# NORMAL PHYSIOLOGY OF GROWTH HORMONE IN ADULTS

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### ABSRACT

Growth hormone (GH) is a 191 amino-acid single chain polypeptide, which is secreted by the somatotrophs in the anterior pituitary. With the recognition of its multiple and complex effects in the early 1960s, the physiology and regulation of GH has become a major area of research interest in the field of endocrinology.

Its secretion is regulated by several factors including GHRH, somatostatin, ghrelin and IGF-1. Apart from its primary function of stimulation of GH secretion, GHRH plays an essential role in pituitary somatotroph development and proliferation. Somatostatin on the other hand is the main inhibitor of GH secretion. Along with its receptors, somatostatin has been extensively studied over the last decade as a treatment for acromegaly. Several somatostatin receptors (SSTR) have been identified, of which SSTR2 and SSTR5 exhibit greater inhibition on the secretion of GH by the somatotrophs. Somatostatin receptor ligands to SSTR2 and 5, such as octreotide, lanreotide and pasireotide, are approved treatment modalities for acromegaly in some countries.

GH acts both directly through its own receptors and indirectly through the induced production of Insulin-like Growth Factor I (IGF-I). The "IGF-1 axis" holds a significant place in the field of with numerous research been done on its pharmacokinetics and endocrinology, pharmacodynamics, affecting different organ systems in humans. Its physiological effects have been demonstrated not only in tissue growth, but also in glucose / lipid metabolism, coronary disease, diabetes mellitus and vascular aging. The use of recombinant IGF-1 in IGF-1 deficiency and insulin insensitivity and the use of IGF-1 receptor inhibitors in the promotion of cellular apoptosis, especially in the management of malignancies, are two other main areas of research in prospect.

GH is a dynamic hormone, which like most other hormones in the human body, varies in concentration and action under the influence of numerous factors. Characteristics including age, physical fitness and body composition play a major role in the level and action of GH in humans and vice versa. It is well recognized for its vital role in glucose and lipid homeostasis and muscle mass function. These effects are of key significance, especially in the current era with increasing availability and use of recombinant growth hormone in the treatment of adults. For complete coverage of this and related area, visit www.endotext.org.

### INTRODUCTION

In his monograph from 1912 on "The Pituitary Gland" Harvey Cushing proposed the existence of a "hormone of growth", and was thereby among the first to indicate that the primary action of this hormone was to control and promote skeletal growth. In clinical medicine growth hormone (GH) (also called somatropin) has until recently primarily been known - and used - for the action suggested by its name - i.e. for the treatment of short stature in hypopituitary children, and for its adverse effects in connection with hyper-secretion as observed in acromegaly. The multiple and complex actions of human GH were, however, acknowledged shortly after the advent of pituitary derived preparation of the hormone in the late fifties - as beautifully reviewed by Maurice Raben in 1962 (1). In the present chapter we will briefly review normal physiology of GH secretion and the effects of GH on intermediary metabolism throughout adulthood. Other important physiological effects of GH will be dealt with in the review of GH replacement in adults.

# GH SECRETION, PHARMACOKINETICS AND PHARMACODYNAMICS

# **Regulation of GH Secretion**

GH is a single chain protein with 191 amino-acids and two disulfide bonds. The human GH gene is located on chromosome 17q22 and comprises five genes. It encodes two distinct GH molecules (22 kDa and 20 kDa) of which 90% in the circulation constitute the 22kDa molecule[1].

GH is secreted by the somatotroph cells located primarily in the lateral wings of the anterior pituitary. There are many critical processes in pituitary organogenesis, including cell migration, proliferation, differentiation, establishing connections between cells/hypothalamus and development of vasculature including the hypophyseal portal system. Several transcription factors such as Pit-1/Pou1 F1 and PROP1 have been identified to play a vital role in the organogenesis of the anterior pituitary and the development of the somatotrophs. Inactivating mutations or deletions in these transcription factor genes lead to under-development of somatotrophs, lactotrophs and thyrotrophs, ultimately leading to anterior pituitary hypoplasia and pituitary hormone deficiency[2].

The morphological characteristics and number of somatotrophs are remarkably constant throughout life, while secretion changes. GH secretion occurs in a pulsatile fashion, and in a circadian rhythm with a maximal release in the second half of the night.

Two hypothalamic hormones regulate GH secretion; Growth Hormone Releasing Hormone (GHRH) with a stimulatory action at the level of gene transcription, and somatostatin with an inhibitory effect

### on the GH secretion from the pituitary gland. (Figure 1)

#### Growth Hormone Releasing Hormone (GHRH)

GHRH is a 44 amino-acid polypeptide produced in the arcuate nucleus of the hypothalamus. These neuronal terminals secrete GHRH to reach the anterior pituitary somatotrophs via the portal venous system, which leads to GH transcription and secretion. Moreover, animal studies have clearly demonstrated that GHRH plays a vital role in the proliferation of somatotrophs in the anterior pituitary, where the absence of GHRH has, in fact, lead to anterior pituitary hypoplasia [3]. The secretion of GHRH is stimulated by several factors including depolarisation,  $\alpha$ 2-adrenergic stimulation, hypophysectomy, thyroidectomy and hypoglycaemia and it is inhibited by somatostatin, insulin-like growth factor-I (IGF-I) and activation of GABAergic neurons.

GHRH acts via a seven trans-membrane G protein-coupled stimulatory cell-surface receptor on the somatotrophs. This receptor has been extensively studied over the last decade leading to the identification of several important mutations. Point mutations in the GHRH receptors, as illustrated by studies done on the *lit/lit* dwarf mice, showed a profound impact on subsequent somatotroph proliferation leading to anterior pituitary hypoplasia [4]. Unlike the mutations in the pit-1 and prop-1 genes, which lead to multiple pituitary hormone deficiencies and anterior pituitary hypoplasia, mutations in the GHRH receptor leads to profound GH deficiency with anterior pituitary hypoplasia. Subsequent to the first GHRH receptor mutation described by Wajnrajch, et al in 1996, an array of familial GHRH receptor mutations have been recognized over the last decade. These mutations, in fact, account for almost 10% of the familial isolated GH deficiencies. An affected individual will present with short stature and a hypoplastic anterior pituitary. However, they lack certain typical features of GH deficiency such as midfacial hypoplasia, microphallus and neonatal hypoglycaemia [5].

#### Somatostatin(SST)

Somatostatin is a cyclic peptide, encoded by a single gene in humans, which mostly exerts inhibitory effects on endocrine and exocrine secretions. Many cells in the body including specialized cells in the anterior periventricular nucleus and arcuate nucleus produce SST. These neurons secrete SST into the adenohypophyseal portal venous system, via the median eminence, to exert its effect on the anterior pituitary. SST has a short half-life of approximately 2 minutes as it is rapidly inactivated by tissue peptidase in humans. The secretion of SST by the hypothalamic neurons is inhibited by high blood glucose and is induced by serum GH/IGF-1 level, exercise and immobilization [6,7].

SST too acts via a seven trans-membrane, G protein coupled receptor and, thus far, five subtypes of the receptor have been identified in humans (SSTR1-5). Although all five receptor subtypes are expressed in the human fetal pituitary, adult pituitary only express 4 subtypes (SSTR1, SSTR2, SSTR3, SSTR5). Out of these four subtypes, somatotrophs exhibit more sensitivity to SSTR2 and SSTR5 ligands in inhibiting the secretion of GH. In fact, the inhibition of both receptors seems to exert a synergistic effect on GH inhibition rather than each receptor individually [8].

#### Other regulators of GH secretion

Various synthetically produced GH releasing compounds and natural hormone ghrelin have been shown to increases the production of growth hormone. Ghrelin is a 28 amino-acid peptide that is the natural ligand for the growth hormone secretagogue receptor. In fact, ghrelin and GHRH have a synergistic effect in increasing circulating growth hormone levels [9]. Interestingly, exogenous ghrelin stimulates food intake and gastric emptying (2). In everyday life it is known that stress, hypoglycaemia and ingestion of protein (high levels of circulating amino acids) stimulates GH secretion, while high levels of glucose and FFA inhibit secretion (Fig 1).





**INSULIN-LIKE GROWTH FACTOR (IGF-1)** 

# Physiology of IGF-1

GH acts both directly through its own receptor and indirectly through the induced production of IGF-I. IGF-1 is a 70 amino-acid peptide, found in the circulation, 99% bound to transport proteins. It is

synthesized both in the liver and in the peripheral tissue, and is an important mediator of GH action. Following the initial discovery of IGF-1 in the late 1950s, Salmon and Daughaday hypothesizes, that GH governs somatic growth by IGF-1 secreted by the liver. However, in the 1980s this hypothesis was modified by the identification of IGF-1 production in most tissues including bone, myoblasts in muscle, erythroid precursors, ovary, kidney and central nervous system. Nevertheless, the liver produces approximately 75% of IGF-1 and is mainly under the regulation of GH while the peripheral production of IGF-1 is regulated by GH as well as tissue dependent paracrine factors. IGF-1 is known as a global and tissue growth factor as well as an endocrine factor. In some tissues IGF-1 acts as a potent inhibitor of cellular apoptosis. In fact, it is well recognized that some tumour cells express abundant IGF-1 receptors to inhibit apoptosis.

Due to its wide array of physiological functions, the "IGF axis" has been a major area of interest over the last two decades, with the identification of two growth factors (IGF-I and IGF-2), seven IGF-binding proteins (IGFBP-1 to 7), and 9 IGFBP-related proteins (IGFBP-RPs). It has been recognized as a key system affecting virtually every organ system in the body, in regulating cell growth and survival.

Interestingly, insulin and IGF-1 share many structural and functional similarities implying that they have originated from the same ancestral molecule. Both molecules could have been part of the cycle of food intake and consequent tissue growth. The IGF-1 gene is member of the insulin gene family and the IGF-1 receptor is structurally similar to the insulin receptor in its tetrametric structure, with 2 alpha and 2 beta subunits [11]. The alpha subunit binds IGF-1, IGF-2 and insulin: however, the subunit has a higher affinity towards IGF-1 compared to IGF-2 and insulin. Although, insulin and IGF-1 share many similarities, during evolution, the functionality of the two molecules has become more divergent, where insulin plays a more metabolic role and IGF-1 plays a role in cell growth.

The IGF-1 receptor is expressed in many tissues in the body. However, the receptor number on each cell is strictly regulated by several systemic and tissue factors including circulating GH, thyroxine, platelet-derived growth factor (PDGF) and fibroblast growth factor. Following the binding of the IGF-1 molecule, the receptor undergoes a conformational change, which activates tyrosine kinase, leading to auto-phosphorylation of tyrosine. Apart from this, the activated receptor phosphorylates "insulin receptor substrate-2" (IRS-2), which in-turn activates the RAS activating protein SOS. This complex activates the mitogen activated protein kinase (MAP kinase) pathway. Thus activation of the MAP kinase pathway becomes vital in the stimulation of cell growth by IGF-1[12].

*In vivo*, IGF-1 is bound almost 100% to a family of binding proteins known as the IGF-binding protein (IGFBP). The IGFBP family has 6 binding proteins (IGFBP 1-6) with a high affinity towards IGF-1and 2, resulting in less than 1% unbound IGF-1. Apart from regulating the free plasma IGF fraction, IGFBPs also play an important role in the transport of IGF into different tissues and extravascular space. IGFBP-3 and IGFBP-2 are the most abundant forms seen in plasma and are saturated with IGF-1 due to its high affinity. 75% of IGF-1 is bound to IGFBP-3. Interestingly, similar to IGF-1, IGFBP-3 production is also regulated by growth hormone. In the plasma, IGFBP-3 is bound to a protein called acid labile subunit (ALS), which stabilizes the "IGFBP 3-IGF 1" complex, prolonging its half-life to approximately 16 hours [13].

IGFBP-1, on the other hand, is present in lower concentration in plasma than IGFBP-2 and 3.

However, due to lower affinity towards IGF-1, IGFBP-1 is usually in an unsaturated state and changing plasma concentrations of IGFBP-1 becomes important in determining the unbound fraction of IGF-1.

# Physiological Effects Of IGF-1

Multiple animal and human studies have analyzed the physiological effects of IGF-1 over the last two decades. Initial studies on hypophysictemised animals showed IGF-1 to promote growth in all tissues with major rate limitation with hypoglycaemia. These studies also demonstrated the anabolic effects of IGF-1 by way of increasing GFR, improving wound healing and reversal of catabolic effects of nutritional deprivation [14].

IGF-1 plays a key role in growth, where it acts not only as a determinant of postnatal growth, but also as an intra-uterine growth promoter. Total inactivation of the IGF-1 gene in mice produced a perinatal mortality of 80% with the surviving animals showing significant growth retardation compared to controls [15]. Human IGF-1 deficiency can be either due to GH deficiency, GH receptor inactivation or IGF-1 gene mutation. Interestingly, infants with congenital GH deficiency and GH receptor mutations present with only minor growth retardation, whereas few reported patients with IGF-1 deficiency, secondary to a homozygous partial deletion of the IGF-I gene, present with severe pre and postnatal growth failure, mental retardation, sensorineural deafness and microcephaly [16,17,18]. The differences in the clinical presentation is most likely due to the fact that patients with GH and GH receptor defects lack the biological effects of both GH and GH-driven IGF-1 with some intact tissue IGF-1, while patients with IGF-1 gene defects lack both GH-driven and tissue-derived IGF-1. This difference in clinical picture sheds light on the significant role played by tissue IGF-1, independent of GH.

More detailed studies on transgenic mice have clearly demonstrated this fact with selective deletion of IGF-1 gene expression only in the liver, showing low serum IGF-1 concentrations with only 6-8% postnatal growth retardation. In contrast, animals with total IGF-1 deletion and only peripherally produced IGF-1 deletion showed marked growth retardation [19]. Therefore, considering growth, both paracrine/autocrine IGF-1 and liver-derived IGF-1 are important in achieving the final adult height.

Apart from its action as a tissue growth, IGF-1 plays an important metabolic role in the body. Several studies have demonstrated that, similar to the action of insulin, IGF-1 increases the peripheral tissue glucose uptake [20]. Skeletal muscle, one of the main peripheral organs involved in glucose metabolism with high expression of IGF-1 receptors, has been shown to up-regulate the number of IGF-1 receptors in the face of insulin resistance [21,22]. Apart from this, IGF-1 has also been shown to suppress hepatic gluconeogenesis, in a dose-dependent manner, along with promoting whole body glucose disposal, implying that it may play a role in improving the insulin sensitivity [23]. Case controls studies done in normal individuals, individual with impaired glucose tolerance and Type 2 diabetes, has shown that plasma IGF-I concentrations, in fact, appeared to be independently associated with insulin sensitivity, accounting for 10.8% of the variation in insulin sensitivity [24].

Along with the discovery of improved insulin sensitivity with IGF-1, there had been a major interest, over the last decade, on the metabolic functions of IGF-1. This has lead to several clinical trials to

assess the enhancement insulin sensitivity in humans by recombinant IGF-1therepy. These trials with recombinant IGF-1, have shown promising results with improved the insulin sensitivity and glycaemic control in patients with severe insulin resistance syndromes and type 2 diabetes mellitus. Maybe surprisingly, some trials have demonstrated improved glycaemic control in type 1 diabetes, when recombinant IGF-1 is co-administered with insulin [25,26,27].

In addition to the association with insulin resistance, another area of growing interest is the link between reduced IGF-1 levels and ischaemic heart disease (IHD). Conti *et al* demonstrated low IGF-1 in individuals with angina pectoris with normal coronary vasculature (syndrome X) [28], while a 15-year population based case-control study in individuals without ischaemic heart disease showed that the group in the lower IGF-1 quartile had a higher risk of IHD during the 15-year follow-up period, with a relative risk of 1.94 (95% CI, 1.03 to 3.66) compared to the high IGF-I quartile group. This is especially interesting as possible confounders such as body mass index, smoking, menopause, diabetes, IGFBP-3, and use of anti-hypertensives were controlled in the analysis [29].

Due to its multiform activites, IGF axis has become an area of extensive study, especially in the fields of oncology, diabetes, obesity, vascular aging and atherosclerosis. Although, the IGF-1 axis has been associated with lipid metabolism, with several in vitro studies suggesting increased free fatty acid uptake in to hepatocytes and adipocyte, the human studies currently available are conflicting and the jury is still out on the direct effect of IGF-1 on lipid metabolism [30,31].

# GH LEVELS: INFLUENCE OF BODY COMPOSITION, PHYSICAL FITNESS AND AGE

With the introduction of dependable radioimmunological assays it was recognized that circulating GH was blunted in obese subjects (3), and that normal aging was accompanied by a gradual decline in GH levels (4). The latter observation led Rudman et al. (5) to the hypothesis that many of the senescent changes in body composition and organ function were related to or caused by hyposomatotropinemia. The term "somatopause" may be considered a paraphrase for Rudmans hypothesis although it remains uncertain who introduced this persuasive term.

Studies done in the late 90s have uniformly documented that hypopituitary adults with severe GHdeficiency are characterized by increased fat mass and reduced lean body mass (LBM) (6). It is also known that normal GH levels can be restored in obese subjects following massive weight loss (7), and that GH substitution in GH-deficient adults normalizes body composition (6).

What remains unknown is the cause-effect relationship between hyposomatotropenimia and senescent changes in body composition. Is the propensity for gaining fat and loosing LBM initiated or preceded by a primary age-dependent decline in GH secretion and action or vice versa?: accumulation of fat mass secondary to non-GH dependent factors (e.g. life style, dietary habits) results in a feedback inhibition of GH secretion

Moreover, little is known about possible age-associated changes in GH pharmacokinetics and bioactivity.

Cross sectional studies done to assess the assosiation between body composition and stimulated GH release in healthy subjects showed that, elderly people (mean age 50 years) had a lower peak

GH response to secretagogues (clonidine and arginine), and females had a higher response to arginine when compared to males. Multiple regression analysis, however, revealed that intraabdominal fat mass was the most important negative predictor of peak GH levels (Fig.2), where as both age, gender and physical fitness were of minor importance. Lean body mass was not significantly associated with GH status in either males or females. (11)



Figure 2. Correlation between intra-abdominal fat mass and 24 hour GH secretion (from ref. 10).

In the same population 24-h spontaneous GH levels were also analyzed by means of deconvolution analysis of samples obtained every 20-minute. Mean GH levels, GH production rate and GH burst amplitude were higher in young people and in females as compared to older people and males, (12). Multiple regression analysis again suggested that intra-abdominal fat mass was the single most important and negative determinant of GH status. Fasting levels of insulin, IGF-I and free fatty acids did not correlate with either estimates of GH status. Surprisingly, LBM exhibited a weak inverse correlation with mean 24-h GH release, but LBM was not associated with other attributes of GH status and was not an independent determinant by multiple regression analysis.

A detailed analysis of GH secretion in relation to body composition in elderly subjects has, to our knowledge, not been performed. Instead serum IGF-I has been used as a surrogate or proxy for

GH status in several studies of elderly men (13-15). These studies comprise large populations of ambulatory, community-dwelling males aged between 50-90 years. Not unexpectedly serum IGF-I declined with age (Fig. 3), but IGF-I failed to show any significant association with body composition or physical performance (13-15).

# GH ACTION: INFLUENCE OF AGE, SEX AND BODY COMPOSITION

Considering the great interest in the actions of GH in adults surprisingly few studies have addressed possible age-associated differences in the responsiveness or sensitivity to GH. In normal adults the senescent decline in GH levels is paralelled by a decline in serum IGF-I, suggesting a down-regulation of the GH-IGF-I axis. Administration of GH to elderly healthy adults has generally been associated with predictable albeit modest effects on body composition and a high incidence of side-effects (17). Whether this reflects an unfavourable balance between effects and side effects in older people or employment of excessive doses of GH is uncertain, but it is evident that older subjects are not resistant to GH. Studies in GH deficient adults with pituitary disease strongly suggest that the dose requirement declines with age. Short-term dose response studies clearly demonstrate that older patients require a lower GH dose to maintain a given serum IGF-I level (18-19), and it has been observed that serum IGF-I increases in individual patients on long-term therapy if the GH dosage remains constant (20). It has also recently been reported that hypopituitary patients above 60 years are highly responsive to even a small dose of GH (21). Interestingly, there appears to be a gender difference in GH deficient adults with men being more responsive in terms of IGF-I generation and fat loss during therapy (22).

The pharmacokinetics and short-term metabolic effects of a near physiological intravenous GH bolus (200 g) were compared in a group of young (" 30 years) and older (" 50 years) healthy adults (23). The area under the GH curve was significantly lower in older subjects, whereas the elimination half-life was similar in the 2 groups, suggesting both an increased metabolic clearance rate (MCR) and apparent distribution volume (Vd) of GH in older subjects. Both MCR and Vd showed a strong positive correlation with fat mass, although multiple regression analysis revealed age to be an independent positive predictor. The short-term lipolytic response to the GH bolus was higher in "young" as compared to "older" subjects, respectively. Interestingly, the same study revealed that the GH binding protein (GHBP) correlated strongly and positively with abdominal fat mass (24).



Figure 3. Changes in serum IGF-I with increasing age. Modified from Jull A et al., J. Clin. Endocrinol. Metab. 1994; 78: 744-752.

"THE SOMATOPAUSE"

It is obvious that the mechanism underlying the so-called somatopause involves other and perhaps more complex mechanisms than the female menopause, which predominantly is caused by gonadal resistance to gonadotropins. A prospective long-term study of normal adults with serial concomitant estimations of GH status and adiposity would provide useful information. Evaluation of GH sensitivity as a function of age, sex and body composition would also be worthwhile. In the mean time the following hypothesis may be proposed (Fig. 4): 1. Changes in life-style and genetic predispositions promote accumulation of body fat with aging 2. The increased fat mass increases FFA availability, inducing insulin resistance and hyperinsulinemia 3. High insulin levels suppress IGFBP-1 resulting in a relative increase in free IGF-I levels. 4. Systemic elevations in FFA, insulin and free IGF- I suppresses pituitary GH release, which further increases fat mass. 5. Endogenous GH is cleared more rapidly in subjects with high amount of fat tissue.

The very strong positive correlation between fat mass and GHBP could suggest that GH is cleared in adipose tissue by a receptor-mediated mechanism. Clearly, future studies are needed to substantiate or refute this simplified model. At present it is equally premature and unwarranted to recommend GH treatment to reverse the age-associated deterioration in body composition and physical performance.



Figure 4. Hypothetical model for the association between GH levels and body composition in adults.

### **METABOLIC EFFECTS OF GROWTH HORMONE**

# **Glucose Homeostasis and Lipid Metabolism**

The involvement of the pituitary gland in the regulation of substrate metabolism was originally detailed in the classic dog studies by Houssay (25). Fasting hypoglycaemia and pronounced sensitivity to insulin were described as salient features of hypophysectomised animals. These symptoms were readily corrected by administration of anterior pituitary extracts. It was also noted that pancreatic diabetes was alleviated by hypophysectomy. Finally, excess of anterior pituitary lobe extracts aggravated or induced diabetes in hypophysectomised dogs.

Luft et al. (26) clearly demonstrated the glycaemic control to deteriorate following exposure to a single supra-physiological dose of human GH in hypophysectomised adults with type 1 diabetes mellitus. Somewhat surprisingly, only modest effects of GH on glucose metabolism were recorded in the first metabolic balance studies involving adult hypopituitary patients (27, 28).

More recent studies on glucose homeostasis in GH deficient adults have generated results, which at first glance may appear contradictory. Insulin resistance may be more prevalent in untreated GH deficient adults (29, 30), whereas the impact of GH replacement on this feature seems to depend on the duration and the dose. Below, some of the metabolic effects of GH in human subjects, with special reference to the interaction between glucose and lipid metabolism, will be reviewed.

### **GH Action In Normal Adults**

Almost forty years ago it was shown that infusion of high dose GH into the brachial artery of healthy adults reduced forearm glucose uptake in both muscle and adipose tissue (31). This was parallelled by a drop in RQ and an increase in muscle uptake of FFA, both of which suggested oxidation of FFA by the muscle. This pattern was opposite that of insulin, and co-administration of insulin and GH resulted in only minimal changes in net fluxes of glucose and FFA across the forearm bed. These studies clearly indicated direct insulin antagonistic effects of GH on muscle and adipose tissue.

The introduction of reliable radioimmunoassays for GH revealed the pulsatile and episodic nature of GH release (32) now known to be generated by alternating secretion of GHRH and SST. A GH pulse is released roughly every second hour with a mean daily secretion of 0.5 mg (33). Apart from a well-known circadian variation in terms of elevated nocturnal GH levels during the early hours of sleep, GH secretion is amplified during fasting and stress, whereas meals suppress GH release.

We studied the metabolic effect of a physiological GH bolus in the post-absorptive state, and demonstrated stimulation of lipolysis following a lag time of 2-3 hours to be the most consistent effect (34). Plasma glucose, on the other hand exhibited only minimal fluctuations, and serum insulin and C- peptide levels remained completely stable. This was associated with subtle reductions in muscular glucose up-take and oxidation, which could reflect substrate competition between glucose and fatty acids (i.e. the glucose/fatty acid cycle). In line with this, sustained exposure to high GH levels induces both hepatic and peripheral (muscular) resistance to the actions of insulin on glucose metabolism together with increased (or inadequately suppressed) lipid oxidation. Apart from enhanced glucose/fatty acid cycling, it has been shown that GH induced insulin resistance is accompanied by reduced muscle glycogen synthase activity (35) and diminished glucose dependent glucose disposal (36). Bak et al. (35) also demonstrated insulin binding and insulin receptor kinase activity from muscle biopsies to be unaffected by GH.

# **Lessons From Acromegaly**

Active acromegaly clearly unmasks the diabetogenic properties of GH. In the basal state plasma glucose is elevated despite compensatory hyperinsulinemia. In the basal and insulin-stimulated state (euglycemic glucose clamp) hepatic and peripheral insulin resistance is associated with enhanced lipid oxidation and energy expenditure (37). There is evidence to suggest that this hypermetabolic state ultimately leads to beta cell exhaustion' and overt diabetes mellitus (38), but a more recent study have demonstrated that the abnormalities are completely reversed after successful surgery (37). Conversely, it has been shown that only two weeks administration of GH in supraphysiological doses (8 IU/day) induces comparable acromegaloid - and reversible - abnormalities in substrate metabolism and insulin sensitivity (39).

# INTERACTION OF GLUCOSE AND LIPID METABOLISM

Relatively few studies have scruntinised the exact sites of action of GH on glucose metabolism. There is no evidence of a net effect of GH on insulin binding to the receptor (35, 40), which obviously implies post receptor metabolic effects. The effect of FFA on the partitioning of intracellular glucose fluxes was originally described by Randle et al. (41). According to his hypothesis (the glucose/fatty acid cycle), oxidation of FFA initiates an up- stream, chain-reaction-like inhibition of glycolytic enzymes, which ultimately inhibits glucose uptake (Fig. 5).



Figure 5. The glucose-fatty acid (Randle) cycle in muscle. Oxidation of fatty acids (FFA) inhibits pyruvate dehydrogenase(PDH). Citrate inhibits phosphofructokinase (PFK). The rise in glucose-6-phosphate inhibits hexokinase. Additional abbreviations: UDP, uridine diphosphate; GLUT 4, Glucose transporter 4.

When considering the pronounced lipolytic effects of GH the Randle hypothesis remains an appealing model to explain the insulin-antagonistic effects of GH glucose metabolism. In support of this experiments have shown that co-administration of anti-lipolytic agents and GH reverses GH-induced insulin resistance. Similar conclusions were drawn from a recent study in GH deficient adults, which showed that insulin sensitivity was restored when acipimox (a nicotinic acid derivative) was co-administered with GH (42). It has, however, also been reported that GH-induced

insulin resistance preceded the increase in circulating levels and forearm uptake of lipid intermediates (43). This early effect of GH on muscular glucose uptake could reflect intra-myocytic FFA release and oxidation and thus be compatible with the Randle hypothesis. It could also imply alternative (early) effects of GH. Moreover, the inhibitory effect of GH on muscle glycogen synthase activity (35) is not readily explained by substrate competition. According to the Randle hypothesis the fatty acid-induced insulin resistance will result in elevated intracellular levels of both glucose and glucose-6-phosphate. By contrast, muscle biopsies from GH deficient adults after GH treatment have revealed increased glucose but low-normal glucose-6-phosphate levels (44). Moreover, NMR spectroscopy studies in healthy adults indicate that FFA infusion results in a drop in the levels of both glucose and glucose-6-phosphate (45). The latter study, which did not involve GH administration, reported that FFA suppressed the activity of PI-3 kinase, an enzyme stimulated by insulin which is considerede essential for glucose transportation into skeletal muscle via translocation of glucose transporter activity (GLUT 4). In a recent study we observed, that GH infusion in healthy subjects, which induced elevated FFA levels and insulin resistance, did not impact insulin-stimulated PI-3 kinase activity (46). Thus, the molecular mechanisms subserving GH-induced insulin resistance remain uncertain.

### **Implications For GH Replacement**

Regardless of the exact mechanisms, the insulin antagonistic effects may cause concern when replacing adult GH deficient patients with GH, since some of these patients are insulin resistant in the untreated state. There is evidence to suggest that the direct metabolic effects on GH may be balanced by long-term beneficial effects on body composition and physical fitness, but some studies report impaired insulin sensitivity in spite of favourable changes in body composition. There is little doubt that these effects of GH are dose-dependent and may be minimised or avoided if an appropriately low replacement dose is used. Still, the pharmacokinetics of s.c. GH administration is unable to mimic the endogenous GH pattern with suppressed levels after meals and elevations only during post absorptive periods, such as during the night. This may be considered the natural domain of GH action, which coincides with minimal beta-cell challenge. This reciprocal association between insulin and GH and its potential implications for normal substrate metabolism was initially recognised by Rabinowitz & Zierler (47). The problems arise when GH levels are elevated during repeated prandial periods. The classic example is active acromegaly, but prolonged high dose s.c. GH administration may cause similar effects. Subcutaneous administration of GH in the evening probably remains the best compromise between effects and side effects (48), but it is far from physiological. We know and understand that hypoglycaemia is a serious and challenging side effect of insulin therapy as a consequence of inappropriately high insulin levels (during fasting). As a corollary, we must realise that hyperglycaemia may result from GH therapy. It is therefore important to carefully monitor glucose metabolism and to use the lowest effective dose when replacing adults with GH.

# EFFECTS OF GROWTH HORMONE ON MUSCLE MASS FUNCTION

The anabolic nature of growth hormone (GH) is clearly evident in patients with acromegaly and vice versa in patients with GH deficiency. A large number of in vitro and animal studies throughout several decades have documented stimulating effects of GH on skeletal muscle growth (49). The methods employed to document in vivo effects of GH on muscle mass in humans have been

exhaustive including whole body retention of nitrogen and potassium, total and regional muscle protein metabolism using labeled amino acids, estimation of lean body mass by total body potassium or dual x-ray absorptiometry (DEXA), and direct calculation of muscle area or volume by computerised tomography (CT) and magnetic resonance imaging (MRI).

### Effects Of GH On Skeletal Muscle Metabolism In Vitro And In Vivo

The clinical picture of acromegaly and gigantism includes increased lean body mass of which skeletal muscle mass accounts for approximately 50 %. Moreover, retention of nitrogen was one of the earliest observed and most reproducible effects of GH administration in humans (1). Thoroughly conducted studies with GH administration in GH deficient children using a variety of classic anthropometric techniques strongly suggested that skeletal muscle mass increased significantly during treatment (49, 50). Indirect evidence of an increase in muscle cell number following GH treatment was also presented (49).

These early clinical studies were paralleled by equally impressive experimental studies in rodent models. GH ad- ministration in hypophysectomised rats increased not only muscle mass, but also muscle cell number (i.e. muscle DNA content) (49). Interestingly, the same series of experiments revealed that work-induced muscle hypertrophy could occur in the absence of GH. The ability of GH to stimulate RNA synthesis and amino acid incorporation into protein of isolated rat diaphragm suggested direct mechanisms of actions, whereas direct effects of GH on protein synthesis could not be induced in liver cell cultures (51). Another important observation of that period was made by Goldberg, who studied protein turnover in skeletal muscle of hypophysectomised rats with 3H-leucine tracer techniques. In these studies it was convincingly demonstrated that GH directly increased the synthesis of both sarcoplasmic and myofibrillar protein without affecting proteolysis (52).

The most substantial recent contributions within the field derive from human in vivo studies of the effects of systemic and local GH and IGF-I administration on total and regional protein metabolism by means of amino acid isotope dilution techniques. Horber and Haymond demonstrated that systemic GH administration for 7 days in normal adults increased whole body protein synthesis without affecting proteolysis (53), and similar data were subsequently obtained in GH deficient adults (54). Fryburg and Barret (55) infused GH (systemically for 8 hours) in normal adults and reported an acute stimulation of forearm (muscle) protein synthesis without any effects on whole body protein synthesis. By contrast Copeland and Nair (56) observed an acute stimulatory effect of GH on whole body protein synthesis, but no stimulatory effect on leg protein synthesis, in a design that also included co-administration of somatostatin to suppress insulin. Finally, Fryburg et al. (57) infused GH into the brachial artery, which was accompanied by a local increase in forearm muscle protein synthesis.

Based on these recent studies it seems that the nitrogen retaining properties of GH predominantly involve stimulation of protein synthesis without affecting (lowering) proteolysis and clues are also provided about the under- lying mechanisms. Theroretically, the protein anabolic effects of GH could be either direct, or mediated through IGF-I, insulin or lipid intermediates. GH receptors are present in skeletal muscle (58), which combined with Fryburgs intra-arterial GH studies, makes a direct GH effect conceivable. An alternative interpretation of Fryburgs data could be that GH stimulates local muscle IGF-I release, which subsequently acts in an autocrine/paracrine manner.

The effects of systemic IGF-I administration on whole body protein metabolism seem to depend on ambient amino acid levels in the sense that IGF-I administered alone suppresses proteolysis (59) whereas IGF-I in combination with an amino acid infusion increase protein synthesis (60). Moreover, intra-arterial IGF-I in combination with systemic amino acid infusion increased protein synthesis (61). It is therefore likely that the muscle anabolic effects of GH at least to some extent are mediated by IGF-I. By contrast, it is repeatedly shown that insulin predominantly acts through suppression of proteolysis and this effect(s) appears to be blunted by co-ad- ministration of GH (62). The degree to which mobilisation of lipids contributes to the muscle anabolic actions of GH has so far not been specifically investigated.

In conclusion several experimental lines of evidence strongly suggest that GH stimulates muscle protein synthesis. This effect is presumably in part mediated through binding of GH to GH receptors in skeletal muscle. This does not rule out a significant role of IGF-I being produced either systematically or locally.

A interesting recent discovery has been that infusion of GH and IGF-I into the brachial artery increase forearm blood flow several fold (57, 63). This effect appears to be mediated through stimulation of endothelial nitric oxide release leading to local vasodilatation (64, 65). Moreover, co-infusion of a nitric oxide inhibitor with IGF-I ap- peared to blunt the stimulatory effect of IGF-I on forearm protein synthesis (64). It thus appears that an IGF-I mediated increase in muscle nitric oxide release accounts for some of the effects of GH on skeletal muscle protein synthesis. These intriguing observations may have many other implications. It is, for instance, tempting to specu- late that this increase in skeletal muscle blood flow contributes to the GH induced increase in resting energy expenditure, since skeletal muscle metabolism is a major determinant of REE (66). Moreover, it is plausible that the reduction in total peripheral resistance seen after GH administration in GHDA is mediated by nitric oxide (65).

# EFFECTS OF GH ADMINISTRATION ON MUSCLE MASS AND FUNCTION IN ADULTS

### WITHOUT GH-DEFICIENCY

As previously mentioned the ability of acute and more prolonged GH administration to retain nitrogen in normal adults has been known for decades and more recent studies have documented a stimulatory effect on whole body and forearm protein synthesis.

Rudman et al. was the first to suggest that the senescent changes in body composition were causally linked to the concomitant decline in circulation GH and IGF-I levels (66). This concept, which is known by some as the somatopause, has recently been reviewed (67), and a number of studies with GH and other anabolic agents for treating the sarcopenia of ageing are currently in progress.

Placebo-controlled GH administration in young healthy adults (21-34 years) undergoing a resistance exercise programme for 12 weeks showed a GH induced increase in LBM, whole body protein balance and whole body protein synthesis, whereas quadriceps muscle protein synthesis rate and muscle strength increased to the same degree in both groups during training (68). In a similar study in older men (67 years) GH also increased LBM and whole body protein synthesis,

without significantly amplifying the effects of exercise on muscle protein synthesis or muscle strength (69). An increase in LBM but unaltered muscle strength following 10 weeks of GH administration plus resistance exercise training was also recorded by Taafe et al. (70). A more recent study of 52 older men (70-85 years) treated with either GH or placebo for 6 months, without concomitant exercise, observed a significant increase (4.4 %) in LBM with GH, but no significant effects on muscle strength (71). Thus no significant clinical benefit from administrating GH to non-GH-deficient senescent patients has been documented yet.

Numerous studies have evaluated the effects of GH administration in chronic and acute catabolic illness. A comprehensive survey of the prolific literature within this field is beyond the scope of this review, but it is noteworthy, that HIV-associated body wasting is a licensed indication for GH treatment in the USA. In this patient category GH treatment for 12 weeks has been associated with significant increments in LBM and physical fitness (72, 73).

# **GROWTH HORMONE SIGNALING IN HUMANS**

GH receptor signaling is a separate and prolific research field by itself as recently reviewed (74). This section will focus on recent data obtained in human models.

Growth hormone receptors have been identified in many tissues including muscle, fat, liver, heart, kidney, brain and the pancreas (75). Activation of receptor-associated Janus kinase (JAK) 2 is considered the critical step in initiating GH signalling. One GH molecule binds to two GHR molecules, and it is believed that preformed, unliganded GHR dimers exist (74). Following GH binding the intracellular domains of the GHR dimer undergo rotation, which is thought to bring together the two intracellular domains each of which bind one JAK2 molecule. This in turn induces cross-phosphorylation of tyrosine residues in the kinase domain of each JAK2 molecule followed by tyrosine phosphorylation of the GHR. Phosphorylated residues on GHR and JAK2 form docking sites for different signaling molecules including signal transducers and activators of transcription (STAT) 1, 3, 5a and 5b (74). STATs bound to the activated GHR-JAK2 complex are subsequently phosphorylated on a single tyrosine by JAK2 after which they dimerize and translocate to the nucleus, where they bind to DNA and act as gene transcription factors. A STAT5b binding site has recently been characterised in the IGF-I gene promoter region, which mediates GH-stimulated IGF-I gene activation (76). Attenuation of JAK2-associated GH signaling is mediated by a family of cytokine-inducible suppressors of cytokine signaling (SOCS) (77). SOCS proteins bind to phosphotyrosine residues on the GHR or JAK2 and suppress GH signaling by inhibiting JAK2 activity and competing with STATs for binding on the GHR. As an example, it has been reported that the inhibitory effect of estrogen on hepatic IGF-I production seems to be mediated via upregulation of SOCS-2 (78).

Data on GHR signaling derive mainly from rodent models and experimental cell lines, although GHinduced acti- vation of the JAK2/STAT5b and the MAPK pathways have been recorded in cultured human fibroblasts from nor- mal human subjects (79). STAT5b in human subjects is critical for GHinduced IGF-I expression and statural growth as demonstrated by the identification of mutations in the STAT5b gene of patients presenting with severe GH insensitivity in the presence of normal GHR (80). GHR signaling in human models in vivo has been reported in a study in healthy young male subjects exposed to an intravenous GH bolus vs. saline (81). In muscle and fat biopsies significant STAT5b tyrosine phosphorylation was recorded 30-60 minutes after GH exposure (81). Significant GH-dependent IGF-I mRNA expression was only detectable in adipose tissue, whereas SOCS-1 and SOCS-3 mRNA expression tended to increase in muscle and fat, respectively (81). There was no evidence of GH-induced activation of PI 3-kinase, Akt/PKB, or MAPK in either tissue. The latter observation is noteworthy in relation to the insulin antagonistic effects of GH.

There is animal and *in vitro* evidence to suggest that insulin and GH share post-receptor signaling pathways (82). Convergence has been reported at the levels of STAT5 and SOCS3 (83) as well as on the major insulin signaling pathway: insulin receptor substrates (IRS) 1 and 2, PI 3-kinase, Akt and extracellular regulated kinases (ERK) 1 and 2 (84, 85). Studies in rodent models suggest that the insulin-antagonistic effects of GH in adipose and skeletal muscle involve a suppression in insulin-stimulated PI3-kinase activity (82, 86). One study assessed the impact of a GH infusion on insulin sensitivity and the activity of PI3-kinase as well as PKB/AKt in skeletal muscle in a controlled design involving healthy young subjects (87). The infusion of GH induced a sustained increase in FFA lev- els and subsequently insulin resistance as assessed by the euglycemic clamp technique. This was, however, not associated with any changes in the insulin-stimulated increase in either IRS-1 associated PI3-kinase or PKB/Akt activity (87). It was subsequently assessed that insulin had no impact on GH-induced STAT5b activation or SOCS3 mRNA expression (88).

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