). When detemir was developed in clinical trials, it was assigned a lower insulin units/mole than the other insulin analogs (which means that more moles of detemir are given for the same number of units of insulin). However, it seems that this potency assignment was not exact. A retrospective comparison in children showed 27 % higher basal insulin doses with detemir compared to glargine, but no other differences between the groups except that a higher proportion with detemir were treated twice daily (Abali, Turan et al. 2014). As a proxy for insulin sensitivity or endogenous insulin production, comparisons of total daily insulin needs in a detemir regimen compared to other basal insulin regimens is not going to be meaningful.

In paper III the reported insulin doses reached a nadir in all treatment groups at 3 months and thereafter doses increased. At 12 months mean basal doses had increased approximately 30 % in NPH and glargine, and almost 50 % in detemir. The time-point for the nadir coincides with the time for the maximal C-peptide levels. This finding speaks in favor of an effect of endogenous insulin secretion on reducing the need of exogenous insulin.

6.3 BODY COMPOSITION

Both in vitro (Kurtzhals, Schaffer et al. 2000) and in vivo clamp studies (Porcellati, Rossetti et al. 2007) show that detemir has a lower lipogenic effect than human insulin and glargine. This could explain why treatment with detemir is associated with less weight gain in most RCTs (Monami, Marchionni et al. 2009). We examined both BMI gain and increased BFP in paper III. In contrast to other studies in children and adolescents (Robertson, Schoenle et al. 2007; Thalange, Bereket et al. 2013), we found no differences between the insulin treatments in any of these aspects. It should be taken into consideration that detemir was given in a larger dose in our study and resulted in better HbA1c. If improvements in HbA1c are not achieved, less effect on weight gain may be expected. In the referred studies, no differences in doses were reported and both NPH and detemir were used either once or twice daily. It has been speculated that both free and albumin-bound detemir can pass into the liver, while only free detemir can pass over the capillary wall. This could possibly result in a greater effect of

detemir on the liver than on peripheral tissue and thereby contribute to why detemir is seen to lead to lesser weight gain (Hordern, Wright et al. 2005).

In paper II we found no differences in weight gain between glargine and NPH, despite the improvement in metabolic control. Meanwhile in paper I we found an increase in weight with the use of glargine and regarded the improvement of glycemic levels as an explanation for this (discussed in chapter 6.1).

6.4 GH-IGF-AXIS

Long-acting insulin analogs, glargine and detemir both have an extended duration (Heise and Pieber 2007) which implies that they may provide improved nightly insulinization. We therefore hypothesized that these insulins could increase IGF-I and decrease IGFBP-1 serum concentrations, in pubertal subjects with type 1 diabetes. We tested this hypothesis in papers I and IV.

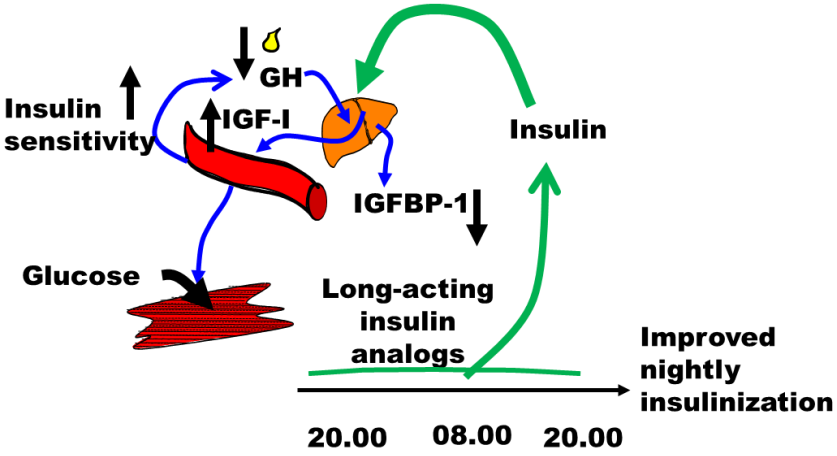

Fig.16. Schematic presentation of the hypothesis of this thesis, that long-acting insulin analogs could provide sustained insulin actions during late night could and normalize changes in GH-IGF-axis .with the use of long-acting insulin analogs.

In paper I we found a 50 % increase in IGF-I in the pubertal subjects after changing to glargine, and this may have contributed to the improved metabolic control. However, despite lower HbA1c with long-acting analogs in pubertal subjects in paper IV, we did not find higher IGF-I SDS in these individuals. While insulin is the main regulator of IGFBP-1 (Brismar, Fernqvist-Forbes et al. 1994), total circulating levels of IGF-I are also dependent on GH status as well as on IGFBP-3, ALS and other IGFBPs. Furthermore, circulating IGF-I levels are shown to be affected by increased IGFBP-3 proteolysis in pubertal subjects with type 1 diabetes (Zachrisson, Brismar et al. 2000).

IGF-I levels in untreated type 1 diabetes are very low due to severe insulin deficiency and are further affected by severely increased insulin resistance, resulting from the catabolic status of untreated type 1 diabetes. In paper IV we found very low IGF-I SDS values in the newly diagnosed children. The mean IGF-I SDS values in the prepubertal individuals were -2 and in the pubertal individuals even lower, at -3. This is in accordance with previous studies

(Bereket, Lang et al. 1995; Shiva, Behbod et al. 2013).The IGF-I SDS levels increased already by the first iv.infusion of insulin and reached its maximum at 2 weeks, although the levels were not normalized. As expected and in line with a previous report from our group (Zachrisson, Brismar et al. 1997), the levels were even more subnormal in the pubertal age group. Surprisingly, even though well treated children have fairly high levels of endogenous insulin production during the first year, as reported in paper III and by others from diagnosis (Greenbaum, Anderson et al. 2009), we demonstrated that they still have IGF-I SDS below zero. Few studies have reported IGF-I SDS, but our findings are in line with a report by Strasser-Vogel et al. (Strasser-Vogel, Blum et al. 1995). In paper I the 12 pubertal subjects had a mean IGF-I SDS of about -2 on NPH and increased after 12 weeks on glargine to -1, still far below the normal mean. We found no suppression of GH secretion at 6 weeks, which suggests that IGF-I might need to reach a completely normal level before inhibiting GH. In line with this Saukkonen et al. found, in a study with combined rhIGF-I and rhIGFBP-3 treatment, that almost a 100% increase in serum IGF-I was needed to decrease GH (Saukkonen, Amin et al. 2004). IGF-I signaling is involved in the development of vascular complications caused by hyperglycemia (Clemmons, Maile et al. 2011), and cardiovascular disease risk factors are inversely related to insulin sensitivity in adolescents with type 1 diabetes (Specht, Wadwa et al. 2013). Therefore it is important that from the beginning of the disease to retrieve normalization of the GH-IGF-I system and thereby increasing insulin sensitivity.

Treatment with rhIGF-I has been shown to have effects that increase glucose uptake and insulin sensitivity, and improve metabolic control in type 1 diabetes (Acerini and Dunger 2000; O'Connell and Clemmons 2002; Simpson, Jackson et al. 2004; Clemmons 2012). In clinical studies, treatment over a longer period with rhIGF-I for individuals with type 1 diabetes have improved metabolic control (Cheetham, Holly et al. 1995; Acerini, Patton et al. 1997; Quattrin, Thrailkill et al. 1997; Quattrin, Thrailkill et al. 2001). These results suggest that the improved metabolic control seen in paper I might be related to the observed increase in IGF-I. A nightly injection of rhIGF-I versus saline was found to increase glucose requirements during an overnight euglycemic clamp in young adult type 1 diabetes subjects (Acerini, Harris et al. 1998). At the same time, a significant suppression of overnight GH secretion was seen, but only for the higher dose of rhIGF-I. Therefore some of the effects of rhIGF-I on glucose metabolism may be related to suppression of GH and not directly to increased levels of IGF-I. In fact, suppression of GH is often considered the more important mechanism. However, O´Conell et al. showed, in a study with a GHR antagonist given to acromegaly patients, that IGF-I can increase insulin sensitivity independently of GH secretion (O'Connell and Clemmons 2002). Despite the increase of IGF-I in paper I, we did not show decreased levels of GH. Although not directly tested, we speculated that lipolysis and gluconeogenesis were unchanged, because of the unchanged GH levels. This is in line with Simpson et al.'s findings in a clamp study where rhIGF-I had no direct effect on lipolysis (Simpson, Jackson et al. 2004).

In this thesis we used IGFBP-1 to assess hepatic insulin actions (Kotronen, Lewitt et al. 2008). In paper I we reported decreased overnight IGFBP-1, 6 weeks after changing from NPH to glargine in 12 pubertal subjects with type 1 diabetes. However we did not find decreased IGFBP-1 20 hours mean, indicating that it was the nightly values of IGFBP-1 that

were mostly reduced. Similarly in paper IV we demonstrated that pubertal subjects treated from diagnosis of type 1 diabetes with long-acting insulin analogs, glargine or detemir had lower 12-month fasting IGFBP-1. These findings demonstrate that long-acting insulin analogs provide increased inhibitory action on hepatic IGFBP-1 production and may also indicate that inhibitory actions on hepatic glucose output are increased. Our findings are in line with earlier studies in adults (Slawik, Schories et al. 2006) and adolescents with type 1 diabetes (Yagasaki, Kobayashi et al. 2010; Ekström, Skogsberg et al. 2013).

The increase in IGFBP-1, reported in paper IV, was only seen in the pubertal subjects treated with long-acting analogs, and in paper I only pubertal individuals were in the study group. Increased hepatic insulin action may be particularly important in puberty when increased GH levels, as shown in paper I, induce hepatic as well as peripheral insulin resistance. IGFBP-1 binds to IGF-I and thereby down-regulates circulating IGF-I activity (Bereket, Lang et al. 1999). In adolescents IGF-I is relatively lower compared to prepubertal children, a finding that may be due to increased insulin resistance (Zachrisson, Dahlquist et al. 2000). The suppression of IGFBP-1 by long-acting insulin analogs, seen in both papers I and IV, indicates that hepatic insulin action/sensitivity is increased, which could also impact on the production of IGF-I (Shishko, Dreval et al. 1994).

IGFBP-1 is directly regulated by insulin and therefore displays a diurnal variation with peak levels fasting in the morning. This may therefore contribute to the glucose rise in dawn by decreasing the bioavailability of IGF-I (Cotterill, Daly et al. 1995; Kobayashi, Amemiya et al. 1997). In paper IV samples for IGFBP-1 determinations were obtained at diagnosis at random times and in the non-fasting state. Despite the non-fasting conditions, it was largely elevated and then quickly decreased following iv.insulin administration. Bereket et al. reported relatively higher IGFBP-1 levels at diagnosis, but they obtained fasting samples from their newly diagnosed patients (Bereket, Lang et al. 1995). They did not report the effect of iv.insulin, which we found to be very efficient in suppressing IGFBP-1. Although the long-acting insulin analogs enhanced the inhibition of IGFBP-1 in the pubertal children, IGFBP-1, fasting samples remained high throughout the 12 months. This is surprising, since most subjects had the capacity to secrete endogenous insulin in response to a MMTT. However, the high IGFBP-1 levels in the fasting state suggest that endogenous insulin may be suppressed by daily insulin therapy.

Higher IGFBP-1 levels were observed in the prepubertal versus pubertal children in paper IV. This is in accordance with previous observations of healthy subjects (Strasser-Vogel, Blum et al. 1995; Juul, Flyvbjerg et al. 1996). It may be a result of higher endogenous insulin secretion and/or higher insulin doses per kg in pubertal children with type 1 diabetes. The role of endogenous insulin, with direct hepatic action on IGFBP-1 production, is supported by our finding of an inverse correlation between C-peptide AUC and IGFBP-1in the relatively newly diagnosed children in paper IV. Multivariate analysis demonstrated that endogenous insulin production was the main predictor of IGFBP-1 at 6 months when C-peptide AUC peaked. The loss of correlation at 12 months may suggest an increasing dependency of IGFBP-1 inhibition on exogenous insulin. Contrary to our finding of increased IGFBP-1 with age, in a study of children and adolescents with type 1 diabetes Strasser-Vogel et al. showed an abolished age dependence in IGFBP-1 (Strasser-Vogel, Blum et al. 1995). This is in agreement with earlier literature (Batch, Baxter et al. 1991). The reason for us to

find an age and puberty dependency of IGFBP-1 is probably due to the finding mentioned above, that when studying subjects with type 1 diabetes with a disease duration of 1 year, most subjects still have relatively well preserved endogenous insulin production and are therefore less dependent on exogenous insulin. Thus IGFBP-1 is more related to age and puberty.

In both papers I and III we showed improved HbA1c with long-acting analogs. In paper IV, when comparing 12-month values of HbA1c in paper III, to IGFBP-1 or IGF-I, there were no correlations, although several other studies have found such correlations (Brismar, Fernqvist-Forbes et al. 1994; Strasser-Vogel, Blum et al. 1995).The lower levels of IGFBP-1 seen with long-acting analogs in both papers I and IV, might have had an effect on the lower HbA1c, by increasing circulating IGF-I bioactivity (Bereket, Lang et al. 1999). Free circulating IGF-I has earlier shown to increase, when IGFBP-1 decreases (Shishko, Dreval et al. 1994; Frystyk 2004). However it is not clear how changes in free circulating IGF-I are related to changes at the tissue level. Because of methodological issues we did not measure IGF-I bioactivity in serum by KIRA or free IGF-I assays. (Bang, Ahlsen et al. 2001). Our group performed a comparison of two different assays for determination of free dissociable IGF-I (fdIGF-I) and found that measuring fdIGF-I by IRMA is of physiological relevance and correlates with concomitant changes in insulin sensitivity (Bang, Thorell et al. 2015) and this method can be used in the future to determine fdIGF-I. Previously our group had also developed a microdialysis technique to access tissue IGF-I concentrations (Berg, Gustafsson et al. 2007), but this method was not feasible with the large number of subjects studied in paper IV and was not used for this purpose in paper I.

In paper IV we found an inverse correlation between IGF-I SDS and C-peptide AUC at 12 months. This was not what we expected, given that hepatic insulin is important for IGF-I production (Daughaday, Phillips et al. 1976; Maes, Underwood et al. 1986), and IGF-I may be involved in the regeneration of the beta cell (Sorensen, Birkebaek et al. 2015). At 12 months, IGF-I SDS was lost as a predictor of C-peptide in a multivariate analysis, where age and pubertal stage predicted C-peptide AUC. In contrast to our findings Bizzarri et al. reported a positive correlation between IGF-I SDS and fasting C-peptide in adolescents on CSII or MIT regimen (Bizzarri, Benevento et al. 2014). They found higher IGF-I SDS in pubertal (mean -1.2) than prepubertal (mean -1.6). Also this contrasts our findings in paper IV and our previous work (Zachrisson, Brismar et al. 1997; Zachrisson, Dahlquist et al. 2000), but it is in line with what others have found (Strasser-Vogel, Blum et al. 1995). Taking data from papers I and IV together, recently diagnosed pubertal subjects have IGF-SDS closer to normal than those with a longer diabetes duration, which suggests that endogenous insulin may still have an impact on the levels of IGF-I.

In paper IV, we did not find any differences in IGF-I SDS or IGFBP-1 between the two long-acting analogs. Ma et al. investigated the acute effect of a single insulin dose on IGFBP-1 AUC 6-12 hours after injection in a crossover study (Ma, Christiansen et al. 2014). They found more suppression of IGFBP-1 levels with detemir versus NPH or glargine. We believe that this finding may depend on the study design and reflect that the AUC was investigated during the peak effect of detemir. Furthermore, the long-term effects may be very different from acute changes.

7 CONCLUSIONS AND FUTURE PERSPECTIVES

The overall results of this thesis support the hypothesis that long-acting analogs, improves short- and long-term metabolic control in type 1 diabetes, by showing improved HbA1c with glargine or detemir in pubertal subjects both changing from previous therapy with NPH and from diagnosis of disease. The abnormalities in the GH-IGF-axis are partly reversed with better suppression of IGFBP-1, a marker of hepatic insulin sensitivity. In patients with previous NPH treatment, a short-term effect of glargine was observed with a 50 % increase in IGF-I suggesting that patients who have lost endogenous insulin can benefit from treatment change to long-acting insulin analogs. The marked increase in IGF-I in these patients did not restore normal IGF-I SDS and consequently did not suppress GH hyper secretion. In contrast to what we hypothesized, there was no impact of glargine or detemir on C-peptide levels or their decline over the first year.

In conclusion, pubertal subjects with type 1 diabetes benefit from improved nightly insulin delivery achieved by long-acting insulin analogs. In order to get the long-term benefits of an optimal treatment, this thesis supports that pubertal children with type 1 diabetes should be treated from diagnosis with glargine or detemir as the first drug of choice for injection therapy. If prepubertal children are started on NPH, the results from this thesis suggest that they should be changed to glargine or detemir when approaching puberty. However, the scientific support for the latter is weaker as it is not based on an RCT. Normal IGF-I and GH levels are likely to be of importance for delaying long-term vascular complications. However, it is unlikely that subcutaneous injection therapy as monotherapy could achieve this goal given the results from this thesis on long-acting insulin analogs and those from a previous thesis from our group, dealing with CSII therapy and the GH-IGF-axis. New approaches will be needed to accomplish this. Some potential future options are outlined below.

- Our studies show that the use of long-acting analogs are beneficial for both improving metabolic control and restoring the changes in the GH-IGF-axis. Whether the ultra-long-acting insulin degludec, which has just been approved for use in children, has beneficial effects on the changes in the GH-IGF-axis needs to be further explored in clinical studies on children and adolescents. The most important factor for success of a new insulin analog may not only be related to challenge of meeting the insulin requirements. It may also require a more hepatophilic insulin, a characteristic that has been reported to be associated with degludec.

- As mentioned, several studies have demonstrated that treatment with rhIGF-I as an adjuvant to intensive insulin therapy reverses the GH-IGF-axis in adolescents with type 1 diabetes. When these studies were first conducted a fear was raised by EMA for the risk of accelation of already started complications, particularly retinopathy. However, it is likely that the development of complications are dependent on IGF-I and that the dependency changes in different developmental stages of the disease. This is suggested by the unravelling of the role of IGF-I in rethinopathy of the premature (ROP) (Hellstrom, Smith et al. 2013). In the early phase of ROP, IGF-I deficiency increases the risk of developing the early changes. Later on, the increase in IGF-I precipitates the later changes. Clemmons and Maile have shown that IGF-I

signalling is involved in diabetic vascular complications via cross-talk with integrin receptors activated by high glucose levels (Clemmons, Maile et al. 2011). It is likely that the inbalance between local and circulating IGF-I levels may explain the difficulties in understanding the role of IGF-I. Possibly, local tissue levels of IGF-I determined by microdialysis relative to circulating levels could be helpful in establishing the relationships as the complications develop.

- CSII, combined with a real-time glucose sensor in a closed loop system with automated data transfer using control algorithms to automatically regulate insulin infusion, the so called "artifical pancreas", has been tested in clinical out patient settings in adults (Kovatchev, Renard et al. 2014) and in children overnight (Nimri, Muller et al. 2014). A closed loop system with diluted insulin used overnight has also been shown to decrease hypoglycemia and glucose variability in young children (Elleri, Allen et al. 2014). This may be as close to the physiological insulin profile that one can get, although the insulin is delivered subcutaneously and will probably not be able to normalize the GH-IGF-axis completely.

- Intraperitoneal insulin delivery provide portal delivery of insulin and has been shown to increase, but not completely normalize IGF-I in several studies (Hanaire-Broutin, Sallerin-Caute et al. 1996; Hedman, Frystyk et al. 2014). Continuous Intra Peritoneal Insulin Infusion (CIPII) is used in clinical practice, either with an implantable pump or a subcuteous port system- Diaport®. A long-term follow up, over 7 years, showed less hypoglycemias, but no difference in HbA1c with CIPII (van Dijk, Logtenberg et al. 2014). CIPII is associated with many complications and with high costs. It is therefore a therapeutic approach for only the most severly ill patients with extreme insulin resistance or in whom autonomaous neuropathy causes severe hypoglycemic unawareness. CIPII has been shown to decrease IGFBP-1 more than with CSII (van Dijk, Logtenberg et al. 2014). CIPII combined with glucose sensing in the peritoneal space may be more efficient regulating infused insulin levels (Burnett, Huyett et al. 2014). Even with such improvements, CIPII is going to be a treatment for a very limited number of patients.

- Intraportal insulin delivery is shown to fully normalize the changes in IGF-I and IGFBP-1. This is only used in research settings, but is the ideal way to deliver insulin.

- Beta cell transplantation can provide portal delivery, if the beta cells are infused into the liver and the insulin is secreated into the portal circulation. This results in higher IGF-I levels than systemic drainage (Frystyk, Ritzel et al. 2008). Again this is going to be a treatment for a limited number of patients.

- In our RCT, the use of long-acting analogs from diagnosis of type 1 diabetes did not improve preservation of beta cell capacity during the first year. Several achievements using different immunmodulators have failed to markedly improve beta cell rest without too serious adverese events. Different combination treatments, with a goal of affecting several aspects of beta cell destruction (Ludvigsson 2014), such as a

combination of an early intensive insulin treatement with a more optimal insulinization and a immunmodulating agent, such as GAD65, and anti inflammatory treatment, may hopefully become effective. Despite this research, there is not currently an established treatment and even in newly diagnosed patients with high endogenous insulin secretion we found low IGF-I levels.

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