GH/IGF and Pro-diabetic Phenotype
2.1
The role of the IGF system in pancreatic \( \beta \)-cell function
(review)
In this review, we highlight recent findings of the roles of various growth factors for pancreas beta cell function with emphasis on the Insulin-like Growth Factors (IGF). Signalling of insulin and the IGF system uses parallel (and interrelated) pathways. There is a complex interplay between insulin receptor-dependent signalling and the signalling of the IGF-system in the development of the islet cells, which has become apparent by various animal knock-out models. Recent knowledge about the influence of human gene polymorphisms relating to islet cell function also sheds more light on the importance of the IGF system for pancreas B-cell function.

Type 2 diabetes mellitus is generally due to a combination of insulin resistance and an impairment in insulin secretion by the pancreatic B-cell (1). Originally, the insulin secretion capacity of the pancreatic B-cell was considered as a communicative process of biological signals, such as derived from meal composition and neural input (2). Insulin secretion is known to adapt to systemic needs for insulin, and in humans systemic insulin profiles mostly reflect peripheral sensitivity to insulin action (3). We will here describe the importance of growth factors, especially insulin-like growth factors I and II, and their signalling pathways for pancreas beta cell growth and function, which have become more clear by the use of animal models, and explore recent findings in humans.

Plasticity of highly differentiated cells
Recent ontological studies show a high plasticity of highly differentiated cells with respect to maturation and differentiation. In contrast of what was thought before, highly differentiated cells have the ability to reenter the cell growth cycle on stimulation with specific growth factors; pancreatic beta cells have been shown to possess these properties (4). It has not been elucidated whether this interval of cell plasticity is limited in time (prenatal or also later in adult life). Barker hypothesized that especially the prenatal period is of importance for the development of functional tissues or organs and that diseases later in adult life (such as hypertension and type 2 diabetes) may have their origin in disturbances of growth and the development of functional tissue capacity (5). This would also imply that a disease later in life may result from a maladaptation due to reduced capacity of functional tissues (6). In support of Barker’s hypothesis, lower birth weight has been shown to be associated with a prevalence of type 2 diabetes (7). Since overt diabetes mellitus results from a failure of pancreatic beta cells to adapt to environmental demands, i.e. insulin insensitivity (1), the effects of growth factors on pancreas beta cells are of growing interest. But also in case of a high plasticity of cells that continue to form functional tissue during adult life, defects in growth factors, such as the insulin-like growth factor system, may potentially give rise to a less adaptive capacity and therefore an increased susceptibility to disease (8). A prerequisite in this situation is an increase of a specific burden, i.e. insulin resistance related to obesity, that needs to be compensated for by functional tissues (i.e. B-cells) which are limited by a reduced capacity, and/or are limited in their adaptive growth.

Growth Factors in Humans
Expression of growth factors
GH, IGF-I and IGF-II are the principal growth factors in humans. Their biological action is dependent from the balance in the total IGF system, that consists of IGF-I, IGF-II and six IGF binding proteins (IGF BP 1-6). The growth hormone (GH) axis is functionally superimposed on the balances that exist in the IGF system. Primary disturbances in the secretion of GH are therefore associated with shifts in the balance of the IGF system. GH is secreted by the adenopi-
tuitary in a 24 hours pulsatile secretion profile, with a peak in the early morning. Other cerebral structures (such as hypothalamus) are involved in the regulation of GH secretion; stimulation by GH Releasing Hormone and ghrelin, and a reduction by somatostatin. GH increases the expression of IGF-I in the circulation (systemic IGF expression) and in many tissues (local IGF expression), while its effects on IGF-II are less clear. Moreover, plasma levels of IGFBP-3 are increased by GH stimulation. IGFBP-3 is the IGF binding protein that binds most of both IGF-I and IGF-II. Due to the fact that IGF-II is bound more avidly by IGFBP3, total plasma IGF-II levels are roughly three times higher than total plasma IGF-I in humans, and the IGF-II pool is therefore larger than the IGF-I pool. IGF-I has a negative feedback on GH secretion.

**Shared receptors for insulin and IGF network**

The distinctive molecules IGF-I, IGF-II and insulin bind to specific cell surface receptors. Due to structural homologies among these molecules, IGF-I also binds to insulin-receptors (but with a lower affinity than insulin), and recto-versa, IGF-I and IGF-II both bind to IGF-1 receptors. In addition to these cross-talks on the cell surface, insulin and IGF-1 receptors share common (post-receptor) intracellular signalling pathways, that were presented in recent reviews (9-12).

The insulin receptor is a transmembranous receptor, consisting of 2 extra-cellular alpha-subunits, that display the epitope for insulin, two membrane spanning beta-subunits and an intracellular tyrosine kinase related part. The IGF-receptor is related to the insulin receptor, with a subsequent 80% structural homology in the kinase domains (10).

Although the receptors are very common in structure, the distributions over the various tissues that are involved in the human insulin resistance syndrome differ. In adipose tissue, no IGF receptors are detected, in contrast to the liver and muscle. These non-equal distributions over several tissues have various implications, and especially in a system that is in imbalance, since signals may be become effective in tissues that are not “allowed” to receive.

**I/IGF signaling**

**Insulin**

Upon binding of insulin to extracellular domains of the insulin receptor, the signal transduction is mediated through intracellular beta-subunits that undergo autophosphorylation of tyrosine residues. After phosphorylation of these submembranous structures, a cascade of phosphorylations of so-called insulin receptor substrates (IRS) starts (13). An intracellular distribution of insulin-like signals is mediated along these IRS substrates. Hitherto, four intracellular IRS (IRS 1-4) proteins have been isolated and the distinctive IRS proteins direct to different intracellular pathways. In short, IRS-1 regulates somatic cell growth and is involved in insulin activity of muscle and adipose tissue (12). IRS-2 is involved in insulin activity mainly in liver, in brain growth, in reproduction, and in pancreas beta-cell growth (14). The pathways that are related to IRS-3 and IRS-4 are still not elucidated, but these IRS proteins are expressed in tissues of neuroendocrine and adipose origin. IRS-3 and IRS-4 disruption gives rise to normal (or even slightly lower) plasma glucose and normal insulin levels (13). Upon phosphorylation of IRS proteins, the Phospho-Inositol 3' Kinase (PI3K) pathway is activated. This pathway consists of multiple subsequent phosphorylations, and gives rise to the expression of insulins effects on glucose homeostasis, such as enhancement of glucose uptake and glycogen synthesis in peripheral skeletal muscle, and inhibition of gluconeogenesis and glycogenolysis in hepatocytes, and inhibition of lipolysis in adipose tissue (9). Moreover, the signal transduction towards more downstream pathways of the PI3K pathway results in additional insulin-related effects, such as on protein synthesis, gene expression, mitogenesis, and cell growth (Table 1). Besides insulin-specific glucose metabolism related pathways (like
PI3K), several other intracellular cascades are activated. After the phosphorylation step of PI3K, several major pathways involve activation of grb2, SOS, RAS, and MAPK, whereas another pathway involves mTOR (mammalian Target Of Rapamycin) and PKB (PhosphoKinase B) activation (10).

**IGF-I**
IGF-I mainly acts via the IGF-1 receptor (IGF-1r). IGF-I has a critical role in fetal and post-natal growth. Knock-out mice homozygous null for either IGF-I or the IGF1R weigh only half the size of the wild-type animals (15). The IGF-1R has structural homology with the insulin receptor, and upon its activation, partly identical transduction pathways as for insulin are activated via IRS-2, followed by activation of PI3K and mTOR, but also of MAPK, both leading to mitogenesis (Table 1).

**IGF-II**
IGF-II can also bind to IGF-1R, but in addition also to the so-called Type-2 insulin-like growth factor receptor (IGF2R, also known as the IGF-6 mannose or IGF-M6 receptor), that has four distinct binding domains, which can bind IGF-II, mannose-6-phosphate containing lysosomal enzymes, retinoic acid, and urokinase-type plasminogen activator receptor (16). This receptor has a role in the degradation of IGF-II, and may function as a tumor suppressor gene (16). In epigenetic phenomena occurring during gametogenesis, leading to silencing of either a maternal or a paternal allele, the expression of IGF-II and IGF-II related genes were recently found to be key factors in the development of tumors, such as Wilm’s tumor and adenocarcinoma of the colon (17). However, also in adjacent colon tissue these epigenetic processes are found, which may suggest a more general field defect. So far, no extrapolation of these epigenetic results with IGF-II have been made to the field of insulin secretion or resistance.

**Relationship with GH**
As stated before, GH is a regulator of expression of IGF-I and IGF BP-3, in both circulation, liver and other tissues. Whether GH also regulates IGF-II production in humans has not been elucidated yet. Exogenous administration of growth hormone to healthy adults during 14 days, did not lead to appreciable changes in plasma IGF-II (18); on the other hand GH appears to stimulate the promoter region of IGF-II gene (19). Interestingly, GH has also cell growth promoting properties without the necessity to induce the local expression of IGFs. GH

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Table 1: Simplified scheme of insulin/IGF signaling. Pathways used only by insulin signaling are given in *italics*; pathways presumably shared by both insulin and IGF are given in **bold**

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Glycogen synthesis</th>
<th>Cell survival</th>
<th>Protein synthesis</th>
<th>Gene expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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**Insulin Receptor / IGF-receptor**

- IRS1 / IRS2
- PI3K
- PKC
- PKB/akt
- GSK3
- mTOR
- p70S6K
- MAPK
- Grb2/SOS
- ras
binding to the GH receptor leads to phosphorylation of the Janus kinase 2 (JAK2), which will activate signal transducer and activator of transcription 5 (STAT 5). STAT 5 will then lead to mitogenesis. GH can therefore stimulate cell growth via various mechanisms, both more directly via its own receptor and/or indirectly via the IGF system. Interestingly, while cross talk between insulin-receptor and IGF receptor dependent mechanisms exist in various levels, there appears to be little or no cross talk between the JAK-STAT and the other pathways, at least in pancreas beta cells (20).

Growth factors and adaptive effects in adult pancreatic B-cells

Animal studies

Pancreatic beta cells are under influence of growth factors not only during the prenatal period. Also later in adult life, the influence of growth factors on the insulin secreting cells is considerable. Especially, knowledge about the involvement of the PI3K cascade in survival of pancreatic beta cells and the positive effect of MAPK activation on gene transcription show relationships between insulin receptor-mediated and IGF receptor-mediated effects with respect to beta-cell growth (9). Although insulin-secreting beta cells are well-differentiated in adult human life (less than 1% of pancreatic beta cells are in mitosis), they are still capable to undergo proliferative changes (9). Beta cell proliferation is stimulated by IGF-I via the activation of the post receptor IRS-2 proteins (21). In animal models, the IRS-2 knock-out mice have been shown to have not only impairment of insulin action, but also of beta cell development, with consequent development of diabetes (22). Those mice have a 60% reduction in pancreas islet cell mass with a relative beta cell deficiency; the number of islets is also half of normal. Disruption of the IRS-1 pathway, however, leads to a mild insulin resistance in peripheral muscle tissue with a two-fold increase in beta cells, that is associated with an hyperinsulinemia (10;12;13). IRS-1 binds with high affinity to calmodulin, a calcium-binding protein involved in insulin-secretion (23). IRS-1 deficient beta cells fail to increase the content of calcium in the cytosolic compartment, while the overexpression of IRS-1 protein increases the calcium levels in INS-1 beta cell lines (13). These observations suggest that a disruption in the IRS-1 pathway results in adaptation by doubling the pancreatic beta cells to overcome the decrease in calcium-driven secretion of insulin. Transgenic mouse models have indicated that subsequent phosphorylation of PI3K, Akt and mTOR are necessary for the transduction of this IGF-I signaling (21;24;25)(Table 1), while glucose has a strong modifying effect (26). Recent studies in mice with a beta-cell specific knockout of the IGF1Receptor indicate, that the mice showed normal growth and development of beta-cells but had a defective glucose-stimulated insulin secretion (with hyperinsulinemia) and had impaired glucose tolerance. These studies indicate that, even if IGFR1 is not crucial for islet cell development, it is of importance for beta cell function (27).

Much less is known about the role(s) of IGF-II in pancreas beta cell mass and function. IGF-II is known to have anti-apoptotic properties. At least part of the effects of IGF-II may relate to its capability to bind to IGF-1 receptors. Recently, it was found that the Goto-Kakizaki (GK rat), which has been widely studied since it is a model for diabetes, displays defective IGF-II synthesis which may cause its insufficient beta-cell development (28). However, transgenic mice overexpressing IGF-II develop frequently diabetes in spite of an increased beta cell mass, possibly due to an increase in glucagon producing alpha cells (13;29). Pancreas cells are capable of producing IGF-II themselves (30).

Other growth factors than IGF are presumably also at play. Fibroblast growth factors (FGF) have been proposed to be implicated already very early in the embryonic pancreas development (31). In-vitro studies suggest that they may influence the intestinal differ-
entification program and the development of the dorsal and/or ventral part of the developing pancreas (32). FGF bind to extracellular FGF-receptors (FGFR) also belonging to the tyrosine kinase family. Recently, it was shown in in vitro studies, that FGF7 could control the development of exocrine pancreatic tissue, while removal of the FGF7 led to proliferation of endocrine tissue (33). Studies with transgenic mouse models, expressing a dominant negative version of FGFR1 show that these animals develop diabetes (34). These animal models have not only a reduced number of beta-cells, but they have also an impaired expression of the glucose transporter 2 (Glut-2), the most prominent glucose transporter of the pancreas and of the liver. Moreover, the expression of PC1/3, prohormone convertase 1/3, which is the enzyme catalyzing the final conversion of the prohormone proinsulin into the actual hormone insulin in the pancreas, is also decreased. Interestingly, the production of FGFs occurs in the beta-cells themselves under the influence of other transcription factors, notably Ipf1/Pdx1, insulin promoter factor1 or pancreatic duodenal homeobox gene 1. Ipf1 is known to stimulate insulin gene transcription and Glut 2 expression (30). In humans, a nonsense mutation of the Ipf1 gene is a known cause of Maturity Onset Diabetes of the Young type 4 (MODY4) (35).

**Human studies**

Recently, several reports mention the effect of disturbances in the expression of the IGF system on beta cell function in humans in relation to glucose metabolism (Table 2). Although still limited to some studies, most of them show an association between plasma levels of the IGF system or the presence of certain common gene polymorphisms and the release of insulin that are estimated by plasma insulin profiles during an oral glucose tolerance test (OGTT). However, plasma glucose levels change constantly during an OGTT. The use of hyperglycemic glucose clamping during which plasma glucose is kept constant, enables studying relationships between beta cell insulin release and influencing factors, such as the IGF system.

<table>
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<th>Variant</th>
<th>Beta Cell Function</th>
<th>Glucose homeostasis</th>
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<tbody>
<tr>
<td>IRS1 Gly972Arg</td>
<td>contradictory findings (46;47) possibly worsening (44)</td>
<td></td>
</tr>
<tr>
<td>IRS2 Gly1057Asp</td>
<td>no effect (47;47;49)</td>
<td></td>
</tr>
<tr>
<td>IGF-I level</td>
<td>no effect (38)</td>
<td>present (36)</td>
</tr>
<tr>
<td>IGF-I gene</td>
<td>no data</td>
<td>no effect (39)</td>
</tr>
<tr>
<td>Promoter IGF-I gene</td>
<td>no data</td>
<td>possibly (40)(low birth weight)</td>
</tr>
<tr>
<td>IGF-1 receptor GAG1013GAA</td>
<td>no effect (39)</td>
<td></td>
</tr>
<tr>
<td>IGF-II level</td>
<td>Stimulating (Twickler)</td>
<td>augmentation glucose (Twickler)</td>
</tr>
<tr>
<td>IGF-II gene</td>
<td>no data</td>
<td></td>
</tr>
<tr>
<td>promoter IGF-II</td>
<td>possibly no effect</td>
<td></td>
</tr>
<tr>
<td>IGF2-APA-I</td>
<td>no data</td>
<td></td>
</tr>
<tr>
<td>IGF2 receptor</td>
<td>no data</td>
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</table>
Relationship with IGF-I

The presence of low plasma IGF-I levels has been associated with an increased risk to develop IGT and type 2 diabetes (36). Both IGT and type 2 diabetes are related to a relative insufficiency of insulin secretion (37). In line with this observation, we also observed a negative correlation of the free IGF-I index with baseline and 2-h glucose response after an OGTT. However, we did not find a relationship of IGF-I with pancreas beta cell function as assessed with hyperglycaemic clamps in normal-glucose-tolerant subjects (38). In view of the above-mentioned findings in IGF1 receptor knock-out mice (27), it could well be that the role of this polymorphism in insulin secretion is more subtle, and would only become apparent during the development of IGT. It is of note, that the development of type 2 diabetes mellitus generally is the result of a combination of disturbances in insulin secretion and in sensitivity (35). Therefore, it could also be that the role of IGF-I in the development of IGT might possibly relate to an effect of IGF-I on peripheral tissue insulin sensitivity. Follow-up studies may be of relevance to clarify this point.

Relation with IGF-II

Very recently, we observed a significant association between both plasma IGF-II and IGFBP-3 levels and measures of beta cell function in subjects who have a normal glucose tolerance; these subjects were on average around 45 years of age, and were mildly obese (Twickler TB, de Sain- van der Velden MGM, van Doorn J, van Haeften TW. Relationship of insulin secretion with plasma Insulin-like Growth Factor-II. Submitted). Obesity (and age to a minor extent) is related to a certain amount of insulin resistance, but may also have an impact on beta-cell function. It is of note that the relationship of IGF-II with insulin secretion was still apparent after correction for obesity and age. It has previously already been shown that insulin secretion is elevated even in subjects with a BMI within normal ranges as compared to lean subjects (3). Our observation that IGF-II levels relate with beta-cell function in mildly obese subjects might therefore indicate a positive effect of IGF-II on beta-cell growth during embryogenesis which is in line with the Barker hypothesis. On the other hand, IGF-II may also have adaptive effects on adult pancreatic beta cells, which would compensate for changes related to increase of age and/or changes in glucose homeostasis due to obesity. However, to our knowledge, no additional data that deals with IGF-II plasma levels and beta cell function at a (very) young age in humans are available.

Gene polymorphisms and the relationship with glucose homeostasis

IGF-I

Several studies in type 2 diabetes patients have looked for the importance of common polymorphisms in genes involved in the IGF system. In a Danish study, DNA analysis of 82 probands of type 2 diabetes families showed no nonsense, frameshift or missense mutations in the IGF-I or IGF-1 receptor genes; however, a number of silent or intron variants were noted (39). The most prevalent polymorphism (GAG1013GAA) of the IGF-receptor was not related to neither birth weight or insulin sensitivity index in a group of 349 healthy subjects. In addition, its prevalence was not higher in subjects with type 2 diabetes mellitus than in subjects with a normal glucose tolerance. Another common polymorphism in the IGF1 promoter region, however, has been reported to influence the weight at birth (40). A low birth weight is related to increased risks of insulin resistance, Impaired Glucose Tolerance and type 2 diabetes mellitus (6;41). Although insulin resistance is generally known to be the most important risk factor for the development of type 2 diabetes, disturbances in insulin secretion are almost always found in type 2 diabetes subjects (42). However, recent preliminary results from our group did not indicate any relationship between the presence of an IGF-1 gene polymorphism and beta cell function, neither in subjects
with normal glucose tolerance nor in subjects with IGT.

**IGF-II**
Little is known about the biological impact of polymorphisms in the IGF-II gene and the expression of a diabetic phenotype. In a recent study in a small group of subjects, a relatively frequent polymorphism of the untranslated part of the IGF2 gene was found to be associated with lower plasma insulin levels after an oral glucose tolerance test. (43). However, in preliminary studies in three groups of subjects we could not find a consistent effect of the polymorphism on either glucose levels after an OGTT or on insulin secretion during hyperglycemic glucose clamps.

**Intracellular IRS proteins**
Recent observations in humans indicate that carriers with a Gly972Arg substitution in IRS-1 proteins have a slightly decreased insulin sensitivity, and a slightly increased prevalence of type 2 diabetes mellitus (13;44). Conformational in-vitro studies show that RIN cells that have an increased expression of this variant have a reduced glucose-induced insulin release (45). Although one series of hyperglycemic clamps describes a marginally decreased beta cell function in humans (46), we were not able to confirm that observation in two larger cohorts (47). Very recently, in vitro studies point to a decreased conversion of proinsulin to insulin in human beta cells with the Gly972Arg variant. However, these data were obtained in cells from only two subjects with the variant, and compared to cells from only two controls (48), while insulin secretory function is known to differ markedly between subjects. Similarly, a common polymorphism (Gly1057Asp) in the IRS2 molecule does not appear to have an appreciable influence on pancreas beta cell function in man neither in subjects with normal glucose tolerance nor in subjects with IGT (46;49).

**Conclusion**
Taken together, animal studies showed a significant impact of growth factors, such as IGF-I and IGF-II, in physiological development of prenatal pancreatic beta cells, and in adaptive properties of beta cells on environmental changes in the postnatal period. In humans, the observation of a relationship between a low birth weight and an increased prevalence of impaired glucose tolerance and type 2 diabetes has prompted research into the effect of the IGF system on adult insulin secretion and the occurrence of derangements of glucose homeostasis. IGF-I levels have been shown to relate to the development of IGT. We have recently found evidence that plasma levels of IGF-II and IGFBP-3 are positively associated with insulin secretion. Others have observed a relationship with a promoter gene polymorphism of the IGF-I gene with (low) birth weight. However, no direct relationship has been found with type 2 diabetes, so far. A major area of further research relates to the question whether the impact of growth factors on the insulin secretion capacity in humans is limited to the prenatal period or whether it is also related to coping processes of the beta cell in the postnatal period.

**Acknowledgments**
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2.2
Insulin-like growth factor-I and low birthweight

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Sir-Nobert Vaessen and colleagues (March 23, p 1036) describe the interesting observation that a polymorphism of the insulin-like growth factor-I (IGF-I) gene is closely associated with low birth weight and an increased incidence of type 2 diabetes mellitus. It is relevant in this context that a phenotypic association between low birthweight and increased incidence of type 2 diabetes is supported by a large Scandinavian study. From their results, Vaessen and colleagues propose that a specific IGF-I polymorphism is related to the extent of plasma IGF-I expression, a key factor in the development of pancreatic insulin-secreting cells. This proposal is supported by previous animal knock-out and transgenic models in which the concentration of plasma IGF-I is a determinant of the development and maturation of the insulin secreting B-cells in fetal life, and consequently affect insulin-secreting properties of the B-cells in adult-life.

Most IGF-I in plasma is bound to IGF-binding protein-3 (IGFBP-3). Due to the heritability of expression of IGF-I and IGFBP-3 (more with respect to IGFBP-3 than IGF-I), we have assessed whether the relation between plasma IGF-I concentration, IGFBP-3 expression in insulin-secreting cells, or both is conserved in adult-life. We did a standard oral glucose tolerance test (75 g glucose), and a hyperglycaemic clamp (10 mmol/L during 180 min) with measurement of first and second (average plasma insulin in 140-80 min period) phase insulin secretion in 53 non-diabetic individuals (mean age 46 years, SD 6; 13 men, 40 women; mean body-mass index 25.9 kg/m², SD 3.8).

Plasma IGF-I concentrations were not related to parameters of insulin secretion, but plasma concentrations of IGFBP-3 were significantly correlated with second-phase insulin secretion (p=0.025) in the clamp, and with baseline (p=0.056) and 120 min plasma insulin after the oral glucose tolerance test (p=0.037). Multiple linear regression showed that the effect of IGFBP-3 on insulin secretion could be accounted for by body-mass index. Thus, IGFBP-3 concentrations are closely related to insulin secretion in the adult pancreas. Several factors, including growth hormone status, age, nutrition, and hepatic function affect plasma concentrations of IGFBP-3. However, the variation between individuals of IGFBP-3 in the circulation of adults seems to be largely determined by a genetic component. Hence, at least to a certain extent, IGFBP-3 concentrations at the tissue level are also affected by genetic factors. IGFBP-3 plays an important part in the modulation (inhibitory or stimulatory) of IGF action at the cellular level.

During fetal development, local concentrations of IGF and IGFBP-3 may affect the balance between cell apoptosis and the maturing of functional B-cells, and hence the insulin secretory capacity in adult life. This may explain the intriguing finding that a relation exists between plasma IGFBP-3 and insulin secretion by the adult pancreas. The apparent effect of body-mass index on this relation remains puzzling. Considered together with the data of Vaessen and colleagues, we suggest that pancreatic B-cell function may be primarily determined early in life.
References


2.3
Plasma IGF-II relates to insulin secretion in man

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To study the relationship between plasma IGF-II and insulin secretion in adult non-diabetic humans.

Methods. We evaluated relationships between plasma IGF-II and insulin secretion in 50 non-diabetic adults, estimated using an oral glucose tolerance test, and a hyperglycemic clamp (10 mmol/L, 180 minutes) with determination of first and second phase insulin secretion. Plasma IGF-II levels were positively associated with fasting plasma glucose (r=+0.37, p=0.024) and fasting insulin (r=+0.33, p=0.021) levels, and with second phase insulin secretion (r=+0.38; p=0.007). Multiple linear regression (with gender, age, BMI and waist-hip ratio as covariates) indicated that plasma IGF-II concentrations contributed significantly to the variance of baseline plasma glucose levels (partial coefficient r=+0.27, p=0.037), and of second phase insulin secretion (partial r=+0.28; p=0.044). Plasma IGF-II levels are significantly related to adult pancreas B-cell function.

Subjects, materials, methods

Subjects
Fifty healthy non-diabetic (OGTT) subjects took part in this study (Table 1). They are part of studies reported in earlier papers (7,8). The local Ethical Committee had approved the study, and informed written consent was obtained from each participant. All subjects had normal values for routine laboratory measurements for hematology, HbA1c (upper normal limit 6.1%), lipids, and kidney, liver, thyroid and adrenal function.

Oral glucose tolerance test (OGTT)
Blood samples for glucose and insulin determinations were taken at baseline and at 30
minute intervals after the oral administration of 75 grams glucose (in 300 ml water), and put on ice.

**Hyperglycemic glucose clamp**
A hyperglycemic glucose clamp was performed during 180 minutes aiming at a glucose level of 10 mmol/l (arterialized blood sampling, 55°C). Blood samples for insulin determination were taken at 2 minute intervals during the first 10 minutes, and at 20 minute intervals thereafter, for determination of first phase (summation from 0 to 10 minutes) and second phase (average plasma insulin levels from 140 to 180 minutes) insulin secretion.

**Laboratory measurements**
Blood glucose was determined immediately with a glucose analyzer (YSI, Yellow Springs, Ohio, USA). Plasma insulin was determined by radioimmunoassay with 125I-labelled insulin (IM 166, RC Amersham, UK). Plasma IGF-II (nmol/L) was determined by a specific RIA, as described previously (9). Plasma IGF-II was also expressed as standard deviation score (SDS) corrected for age and gender, as reported previously (9).

**Statistical analysis**
Data are presented as mean with SD. Linear correlations of plasma IGF-II, and its SDS, with plasma glucose and insulin levels before and after the OGTT, and with first and second phase secretion during the clamp were investigated. Multiple linear regression (MLR) analysis was also performed with the use of age, gender, body mass index (BMI) and waist-hip (WH-) ratio, as covariates.

**Results**
Plasma IGF-II levels were positively correlated with baseline plasma glucose (p=0.004), and with baseline plasma insulin levels (p=0.021) (Table 2).

Multiple linear regression indicated that plasma IGF-II contributed significantly to the variance of basal glucose (partial coefficient r=+0.27, p=0.037), but not to that of plasma insulin levels.

In the univariate analysis, no significant associations were found between the first phase insulin secretion and IGF-II, while second phase insulin secretion was positively related to plasma IGF-II levels (p=0.007) (Table 2).

Multiple linear regression analysis indicated

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Table 1: Baseline characteristics of 50 subjects, fasting, 30 minute and 120 minute plasma glucose and insulin levels after an OGTT, and first and second phase insulin secretion as determined during a 3 hour hyperglycemic clamp (10 mmol/L, 180 minutes) in 50 healthy subjects. Data is Mean ± SD.

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Gender (f/m)</td>
<td>38/12</td>
</tr>
<tr>
<td>Age (year)</td>
<td>46.2 ± 6.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 3.5</td>
</tr>
<tr>
<td>Waist-Hip ratio</td>
<td>0.82 ± 0.07</td>
</tr>
<tr>
<td>Basal plasma Glucose (mmol/L)</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>120 min plasma Glucose (mmol/L)</td>
<td>6.7 ± 1.6</td>
</tr>
<tr>
<td>Basal plasma Insulin (pmol/L)</td>
<td>41.3 ± 20.3</td>
</tr>
<tr>
<td>30 min plasma Insulin (pmol/L)</td>
<td>303 ± 182</td>
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<td>120 min plasma Insulin (pmol/L)</td>
<td>337 ± 282</td>
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<td>First phase (pmol/L * 10 min)</td>
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<td>Second phase (pmol/L)</td>
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</tr>
<tr>
<td>Plasma IGF-II (nmol/L)</td>
<td>54.4 ± 9.9</td>
</tr>
</tbody>
</table>
that BMI has the largest impact on second phase secretion (partial coefficient $r = 0.38$, $p=0.015$). Plasma IGF-II levels contributed significantly to the variance of second phase insulin secretion ($r = +0.28$, $p=0.044$).

In general, the use of Standard Deviation Scores (SDS) for univariate and multiple linear regression led to the same results (Table 2).

## Discussion

To our knowledge, the present studies are the first to evaluate the relationships between IGF-II and pancreas B-cell function. They indicate that plasma IGF-II levels and second phase insulin secretion are related, also after correction for age, gender, BMI, and waist to hip ratio. Pancreas beta cell function is closely and negatively related with insulin sensitivity: in obesity, insulin sensitivity is decreased with a marked increase in the pancreatic beta-cell function (up till two- to three-fold) (6). Therefore, body weight is a major determinant of beta-cell function. In a preliminary report, we already showed that the relationship of IGFBP-3 with beta cell function is mainly due to an interaction with BMI (5).

The IGF system (and thus IGF-I and IGF-II) in both transgenic rat and mice models, is closely involved in the development of the fetal pancreas. The present observation is the first supporting this relationship in humans. Both IGF-I and IGF-II affect positively proliferation and maturation of developing B-cells, although partly through different receptors. In both fetal and neonatal life, the synthesis of IGF-II, is a common feature of isolated human islets cells (2). The role of IGF-II in the pancreas of the fetus and neonate is likely to be that of a paracrine or autocrine mitogen and anti-apoptosis agent, acting through the type 1 IGF-receptor.

Human plasma IGF-II levels increase moderately during early childhood and remain on a stable level during adult life, reaching plasma values exceeding those of plasma IGF-I more three-fold. Since the estimated heritability of plasma IGF-II concentrations is around 66%, there must be important genetic traits for the expression of IGF-II (10). Moreover, the expression of IGF-II specific receptors in fetal life is higher than IGF-I receptors (2), and it is thus plausible that the impact of (changes in) IGF-II on the definite functional properties of the pancreatic cells is important. (1).

We conclude that beta cell function is related with IGF-II, independent from other known key factors such as obesity. Although it is

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**Table 2:** Linear correlation coefficients of relationships of IGF-II with fasting and post-glucose load glucose and insulin levels (OGTT), and first and second phase insulin secretion parameters as determined with a hyperglycemic clamp (10 mmol/L, 3 hours) in 50 non-diabetic subjects. SDS IGF-II denotes Standard Deviation Score for IGF-II according to previously determined SDS IGF-II values for age, gender and BMI.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IGF-II</th>
<th>SDS IGF-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose</td>
<td>+0.37</td>
<td>+0.38</td>
</tr>
<tr>
<td>120 min Glucose</td>
<td>+0.05</td>
<td>+0.08</td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>+0.33</td>
<td>+0.34</td>
</tr>
<tr>
<td>30 min Increment Insulin</td>
<td>+0.19</td>
<td>+0.19</td>
</tr>
<tr>
<td>120 min Insulin</td>
<td>+0.18</td>
<td>+0.21</td>
</tr>
<tr>
<td>First Phase</td>
<td>-0.11</td>
<td>-0.15</td>
</tr>
<tr>
<td>Second Phase</td>
<td>+0.38</td>
<td>+0.35</td>
</tr>
</tbody>
</table>

$^a p<0.05$, $^b p<0.02$, $^c p<0.01$
tempting to speculate that properties of the insulin secreting cells are determined in early life, obesity has also a marked influence on actual B-cell function. Therefore, IGF-II may have a role in B-cell adaptation to obesity (induced insulin resistance) during adult life. Consequently, a thorough analysis of the IGF system, from fetal to adult life, with respect to the properties of the insulin secreting cells may improve the understanding of (adult) insulin secretion, and possibly also shed new light on the disturbances of B-cell function related to the development of type 2 diabetes mellitus.

Acknowledgements

TW van Haeften has received a grant by the Dutch Diabetes Research Foundation (Amersfoort, The Netherlands) for these studies. ThB Twickler is research fellow of the National Institute of Health and Medical Research (INSERM) France.

References

2.4
Fasting Plasma IGF-1 Levels in AGHD Predict the Level of Insulin Resistance after initiation of rhGH Therapy
Recently, clinical concern arose about the increased IR in short-term substitution studies with recombinant growth hormone (rhGH) in adult-onset growth hormone deficiency (AGHD) patients. In several studies, the incidence of diabetes mellitus type II is higher in GH substituted patients with adult and/or childhood onset GHD (1) and it is known for a long time that an increased IR is associated with increased cardiovascular morbidity and mortality (2). On the opposite, increasing clinical evidence showed that premature atherosclerosis is a clinical feature in the AGHD syndrome and that rhGH substitution improved the initially increased femoral intima media thickness (IMT) and endothelial dysfunction (measured with Flow Mediated Dilation; FMD) (3-6). If IR increase after rhGH substitution, the beneficial effect of rhGH upon the cardiovascular parameters could consequently be counteracted. Nowadays, the substitution of rhGH is only according to sex and age (7;8) without a regular follow-up of a subsequent IR.

In this short communication, we report the observation that baseline plasma IGF-1 level in AGHD could prospect the insulin sensitivity that result after 6 months of rhGH substitution.

Subjects and Methods

Subjects
Eleven adult GHD patients (9 men and 2 women), aged 49 ± 5 years, BMI 28.3 ± 3.1 kg/m² (Mean ± SD) participated in this intervention study. After being optimally substituted for other deficient pituitary hormones, the patients were treated with daily subcutaneous rhGH during 6 months. Before the start and after six months of rhGH substitution venous blood was drawn to analyse. Dosages of rhGH were titrated upon the adjusted plasma IGF-1 levels, according to their age and sex. The daily administered rhGH was similar in all patients (between 0.8 and 1.1 IU/day). None of the patients had additional treatment in the study period, had a positive family history for type II Diabetes or suffered from renal and/or liver disease. All patients obtained written information about the protocol (approved by the ethical committee) and from all patients an informed consent was obtained.

Methods
In fasting conditions, plasma glucose, insulin and IGF-1 concentrations were assessed before and after rhGH replacement. The IR was estimated by calculating HOMA index (fasting insulin times fasting plasma glucose divided by 22.5) (9). Measurement of insulin and IGF-1 were performed in plasma samples with a radio-immuno assay (10).
Statistical analysis
Data are presented as median (range). Effects of rhGH substitution in AGHD patients were analyzed by a paired (two tailed) t-test. Pearson’s correlation or Spearman’s rank correlations were applied to evaluate relationships between parameters. Correlations are mentioned in the text if they reached statistically significance. Differences between before and after rhGH treatment are expressed as delta. A P value of 0.05 was considered significant. Statistical analysis was performed with Sigma Stat (Jandel Corporation).

Results
The increase in insulin secretion (C-peptide was also increased) was not sufficient to prevent the small but statistically significant increase in fasting glucose (4.6 vs. 5.1 mmol/L) (Table 1). Although, the plasma insulin levels increased it did not reach the level of significance. Overall insulin resistance as determined by HOMA index, increased after rhGH. As expected, a significant increase in plasma IGF-1 levels was observed after treatment with rhGH. Plasma IGF-1 levels, before rhGH substitution, were negatively correlated with the delta HOMA index (delta HOMA index = -0.01 * (basal IGF-1 level) + 1.96; r = 0.56) (Figure 1).

The delta plasma IGF-1 levels were positively correlated with the delta HOMA index (delta HOMA index = 0.01*(delta IGF-1) - 0.40; r = 0.73).

Table 1: Effect of six months rhGH treatment on various plasma parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>on rhGH treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.6 (4.0-6.0)</td>
<td>5.1 (4.6-6.0)</td>
</tr>
<tr>
<td>Insulin (mE/L)</td>
<td>6 (3-17)</td>
<td>9 (2-21)</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.3 (0.7-4.5)</td>
<td>1.8 (2.6-5.6)</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>102 (30-182)</td>
<td>212 (180-280)</td>
</tr>
</tbody>
</table>

Results are Median (range). * P<0.05.
Discussion

In the present study, we showed that pre-treatment plasma levels of IGF-1 in AGHD patients could partially account for the definite change in HOMA index (basal insulin sensitivity) after rhGH therapy \( (r^2 = 0.32) \). Moreover, a decrease in insulin sensitivity in AGHD patients during treatment was associated with the incremental in plasma IGF-1 levels. Knowledge of plasma profiles of IGF-1 before and during rhGH substitution could therefore help in the individual titration of rhGH, and may optimize beneficial effects of rhGH treatment.

An increase in HOMA-index, due to elevation of both the plasma glucose and insulin levels, observed after rhGH substitution, reflect a decrease in insulin sensitivity. Short-term rhGH infusion results in hyperinsulinaemia, in impairment of insulin to suppress the hepatic de-novo glucose production and in stimulation of the peripheral glucose uptake and oxidation in skeletal muscles \( (11;12) \). Decreased insulin sensitivity is already described to be an independent cardiovascular risk factor, even in non-diabetic populations \( (13) \). The relation between hyperinsulinemia and cardiovascular mortality was confirmed in a large population during a 15-year follow-up study. In that study, the calculated HOMA index in subjects who died from cardiovascular disease was 2.11 \( (14) \). As previously noted the AGHD syndrome is associated with an increased mortality. While in the present study only one untreated patient exhibit a HOMA index above 2, the HOMA index was >2 in five patients on rhGH treatment. Therefore, the HOMA index in AGHD that results from GH substitution needs to be of concern during clinical follow-up.

In this study, lower plasma IGF-1 levels at the start of rhGH substitution result in more IR in rhGH treated AGHD patients. Plasma IGF-1 levels are for 35% genetically determined. A possible explanation could be a less intrinsic insulin secretion capacity by the pancreas due to initially low plasma IGF-1 levels. Prenatal, the development of pancreatic \( \beta \) cells is influenced by IGF-1; low plasma IGF-1 in the prenatal period result in a lower insulin secreting capacity. IGF-1 is an important regulator in the differentiation and proliferation of the pancreatic \( \beta \) cell \( (15) \). From index subjects and family members with IR, a genetically determined low IGF-1 expression was associated with a 7.5 fold increased risk for diabetes \( (95 \text{ percent confidence interval of odds ratio} 2.8 \text{ to} 16.2 \) \( (16) \). Therefore, the increase in IR after rhGH substitution may be interpreted as an in-born capacity reduction of the pancreatic gland that is not capable to oppose the increased glucose load (possibly due to increased gluconeogenesis that is related to rhGH substitution) in the general circulation. Therefore, the enhanced insulin response of the pancreatic \( \beta \) cell may be primarily considered to be genetically determined and only in a secondary manner, after the substitution of rhGH and the consequent elevation of the systemic glucose load will lead to IR.

From our results, one could consequently derive that less concern about IR is needed in these AGHD patients with higher plasma baseline IGF-1 levels. In line with this, AGHD patients with lower baseline IGF-1 levels will possibly need additional treatment to lower the insulin resistance by for example drugs like biguanides (Metformin) or insulin therapy. The increase in IR is also associated with the IGF-1 elevation after rhGH substitution and this observation advocate an optimal titration of rhGH substitution with special attention to the AGHD patients with initially lower plasma IGF-1 levels.

In conclusion, the increase of insulin resistance in AGHD after substitution of rhGH could be optimally managed with special attention to the baseline plasma IGF-1 levels.
Acknowledgements

Financial grant was obtained from NOVO-Nordisk B.V., Alphen a/d Rijn, the Netherlands.

References

2.5

Endogenous glucose production rate during GH therapy in adult-onset growth hormone deficiency is maintained due to an elevated contribution of gluconeogenesis

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GH therapy in adult-onset growth hormone deficiency (AGHD) is associated with changes in glucose homeostasis. The circulating availability of glucose results from gluconeogenesis (GNG) and glycogenolysis (GL). In order to investigate GNG and GL in AGHD, GNG and GL in AGHD patients (n=5) were analyzed, before and after 6 and 12 months of GH therapy, in comparison to mild obese healthy volunteers (n=5), matched for age, sex and BMI. The total glucose turnover was estimated after intravenous infusion of 6,6 2H2 glucose, with a fractional contribution of gluconeogenesis after oral ingestion of 2H2O. The contribution of GNG via pyruvate pathway was determined through 2H enrichment in C6/body water, and via pyruvate + glycerol pathways through 2H enrichment in C5/body water. Glycogenolysis was calculated as the difference between glucose production and gluconeogenesis. On baseline, fasting plasma levels of insulin, glucose, C-peptide and HOMA did not differ between AGHD patients and control subjects. During GH therapy, insulin sensitivity decreased, but not significant. Total glucose turnover in AGHD was not increased compared to control subjects and remained constant during GH therapy. Pretreatment, GL in AGHD was increased (and inverse for GNG), compared to control subjects. Due to GH therapy, the percentage contribution of GL decreases (from 34% on baseline to 25% on rhGH (+12)), in favor for GNG (with pyruvate as a major substrate). Plasma glutamate levels increased during GH therapy. GNG was inversely associated with plasma IGF-2 levels (r = -0.61, p< 0.01).

Insulin sensitivity in AGHD patients tends to decrease during GH therapy. The total glucose turnover in AGHD patients remained stable, GL continue to be the major source of glucose in the postabsorptive phase, before and during GH therapy (in comparison to mild obese control subjects). Simultaneously, an increase in GNG (with pyruvate as a major precursor) is observed during GH therapy that may explained by an increase in fatty acid oxidation due to GH lipolytic effects.

Although results are conflicting, most reports note distinctive changes in glucose homeostasis in AGHD patients during GH therapy, such as a decrease in insulin sensitivity (1-3). Consequently, this may indicate a reduced availability of glucose for tissue metabolism (if not compensated by higher plasma insulin levels). An impaired glucose metabolism, and especially glycolysis, will limit cognitive functions, physical activity, and other glucose dependent processes. In humans, optimal plasma levels of endogenously derived glucose (in a range of 5 to 8 mmol/L) are maintained in a well-regulated balance in GNG, GL and glycolyse. In order to understand better the consequences of GH therapy on the molecular level of glucose metabolism, thorough analysis of both GNG and GL, with respect to EGP in AGHD patients, may therefore be of interest.

So far, no studies with a direct assessment of GNG and GL in AGHD patients are performed, or the effects of GH therapy on it. Moreover, the previous used methods to analyze GNG were insufficient and were an important limitation in the progress of knowledge about GNG and GL in humans. In healthy subjects who receive a 12 h GH infusion, Butler et al (4) measured carbon dioxide incorporation into glucose as quantitative estimation of GNG. However, this method is not sufficient, mainly due to dilution of marked labels at the oxalo-acetate level. This dilutive effect can be avoided by recent developed method that uses deuterium incorporation into glucose (after oral
administration of \( ^2\text{H}_2\text{O} \) that provides whole body estimate of total GNG (and by calculation GL).

In this study, we aim to analyze in AGHD patients the contribution of GNG and GL, in the total EGP compared to that in matched healthy control subjects, and in AGHD patients during 6 and 12 months GH therapy.

**Subjects and methods**

**Patients**
The study population consisted of 5 male patients with AO-GH deficiency with a mean age of 50 years (range 44-58) and a mean body mass index of 29.2 kg/m² (range 25.0-33.8 kg/m²). Patients were recruited from the department of endocrinology in UMC Utrecht, the Netherlands. The origin of the GH deficiency was panhypopituitarism after neurosurgery for a pituitary adenoma (time interval after surgery was at least 6 months). GH deficiency was confirmed by at least two GH stimulation tests. All patients were substituted for additional deficient pituitary axes, such as with thyroxin, corticosteroids, sex hormones and 3 patients receive desmopressin. Patients were only included in this study, if these hormones were optimally substituted. None of them were known with diabetes. The clinical characteristic are shown in table 1.

After inclusion and baseline measurements (including stable isotope studies), GH hormonal replacement (Norditropin, Novo, Nordisk) was started. After 6 months and 12 months GH therapy, the patients were subjected to a study day for the second time and third time, respectively. One patient could not complete the whole study because of surgery after 8 months of rhGH therapy. A weight maintaining diet has been described for three days prior to the study day to both the patients and control subjects. While on diet, excessive exercise and alcohol intake was not allowed.

The dietary compliance was evaluated from a diary record, which reports three days’ diet prior to investigation in the metabolic ward. At baseline, the total energy intake in AGHD was 2088 ± 228 kcal/day, compared to 2150 ± 151 in control subjects (not significantly different). Moreover, GHD patients consumed 42 ± 2 % of carbohydrates, 36 ± 2 % fat and 21 ± 2 % protein (percentages were similar in control subjects). The patients following GH treatment (6 months) consumed a total of 2513 ± 188 kcal/day, 43 ± 3 % carbohydrates, 36 ± 2 % fat and 21 ± 4 % protein. The patients following long term GH treatment (12 months) consumed a total of 2122 ± 188 kcal/day with a 44 ± 0 % carbohydrates, 36 ± 1 % fat and 21 ± 1 % protein.

**Control subjects**
The control subjects consisted of 5 men with an average age of 52 years (range 45-58) and BMI of 28.2 kg/m² (range 24.0-29.4 kg/m²). The control subjects consumed 45 ± 1 % of carbohydrates, 35 ± 1 % fat and 19 ± 1 % protein.

The institutional Ethical committee approved the study protocol and each subject gave his or her informed written consent to participate.

**Methods**
Recently, a method has been developed in which Deuterium oxide \( ^2\text{H}_2\text{O} \) can be used as tracer to measure gluconeogenesis (5,6). Briefly, the principle is as follows: \(^2\text{H}\) is bound to the C3 of Phosphoenolpyruvate (PEP) that becomes eventually the C6 of glucose. GNG from glycerol also results in labeling of the C5 of glucose via incorporation of the label into C2 of glyceraldehyde-3-P, which equilibrates with dihydroxyacetone-3-P. Because of rapid cycling between Glucose-6-P and fructose 1,6-P, there is an addition of hydrogen from body water to C2 by both GNG and glycolgenolysis. Enrichment at C2 of glucose was approximately equal to the enrichment in body water in the...
both control subjects (5;5;7), in patients with malaria falciparum and cirrhosis (8;9) and in subjects in whom effects of fatty acids elevation on GNG was examined (10). Thus, enrichment of the deuterium bound to carbon 6 of glucose to that in body water following $^2$H$_2$O administration equals the fraction of glucose formed by gluconeogenesis via pyruvate while enrichment of the deuterium bound to carbon 5 of glucose to that in body water equals the fraction of glucose formed by gluconeogenesis including glycerol. Use of non-recycling [6,6 $^2$H$_2$] glucose label provide a value for total hepatic glucose production. Because plasma deuterated glucose is analyzed, there is no discrimination between renal and hepatic GNG and thus whole body gluconeogenic fraction is measured.

Experimental design
One day before the study day, all subjects collected 24 hours urine specimen, which was analyzed for nitrogen (N) and for background of body water. Before the study day, participants have their last oral intake at 6.00 P.M. On the study day, subjects were admitted to the University Medical Center Utrecht, between 07.00 and 07.30 am. To avoid physical activity during the study, subjects came by bus or by car and were transported in a wheel chair in the hospital. Body weight was measured and a dorsal hand vein was cannulated for “arterialized” venous blood sampling (drawn from a hand vein in a heated box (62°C). Blood samples were drawn for measurement of basal enrichments and other parameters for insulin sensitivity (glucose, glucagon, HbA1c, insulin, C-peptide), additional hormones (free T4, free T3, cortisol), the IGF system (IGF-1, IGF-2, IGFBP-1, IGFBP-3), the precursors for GNG (pyruvate, lactate, alanine, glutamate, glutamine) and the lipolytic products (free fatty acids, glycerol).

A cubital vein in the contralateral arm was cannulated for the infusion of 6,6 $^2$H$_2$ glucose which started at 10.00 AM. At $t=10.00$ a priming 6,6 $^2$H$_2$ glucose 98% (26.4 µmol/kg prime) and a maintenance dose of 0.33 µmol/kg/hr min was given for two hours to determine the total rate of glucose appearance (Ra). Both catheters were flushed with heparin. During the study day, no food was given and subjects were allowed to drink water that was enriched to 0.5% with $^2$H$_2$O to maintain steady state. To achieve an enrichment of $^2$H in body water of approximately 0.5%, they were given orally $^2$H$_2$O (1g/kg body water) (>99.8 % enriched; Cambridge Isotopes, Andover, MA) at $t=8.00$, 8.30, 9.00, 9.30 en 10.00 AM. The next two hours were allowed for equilibration of the $^2$H$_2$O. After emptying the bladder at $t=12.00$, urine have been collected between $t=12.00$ and $t=13.00$. At $t=10.00$ AM, a blood sample for background enrichment was drawn. At $t=12.00$, 12.15 and 12.30 hr heparin blood samples were drawn from “arterialized” venous blood for the measurement of deuterium enrichments in blood glucose. After the last blood sample, the intravenous lines were removed and the subjects were given a regular meal.

Standard Analytical procedures
Plasma glucose was measured with standard laboratory methods on a Vitros 950 (Johnson & Johnson, Clinical Diagnostics, NY, USA). HbA1c was measured using a high-performed liquid chromatography method. Free T4 was measured with a full automatic competitive micro particle chemoluminescence immuno-assay. Free T3 was measured using an immuno enzymetric assay (a competitive ELISA using streptavidin technology performed on a ES300, Roche Diagnostics GmbH, D-68298 Mannheim). Glucagon was measured after alcohol extraction followed by a competitive radiommmunoassay using a polyclonal antibody raised in rabbit against Pancreas Glucagon. Cortisol was measured with immuno chemiluminiscence. IGF1 was measured with an immuno chemolumniscence (Nichols Institute Diagnostics, San Juan Capistrano, USA). Concentrations of plasma IGF-2, IGFBP-3 (mg/L) and IGFBP-
1 (µg/L) were determined by specific RIAs, as described previously (11). Insulin was measured with a competitive radioimmunoassay using a polyclonal anti-insulin-antibody (CARIS46), 125I-Insulin (IM166, Amersham Nederland bv) as a tracer and Humuline (YV2632 AMV Lilly, Indpls,USA) as a standard. C-peptide was measured with a competitive radioimmunoassay (MD315, Euro-Diagnostica, Malmö, Sweden). Pyruvate, FFA was measured on a Cobas Fara (Roche, Germany) using an enzymatical method. Lactate and glycerol were measured with standard laboratory method on a Hitachi-911 (Roche, Germany). Plasma alanine, glutamate and glutamine concentrations were measured on a Biochrom 20 automatic amino acid analyzer (Pharmacia Biotech, England).

**Body composition measurements**

Body height was measured to the nearest 1.0 cm by using a wall mounted stadiometer, and body weight to the nearest 0.05 kg. BMI was calculated as weight (kg) divided by height squared (m²). Body fat was assessed by bioelectric impedance analysis (BIA) (tetrapolar BIA-101 analyzer: RJL-Systems, Detroit), based on resistance and reactance measurements. Resistance and reactance were measured (in ) after application of an alternating current of 800 µA at 50 kHz with the electrodes placed as described by Lukaski et al (12). Body fat was calculated by using the manufacturer-supplied equation.

**Indirect calorimetry**

At t=0.900 A.M., ventilation, oxygen consumption and carbon dioxide production were calculated breath to breath (Oxycon Sigma (Mijnhardt, Jaeger)). Gas analyses were automatically performed by using room air as a reference. Subjects were asked to breath normally. Data were recorded at 30 seconds interval during 30 minutes. Before measuring, two minutes of adaptation period were introduced. Net glucose and lipid oxidation rates were estimated with the use of former described method (13) and protein oxidation rates were estimated by the assessment of serum nitrogen (N) concentrations and urinary excretion rates of N.

**Nitrogen measurement**

The nitrogen was converted to ammonia by wet oxidation according to the Kjeldahl technique in a digestion mixture of sulfuric acid, sodium sulphate and mercuric sulphate.

**Procedures for measuring glucose metabolism**

In order to measure glucose kinetics 5 ml plasma for enrichment on C5 and 2 ml to determine enrichment on C6 was immediately deproteinized using equal volumes of prechilled 10% perchloric acid and stored at -20°C. The other samples were immediately measured or stored below -20°C. The deuterium enrichment in bound to carbon 5 and 6 of blood glucose was determined as described elsewhere (5;6) with some slight modifications. In short, supernatant obtained after deproteinizing blood samples was passed through a mixed cation of AG1-X8 (formate form) and AG 50 w-X8 (H+). Glucose in the effluent was isolated with use of high pressure liquid chromatography using a Aminex HPX-87c column (Bio-Rad, the Netherlands) with water at 80°C as solvent. In order to isolate C6, 0.5-1mg was oxidized with periodate to form formaldehyde. This was converted to hexamethylenetetramine (HMT). Enrichment at C5 of a portion of glucose is determined chemically by conversion of glucose to xylose with removal of C6. The xylose is oxidized with periodate to form formaldehyde. Plasma glucose enrichment was determined by gas chromatography mass spectrometry (GC-MS) on a Hewlett-Packard HP 5890 type II gas chromatograph interfaced to a HP 5989B mass spectrometer. The gas chromatograph was equipped with a coating CP Sil 19CB capillary column (Chrompack, Bergen op Zoom, the Netherlands). Injection (2 µl) was performed in a split mode (1:20). The flow rate of carrier gas (helium) was 1 ml/min. Injector temperature was 240°C and oven temperature was programmed starting at 210°C for 1 minute,
then increased from 210°C to 280°C at 25°C/min and maintained at 280°C for three minutes. The HMT eluted at approximately 4.5 minutes. The source and quadruple temperature were 250°C and 150°C, respectively. Fragments 140, 141 and 142 were monitored and enrichment was determined using a calibration curve whereby measured ion abundance ratios are correlated with enrichment of standards of known isotopic composition. The distribution of different masses in HMT was used to calculate deuterium enrichments by mass isotope distribution analysis (14). Comparing both methods revealed a correlation of \( r^2 = 0.995 \) \( (y = 0.908x - 0.0061, n = 54) \). Since we observed that the tracer/tracee measurements are affected by the quantity of HMT analyzed, an initial “pre-run” was performed to determine relative sample concentrations. The second run was performed after adding appropriate volumes of solvent to each vial in order to narrow sample concentration within the concentration measured within the calibration curve. All samples were measured in duplicate, with a CV < 3%.

The enrichment in urinary water was determined after reaction with calcium carbide to form acetylene (15). The \( m/z \) signal ratio for acetylene measurements were performed in triplicate (different vials) with a CV% < 4%.

Calculations and statistics

To measure glucose production, an infusion of \( [6,6 \text{ } ^2\text{H}_2] \text{ glucose} \) is given simultaneously with \( ^2\text{H}_2\text{O} \). The percentages of the HMT molecules, with two \( ^2\text{H} \) bound to C6, are determined and are measured as percentage of the molecules of molecular mass 142 \( (m+2) \). Glucose production is calculated as:

\[
\text{Glucose production} = \frac{\text{Enrichment}}{\text{Calibration curve}} \times \text{Metabolic rate}
\]

Table 1: Clinical and fasting biochemical characteristics of GHD patients before and after respectively 6 and 12 months of rhGH replacement therapy compared with age, sex and BMI matched control subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months rhGH</th>
<th>12 months rhGH</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 2</td>
<td>50 ± 2</td>
<td>50 ± 1</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>96.2 ± 5.1</td>
<td>97.4 ± 6.9</td>
<td>95.7 ± 6.5</td>
<td>85.3 ± 3.6</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.2 ± 1.7</td>
<td>29.5 ± 1.9</td>
<td>29.8 ± 2.4</td>
<td>28.2 ± 1.1</td>
</tr>
<tr>
<td>FM (Kg)</td>
<td>23 ± 3</td>
<td>22 ± 3</td>
<td>23 ± 4</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>FFM (Kg)</td>
<td>73 ± 5</td>
<td>75 ± 5</td>
<td>73 ± 5</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>FM (%)</td>
<td>24 ± 3</td>
<td>23 ± 3</td>
<td>23 ± 4</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>76 ± 3</td>
<td>77 ± 3</td>
<td>77 ± 4</td>
<td>75 ± 2</td>
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<tr>
<td>WHR</td>
<td>0.96 ± 0.03</td>
<td>0.94 ± 0.04</td>
<td>0.93 ± 0.05</td>
<td>0.94 ± 0.02</td>
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<tr>
<td>RQ</td>
<td>0.80 ± 0.02</td>
<td>0.79 ± 0.02</td>
<td>0.78 ± 0.02</td>
<td>0.79 ± 0.03</td>
</tr>
<tr>
<td>M (kcal/24hr)</td>
<td>1038 ± 78</td>
<td>1269 ± 139</td>
<td>1242 ± 191</td>
<td>1138 ± 95</td>
</tr>
</tbody>
</table>

Data represent Mean ± SEM. BMI = Body mass index, FM = Fat mass, FFM = Fat free mass, WHR = waist-hip ratio, RQ = respiratory quotient, M = Metabolic rate.

Data represent mean ± SEM
Infusion rate (0.33 μmol/kg/min)/ enrichment (M+2) at blood glucose C-6. The fraction of blood glucose formed by GNG from pyruvate was calculated by one half of the enrichment at carbon 6 (M+1) divided by the enrichment in body water. A factor of 0.5 is used since two hydrogen atoms are bound to carbon 6. The percent contribution of GNG from pyruvate + glycerol to plasma glucose was calculated as the ratio of the enrichment at C5/enrichment at body water in each subject. The rate of GNG from both pyruvate and glycerol and pyruvate was calculated by multiplication of the total glucose production by fractional GNG. Glycogenolysis was calculated from the difference between the rates of total glucose production and GNG (from glycerol and pyruvate). Data are represented as mean ± SEM, expressed as %, μmol/kg/min and μmol/kg FFM/min. Statistically significance (which was set at p<0.05) of difference was tested using a paired t-test for patients and a two sample t-test was used to compare patients with control subjects. Log transformation was used in non-normally distributed parameters.

Results

Body composition
Body weight (kg), BMI (kg/m²), WHR and Fat Free Mass (FFM) were not significantly different between AGHD patients (on baseline, rhGH (+6) and rhGH(+12)), and control subjects (table 1).

Indirect calorimetry
The resting metabolic rate in AGHD tended to increase from 1038 ± 78 kcal/24 h to 1269 ± 139 kcal/24 h after 6 months to 1242 ± 191 kcal/24 h after 12 months rhGH therapy (table 1). No difference in resting metabolic rate was found at baseline in AGHD, compared to control subjects. Glucose oxidation was higher than fat oxidation in both AGHD at baseline and control subjects. The oxidation of fat in AGHD patients increased during GH therapy, with a decrease in glucose oxidation, however not significant (table1).

Circulating metabolites and hormones

Glucose and Insulin
Fasting plasma glucose levels in AGHD were not increased, as compared to BMI matched control subjects. GH therapy tended to increase plasma glucose levels (table 2). Insulin, C-peptide, pyruvate and HOMA-ratio were not significantly different between patients (before and during GH therapy) and control subjects (table 2). Glycosylated hemoglobin (Glyc-HB) was unchanged during GH therapy (table 2). Fasting insulin levels in AGHD tended to increase during GH therapy, but at baseline no difference with control subjects was found. Fasting plasma insulin concentrations correlated positively with the degree of fat mass (Kg) (r= 0.75, p<0.0001; grouped results of all participants).

Hormones
No change in plasma free T4 and free T3 levels in AGHD was found during GH therapy. Plasma glucagon and cortisol levels tend to be higher in GH treated AGHD patients compared to control subjects, but no significant difference was found.

IGF system
The plasma levels of IGF-1, IGF-2 and IGFBP-3 were lower in AGHD patients than in the control subjects. The IGF-1 concentration was significantly higher at 6 months and at 12 months rhGH therapy, compared to pretreatment levels (p<0.05), and significantly higher at 12 months rhGH therapy, compared to 6 months rhGH treatment (p<0.01) (table 2). Both IGF-2 and IGFBP-3 increased during GH therapy.

Glucose precursors
FFA (table 2) as well as the other gluconeogenic substrate precursors, such as lactate, alanine, glutamine and glycerol (table 3), were not significantly different between AGHD patients and control subjects and
these parameters did not change during GH therapy. In contrast, glutamate was significantly higher (P<0.05) at study entry, compared with control subjects, and sustained higher during GH therapy for 6 and 12 months (table 3). There was a significant decrease in the urinary N excretion after 6 months therapy (but not after 12 months GH therapy due to higher variance), compared to pretreatment levels.

**Glucose metabolism**

The postabsorptive rates of endogenous glucose production, GNG and glycogenolysis are presented in table 4. There was no difference between AGHD patients and control subjects in endogenous glucose production, expressed as µmol/kg/min or as µmol/kg FFM/min. During GH therapy, total glucose rate remained constant. GL, expressed as µmol/kg FFM/min, was significantly increased in pretreatment GH period (2.7 ± 0.3 µmol/kg

---

**Table 2:** Fasting biochemical characteristics of GHD patients before and after respectively 6 and 12 months of rhGH replacement therapy compared with age, sex and BMI matched control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>12 months</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.3</td>
<td>5.2 ± 0.2</td>
<td>5.6 ± 0.5</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>Gly-Hb (%)</td>
<td>5.8 ± 0.1</td>
<td>5.6 ± 0.2</td>
<td>5.6 ± 0.1</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Free T4 (pmol/L)</td>
<td>14 ± 2</td>
<td>15 ± 1</td>
<td>15 ± 3</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Free T3 (pmol/L)</td>
<td>6.2 ± 0.4</td>
<td>7.8 ± 1.0</td>
<td>6.9 ± 0.7</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>Glucagon (pmol/L)</td>
<td>29 ± 4</td>
<td>32 ± 6</td>
<td>34 ± 18</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>Cortisol (µmol/L)</td>
<td>0.60 ± 0.09</td>
<td>0.61 ± 0.12</td>
<td>0.61 ± 0.06</td>
<td>0.45 ± 0.10</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>117 ± 25</td>
<td>198 ± 27</td>
<td>154 ± 23</td>
<td>133 ± 12</td>
</tr>
<tr>
<td>IGFBP-1 (µg/L)</td>
<td>33 ± 7</td>
<td>28 ± 7</td>
<td>42 ± 25</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>IGFBP-3 (mg/L)</td>
<td>1.35 ± 0.15</td>
<td>1.62 ± 0.06</td>
<td>1.76 ± 0.29</td>
<td>1.86 ± 0.18</td>
</tr>
<tr>
<td>IGF-2 (ng/ml)</td>
<td>301 ± 27</td>
<td>369 ± 36</td>
<td>330 ± 25</td>
<td>382 ± 23</td>
</tr>
<tr>
<td>Insuline (mE/L)</td>
<td>8 ± 3</td>
<td>12 ± 5</td>
<td>13 ± 7</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>C-peptide (amol/L)</td>
<td>0.92 ± 0.19</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>0.97 ± 0.12</td>
</tr>
<tr>
<td>Pyruvate (µmol/L)</td>
<td>83 ± 14</td>
<td>70 ± 7</td>
<td>74 ± 7</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>FFA (µmol/L)</td>
<td>1095 ± 172</td>
<td>1255 ± 73</td>
<td>1244 ± 253</td>
<td>1513 ± 341</td>
</tr>
<tr>
<td>HOMA-ratio</td>
<td>1.9 ± 0.7</td>
<td>3.0 ± 1.2</td>
<td>3.6 ± 2.3</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>N (g/day)</td>
<td>12.9 ± 0.8</td>
<td>11.7 ± 0.8</td>
<td>10.5 ± 2.9</td>
<td>11.6 ± 0.8</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM

* NC: data onbreek nog (infusie 21)

* p<0.05 compared to basal situation

*# p<0.01 compared to 6 months treatment

*# p <0.05 compared to control subjects
FFM/min; p<0.01), and after 6 months therapy (2.5 ± 0.3 µmol/kg FFM/min; p<0.05), compared to control subjects (1.5 ± 0.1 µmol/kg FFM/min). The percent contribution of GNG from pyruvate to glucose production was significantly (P<0.05) lower at basal situation (44.6 ± 2.4 %) compared to control subjects (55.7 ± 3.2%). This is also true when expressed in µmol/kg FFM/min although this was not statistically different (table 4). The percent contribution of GNG from pyruvate + glycerol to glucose production was statistically lower at baseline and at 6 months treatment compared to control subjects (P<0.05) and gradually increased during rhGH treatment; 66.7 ± 3.1 at baseline to 68.5 ± 3.8 and 75.6 ± 1.9 at 6 and 12 months of rhGH treatment respectively. However, when expressed as µmol/kg FFM/min it was not significantly different. In the whole data set, GNG (µmol/kg FFM/min) was negatively associated with plasma IGF-2 levels (R=-0.605, p=0.006) (fig.1).

Table 3: Plasma GNG precursors levels in GHD subjects and on respectively 6 and 12 months treatment on rhGH

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>12 months</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.6 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Alanine (µmol/L)</td>
<td>304 ± 16</td>
<td>324 ± 27</td>
<td>335 ± 60</td>
<td>318 ± 14</td>
</tr>
<tr>
<td>Glutamate (µmol/L)</td>
<td>125 ± 12</td>
<td>114 ± 11</td>
<td>124 ± 12</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>Glutamine (µmol/L)</td>
<td>451 ± 33</td>
<td>400 ± 78</td>
<td>482 ± 17</td>
<td>517 ± 23</td>
</tr>
<tr>
<td>Glycerol (mmol/L)</td>
<td>0.16 ± 0.05</td>
<td>0.18 ± 0.03</td>
<td>0.24 ± 0.05</td>
<td>0.13 ± 0.04</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM
@ p<0.05 compared to control subjects

Table 4: Glucose turnover of GHD patients before and after respectively 6 and 12 months of rhGH replacement therapy compared with age, sex and BMI matched control subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>12 months</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Ra (µmol/kg/min)</td>
<td>10.5 ± 0.3</td>
<td>10.8 ± 0.5</td>
<td>9.5 ± 0.7</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>Glucose Ra (µmol/kg FFM/min)</td>
<td>8.0 ± 0.5</td>
<td>8.4 ± 0.7</td>
<td>7.3 ± 0.9</td>
<td>7.6 ± 0.4</td>
</tr>
<tr>
<td>GNG Pyruvate (%)</td>
<td>44.6 ± 2.4</td>
<td>47.4 ± 1.2</td>
<td>53.2 ± 4.2</td>
<td>55.7 ± 3.2</td>
</tr>
<tr>
<td>GNG Pyruvate (µmol/kg FFM/min)</td>
<td>3.6 ± 0.2</td>
<td>4.0 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>GNG pyruvate + glycerol (%)</td>
<td>66.7 ± 3.1</td>
<td>68.5 ± 3.8</td>
<td>75.6 ± 1.9</td>
<td>79.8 ± 2.2</td>
</tr>
<tr>
<td>GNG Pyruvate +glycerol (µmol/kg FFM/min)</td>
<td>5.4 ± 0.5</td>
<td>5.7 ± 0.4</td>
<td>5.5 ± 0.8</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Glycogenolysis (µmol/kg FFM/min)</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

@ p<0.05 compared to control subjects.
# P<0.01 compared to control subjects.
Discussion

Endogenous glucose production in AGHD patients is maintained on a stable level due to an increase in gluconeogenesis during GH therapy. Probably, this increase in GNG during GH therapy is the consequence of a decrease in insulin sensitivity. On the other hand, less potency to compensate the decrease in insulin sensitivity (by components of the IGF system, such as IGF-2) during GH therapy may be another explanation. Animal models with defects in the expression of IGF-2 receptors or with defects in the related IRS-2 pathway showed no capacity in the compensation for a prodia-betic phenotype (16;17). Indeed, in our study pooled plasma IGF-2 levels were found to be negatively associated with pooled GNG.

Although several earlier reports noted decreased insulin sensitivity during GH therapy (18;19), a recent report found that the higher start dosages of GH in those early days of GH treatment were related to these negative effects on glucose homeostasis (20). Even opposite effects of GH therapy on insulin sensitivity were found; a study with a hyperinsulinemic-euglycemic clamp in AGHD patient showed that 7 years of GH treatment may prevent from an age-related decline in insulin sensitivity (19). Indeed, in this study no increase in HOMA index was observed, although a tendency existed for higher levels of insulin during GH therapy. In one study, we compared AGHD patients with BMI- and age matched control subjects on baseline, and insulin sensitivity was in the mild obese control subjects higher than in AGHD patients (although not significant).

The metabolic rate in AGHD patients increased slightly, but not significantly, during GH therapy. In previous studies, rest metabolic rate (RMR) in AGHD patients increased after 3 to 6 months of GH therapy, and the change in RMR could be explained for about 60% by the change in FFM (21;22). Moreover, a decrease in FFM in AGHD patients was observed in the first 6 weeks of the 24 weeks GH therapy, with a simultaneous increase in RMR (23). In line with this, we also found no change in FFM during GH therapy. The glucose oxidation, as a principal energy source in humans, (as estimated by RQ measurements) is about 60% in both AGHD patients and control subjects with only a small decrease during GH therapy. Fat oxidation in AGHD patients increased during GH therapy, but not significant. Recombinant GH substitution in fasting healthy volunteers increased oxidation of fatty acids with a related decrease in serum alanine levels (24). Our observations are in concordance with Hoffman et al, who showed no difference in energy expenditure (by indirect calorimetry) and fuel utilization (fat and glucose oxidation) between AGHD patients and control sub-

In this study, total glucose turnover was not different between AGHD patients and control subjects. This observation is in line with previous reports that explore post absorptive state glucose metabolic clearance rate and glucose turnover in AGHD (26), also after start of GH therapy no significant change in glucose turnover was found (27). Additionally, so far, no other study showed that the percentage of GNG is markedly decreased in pretreatment AGHD patients in comparison to mild obese control subjects. On the other hand, GL in AGHD was increased. In physiological conditions, a decrease in GNG is directly related to an increase in glycogenoly-sis to prevent harmful hypoglycemic events. This inverse relationship between GNG and GL reflect an autoregulatory pathway, that activates GL in response to a decreased level in GNG. Since control subjects were matched for BMI, the difference in GNG can consequently not explained by the mild obe-
sity. Other factors, probably induced by GH and translated towards the IGF system, may be more adjusted. While previous reports mention normal insulin sensitivity in GH
deficient subjects, some other studies report an increased insulin resistance in obese GH deficient subjects (28), and an impaired glucose tolerance, in especially female GH deficient subjects. GNG is under influence of the hormone glucagon, that slightly increases in parallel with GNG in this study. The increased amounts of lipolytic products, such as glycerol and fatty acids, during GH therapy will decrease the entry of citrate in the Kreb cycle, and favours acetyl-Co A to the oxaloacetate-malate reaction with formation of GNG substrate. In line with conditions that are associated with an accelerated lipolysis, such as a prolonged fasting period (29) and in diabetes mellitus (30) glycerol acts as a precursor in gluconeogenic processes. Although, FFAs serve as a substrate for de novo glucose synthesis (31), high FFA levels also inhibit insulin action on the receptor level. Consequently, activation of glycogen synthase by insulin is inhibited (32).

The significant decreased GNG in AGHD patients may also be due to failure of the expression of rate determining enzymes in GNG, such as phosphoenol pyruvate carboxy kinase, Gluc-6-phosphatase and fructose 1,6 biphosphatase, or by reduced supply of amino acids that serve as glucose precursors in GNG. In AGHD patients, severe defects of in vivo insulin sensitivity and skeletal muscle intracellular glucose phosphorylation and glycogen synthase activity persist with 24 months rhGH therapy (approximately 0.22 IU/kg week) (33,34). Although limited amounts of glycogone in AGHD patients are present, GL contributed most to EGP. After start of GH therapy, the increase in GNG in AGHD is mostly related to pyruvate as a C3 GNG precursor. The amino acid alanine in humans provides most of the substrate, that is needed to form pyruvate with subsequent GNG (Felig cycle), and plasma levels of alanine in AGHD patients tend to increase during GH therapy. GH has a nitrogen sparing effect; and this related to a decrease in N urine and an increase in glutamate efflux from the liver (35,36). Indeed, plasma glutamate levels in AGHD patients were higher and a decrease of N in 24 hour collected urine was found during GH therapy. Interesting, the plasma glutamate levels in AGHD patients were already increased before treatment, and this suggests that not only GH induces the glutamate efflux. Although baseline plasma levels of alanine (most important GNG substrate) and lactate were comparable in AGHD patients and control subjects, the plasma glutamine levels were decreased in GHD patients and glutamate significantly increased in those on therapy (table 3). In terms of adding new (non-glucose derived) carbons to the plasma glucose pool, glutamine appears to be as important as lactate (37). In humans in a postabsorptive condition, glutamine is an important precursor for renal GNG which contributes 20% to 25% of the whole body glucose production (38).

Taken together, in comparison with control subjects that are matched for BMI, the level of peripheral insulin resistance, the glucose production rate and level of glucose oxidation are comparable to AGHD patients, before and during GH therapy. However, the contribution of GNG as part of total glucose production rate in AGHD patients increase with alanine, pyruvate and glutamine (with a possible increase of renal GNG) as probable precursors, in parallel with a decrease in urinary nitrogen excretion (that is a nitrogen sparing effect of GH). The negative relationship between GNG and plasma IGF-2 levels is not elucidated yet, but suggests an interesting relationship, also in humans, between components of the IGF system and glucose homeostasis.

Acknowledgements

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ry) for the assessment of VCO₂ was outstanding. Dr PS van Dam is gratefully thanked for selection and clinical follow up of some of the participating AGHD patients. ThB Twickler is a postdoctoral visiting research fellow (Poste Vert) of National Institute of Science and Medical Research (INSERM) in France, and receiver of a travel fellowship of the International Atherosclerosis Society (IAS), Dutch Association of Science (NWO) and the foundation “De Drie Lichten”. Novo Nordisk BV, Alphen a/d Rijn, the Netherlands gave financial support to this study.

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