Endotext

NORMAL PHYSIOLOGY OF GROWTH HORMONE IN ADULTS

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ABSTRACT

Growth hormone (GH) is an ancestral hormone, secreted episodically from somatotroph cells in the anterior pituitary. Since 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. In adulthood its main role is to regulate metabolism. Pituitary synthesis and secretion of GH is stimulated by episodic hypothalamic secretion of GH releasing factor and inhibited by somatostatin. Insulin-like Growth Factor I (IGF-I) inhibits GH secretion by a negative loop at both hypothalamic and pituitary levels. In addition, age, gender, pubertal status, food, exercise, fasting, sleep, and body composition play important regulatory roles. GH acts both directly through its own receptors and indirectly through the induced production of IGF-I. Their effects may be synergic (stimulate growth) or antagonistic as for the effect on glucose metabolism: GH stimulates lipolysis and promotes insulin resistance, whereas IGF-I acts as an insulin agonist. The bioactivity of IGF-I is tightly controlled by a multitude of IGF-I binding globulins. The mechanisms to explain the insulin antagonist effect of GH in humans are causally linked to lipolysis and ensuing elevated levels of circulating free fatty acids (FFA). The nitrogen retaining properties of GH predominantly involve stimulation of protein synthesis, which could be either direct or mediated through IGF-I, insulin, or lipid intermediates. In this chapter the normal physiology of GH secretion and the effects of GH on intermediary metabolism throughout adulthood, focusing on human studies, are presented.

INTRODUCTION

Harvey Cushing proposed in 1912 in his monograph "The Pituitary Gland" the existence of a "hormone of growth" and was thereby among the first to indicate that the primary action of growth hormone (GH) was to control and promote skeletal growth. In clinical medicine GH (also called somatotrophin) was previously known for its role on promoting growth of hypopituitary children, and for its adverse effects in

connection with hypersecretion 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 reviewed by Raben in 1962 (1).

In the present chapter we will briefly review the normal physiology of GH secretion and the effects of GH on intermediary metabolism throughout adulthood. Other important physiological effects of GH are presented in the review on GH replacement in adults.

GROWTH HORMONE

GH is a single chain protein with 191 amino-acids and two disulfide bonds. The human GH gene is located on chromosome 17q22 as part of a locus that comprises five genes. In addition to two GH related genes (GH1 that codes for the main adult growth hormone, produced in the somatotrophic cells found in the anterior pituitary gland and, to a minor extent, in lymphocytes, and GH2 that codes for the placental GH), there are three genes coding for chorionic somatomammotropin (CSH1, CSH2 and CSHL) (also known as placental lactogen) genes (2,3). The GH1 gene encodes two distinct GH isoforms (22 kDa and 20 kDa). The principal and most abundant GH form in pituitary and blood is monomeric 22K-GH isoform, representing also the recombinant GH available for therapeutic use (and subsequently for doping purposes) (3). Administration of recombinant 22K-GH exogenously leads to a decrease in the 20K-GH isoform and testing both isoforms is used to detect GH doping in sports (4).

As already mentioned, GH is secreted by the somatotroph cells located primarily in the lateral wings of the anterior pituitary. A recent single cell RNA sequencing study performed in mice showed that GH-expressing cells, representing the somatotrophs, are the most abundant cell population in adult pituitary gland (5). The differentiation of somatotroph cell is governed by the pituitary transcription factor 1 (Pit-1). Data in mice suggests that the pituitary holds regenerative competence, the GH-producing cells being regenerated from the pituitary's stem cells in young animals after a period of 5 months (6).

Physiological Regulation of GH Secretion

The morphological characteristics and number of somatotrophs are remarkably constant throughout life, while the secretion pattern changes. GH secretion occurs in a pulsatile fashion, and in a circadian rhythm with a maximal release in the second half of the night. So, sleep is an important physiological factor that increases GH release. Interestingly, the maximum GH levels occur within minutes of the onset of slow wave sleep and there is marked sexual dimorphism of the nocturnal GH increase in humans, constituting only a fraction of the total daily GH release in women, but the bulk of GH output in men (7).

GH secretion is also gender, pubertal status, and age dependent (Figure 1) (8). Integrated 24 h GH concentration is significantly greater in women than in men and greater in the young than in the old adults. The serum concentration of free estradiol, but not free testosterone, correlates with GH and when correcting for the effects of estradiol, neither gender nor age influence GH concentration. This suggests that estrogens play a crucial role in modulating GH secretion (8). During puberty, a 3-fold increase in pulsatile GH secretion occurs that peaks around the age of 15 years (yr) in girls and 1 yr later in boys (9).

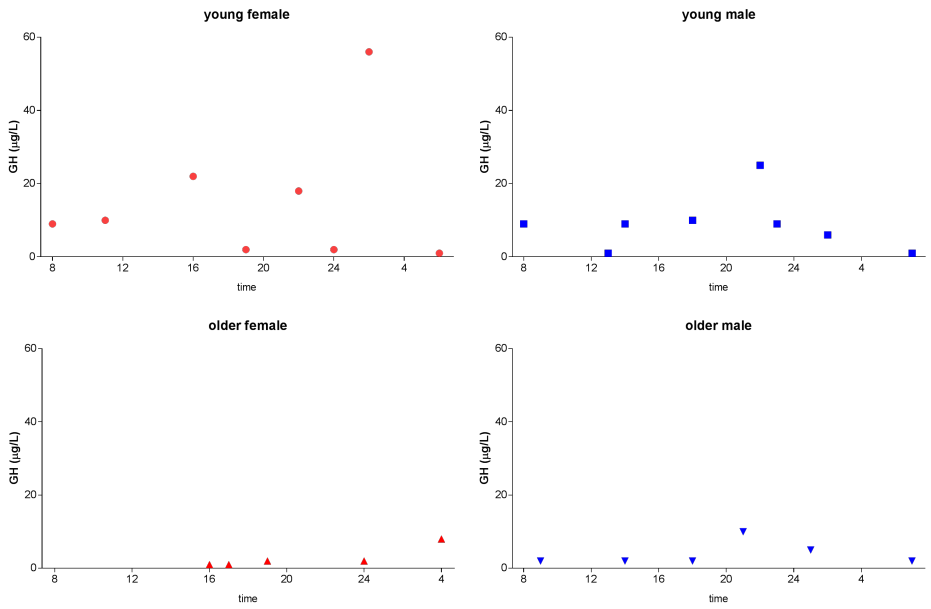

Figure 1. The secretory pattern of GH in young and old females and males. In young individuals the GH pulses are larger and more frequent and females secrete more GH than men (modified from (8)).

Pituitary synthesis and secretion of GH is stimulated by episodic hypothalamic hormones. Growth hormone releasing hormone (GHRH) stimulates while somatostatin (SST) inhibits GH production and release. GH stimulates IGF-I production which in turn inhibits GH secretion at both hypothalamic and pituitary levels. The gastric peptide ghrelin is also a potent GH secretagogue, which acts to boost hypothalamic GHRH secretion and synergize with its pituitary GH-stimulating effects (Figure 2) (10). Interestingly, recently germline or somatic duplication of GPR101 constitutively activates the cAMP pathway in the absence of a ligand, leading to GH release. Although GPR101 physiology is unclear it is worth mentioning it since it clearly has an effect on GH physiology (11).

In addition, a multitude of other factors may impact the GH axis most probably due to interaction with GHRH, somatostatin, and ghrelin. Estrogens stimulate the secretion of GH but inhibit the action of GH on the liver by suppressing GH receptor (GHR) signaling. In contrast, androgens enhance the peripheral actions of GH (12). Exogenous estrogens potentiate pituitary GH responses to submaximal effective pulses of exogenous GHRH (13) and mutes inhibition by exogenous SST (14). Also, exogenous estrogen potentiates ghrelin action (15).

GH release correlates inversely with intraabdominal visceral adiposity via mechanisms that may depend on increased FFA flux, elevated insulin, or free IGF-I.

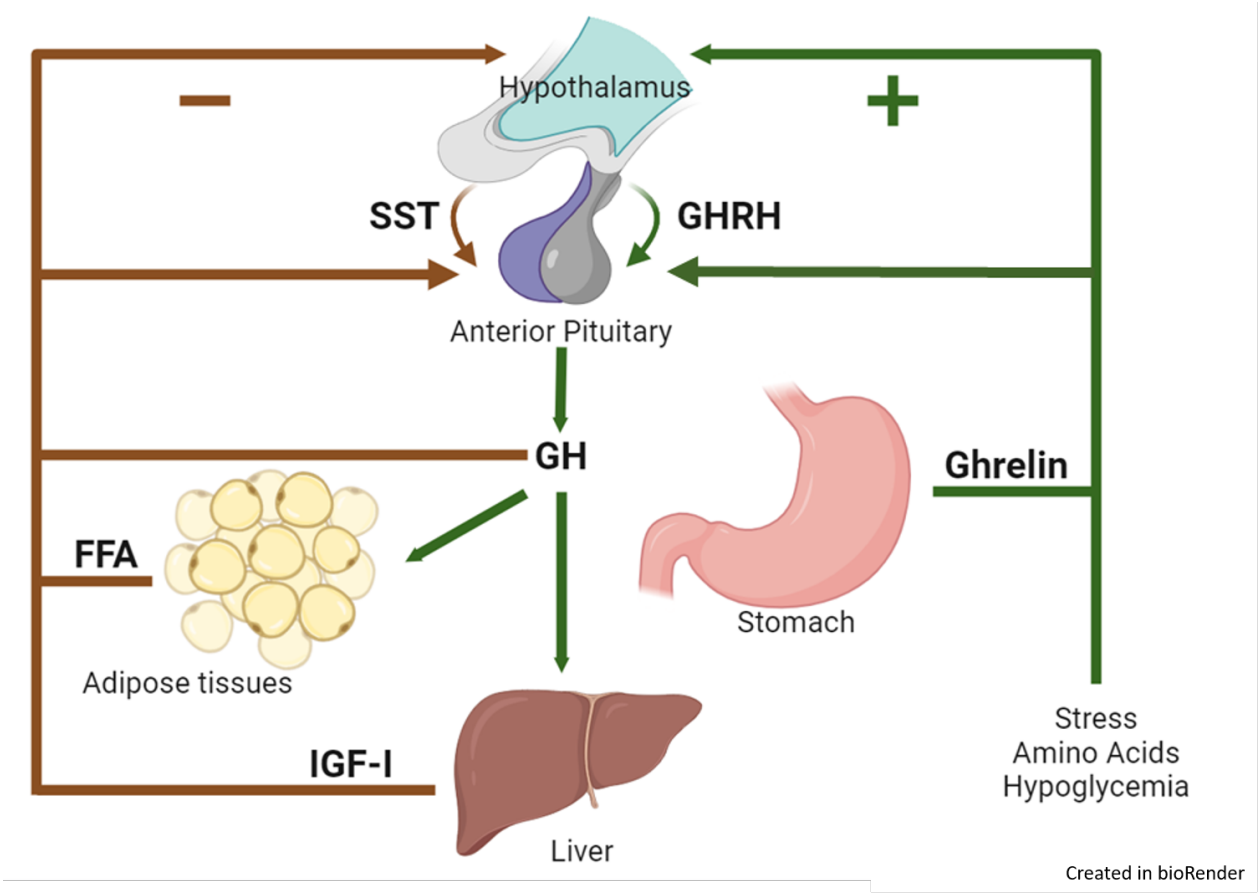

Figure 2. Factors that stimulate and suppress GH secretion under physiological conditions.

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 demonstrated that GHRH plays a vital role in the proliferation of somatotrophs in the anterior pituitary, whereas the absence of GHRH leads to anterior pituitary hypoplasia (16). In addition, GHRH upregulates GH gene expression and stimulates GH release (17). The secretion of GHRH is stimulated by several factors including depolarization, α2-adrenergic stimulation, hypophysectomy, thyroidectomy, and hypoglycemia and it is inhibited by somatostatin, IGF-I, and activation of GABAergic neurons.

GHRH acts on the somatotrophs via a seven trans-membrane G protein-coupled stimulatory cell-surface receptor. 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 (18). 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. Subsequently to the first GHRH receptor mutation described in 1996 (19) an array of familial

GHRH receptor mutations have been recognized over the last decade. These mutations 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 hypoglycemia (20).

SOMATOSTATIN (SST)

SST 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 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 stimulated by serum GH/IGF-I level, exercise, and immobilization (21).

SST 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 a synergistic manner (22).

GHRELIN

Ghrelin is a 28 amino-acid peptide that is the natural ligand for the GH secretagogue receptor. In fact, ghrelin and GHRH have a synergistic effect in increasing circulating GH levels (7). Ghrelin is primarily secreted by stomach and may be involved in the GH response to fasting and food intake.

Clinical Implications

GH LEVELS- INFLUENCE ON BODY COMPOSISTION, PHYSICAL FITNESS, AND AGE

With the introduction of dependable radioimmunological assays, it was recognized that circulating GH is blunted in obese subjects, and that normal aging is accompanied by a gradual decline in GH levels (23,24). It has been hypothesized that many of the senescent changes in body composition and organ function are related to or caused by decreased GH (25), also known as "the somatopause".

Studies done in the late 90s have uniformly documented that adults with severe GH deficiency are characterized by increased fat mass and reduced lean body mass (LBM) (26). It is also known that normal GH levels can be restored in obese subjects following massive weight loss (27), and that GH substitution in GH-deficient adults normalizes body composition. What remains unknown is the cause-effect relationship between decreased GH levels and senescent changes in body composition. Is the propensity for gaining fat and losing lean body mass initiated or preceded by a primary age-dependent decline in GH secretion and action? Alternatively, accumulation of fat mass secondary to non-GH dependent factors (e.g., lifestyle, 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 association between body composition and stimulated GH release in healthy subjects show that, adult people (mean age 50 yr) have 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, reveal that intra-abdominal fat mass is the most important and negative predictor of peak GH levels as previously mentioned (Figure 3) (28). In the same population, 24-h spontaneous GH levels also predominantly correlated inversely with intra-abdominal fat mass (29).

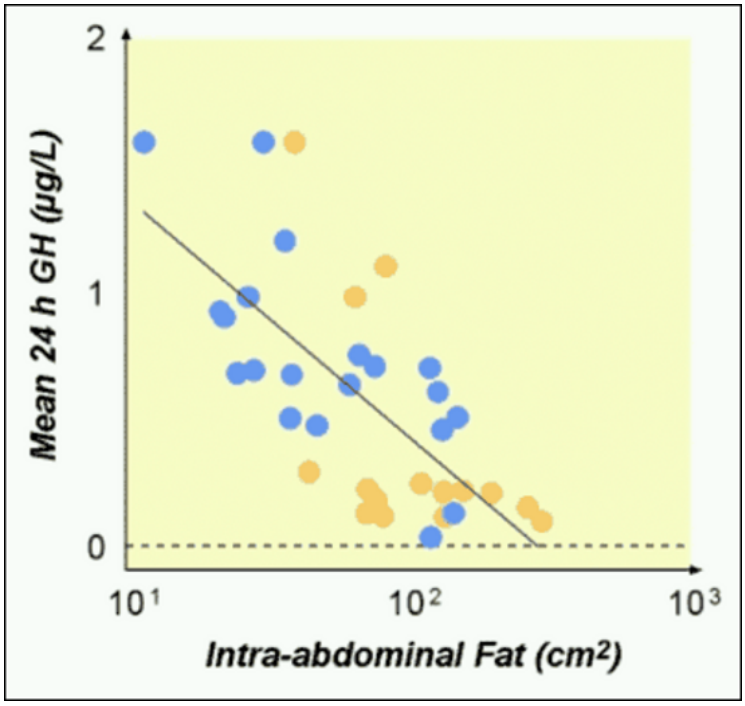

Figure 3. Correlation between intra-abdominal fat mass and 24-hour GH secretion.

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 (30-32). These studies comprise large populations of ambulatory, community-dwelling males aged between 50-90 yr. As expected, the serum IGF-I declined with age (Figure 4), but IGF-I failed to show any significant association with body composition or physical performance.

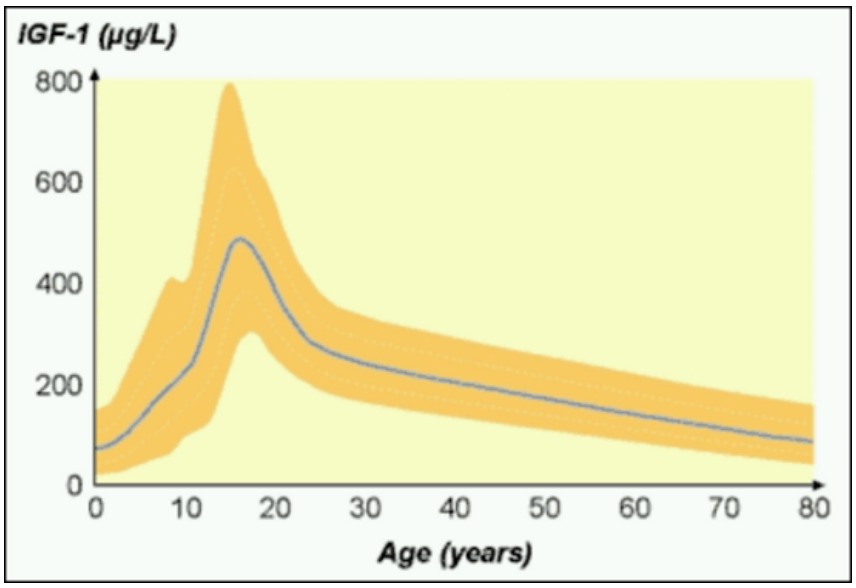

Figure 4. Changes in serum IGF-I with age; modified from (33).

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 paralleled 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 side effects in terms of fluid retention and modest insulin resistance (34). Whether this reflects an unfavorable 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. Short-term dose response studies clearly demonstrate that older patients require a lower GH dose to maintain a given serum IGF-I level (35,36), and it has been observed that serum IGF-I increases in individual patients on long-term therapy if the GH dosage remains constant. Moreover, patients with GH deficiency older than 60 yr are highly responsive to even a small dose of GH (37). Interestingly, there is a gender difference in response to GH treatment with men being more responsive in terms of IGF-I generation and fat loss during therapy, most probably due to men having lower estrogen levels that negatively impact the effect of GH on IGF-I generation in the liver (38).

The pharmacokinetics and short-term metabolic effects of a near physiological intravenous GH bolus (200 micrograms) were compared in a group of young (30 yr) and older (50 yr) healthy adults (39). The area under the GH curve was significantly lower in older subjects, whereas the elimination half-life was similar in the two groups, suggesting both an increased metabolic clearance rate and apparent distribution volume of GH in older subjects. Both parameters showed a strong positive correlation with fat mass, although multiple regression analysis revealed the 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. Interestingly, the same study showed that the GH binding protein (GHBP) correlated strongly and positively with abdominal fat mass (40).

A prospective long-term study of normal adults with serial concomitant estimations of GH status and

adiposity would provide useful information about the cause-effect relationship between GH status and body composition as a function of age. In the meantime, the following hypothesis is proposed (Figure 5): 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 IGF binding protein (BP)-1 resulting in a relative increase in free IGF-I levels; 4. Systemic elevations of 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.

At present it is not justified to treat the age-associated deterioration in body composition and physical performance with GH also due to concern that the ensuing elevation of IGF-I levels may increase the risk for the development of neoplastic disease.

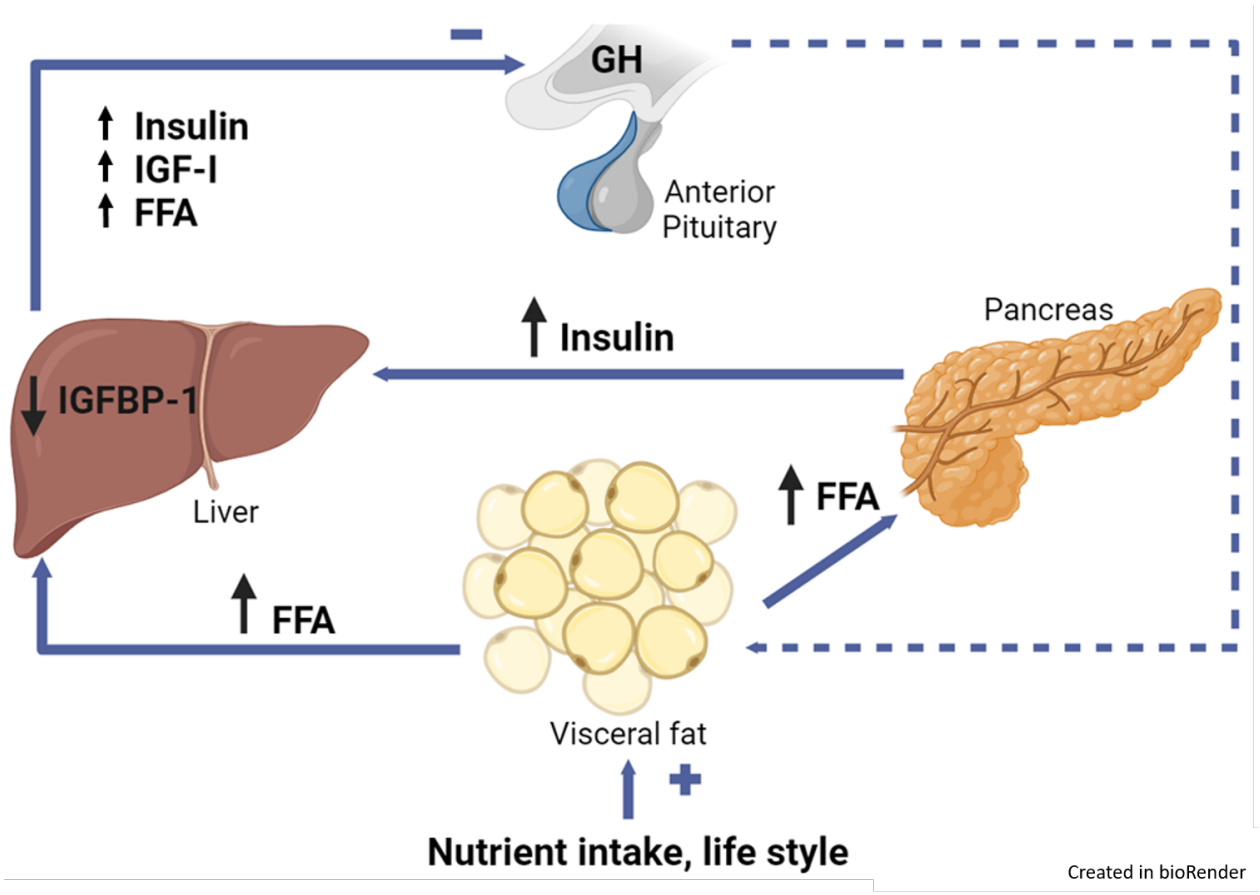

Figure 5. Hypothetical model for the association between low GH levels and increased visceral fat adults.

LIFE- LONG GH DEFICIENCY

A real-life model for the GH effects in human physiology is provided by the subjects with life-long severe reduction in GH signaling due to GHRH or GHRH receptor mutations, combined deficiency of GH, prolactin, and TSH, or global deletion of GHR. They show short stature, doll facies, high-pitched voices, central obesity, and are fertile (41). Despite central obesity and increased liver fat, they are insulin

sensitive, partially protected from cancer, and present a major reduction in pro-aging signaling and perhaps increased longevity (42). The decrease in cancer risk in life-long GH deficiency together with reports on the GH permissive role for neoplastic colon growth (43), preneoplastic mammary lesions (44), and progression of prostate cancer (45) demands, at least, a careful tailoring of GH substitution dosage in the GH deficient patients. However, recent evidence suggests that the GH produced locally by the colon tumor cells, and not pituitary GH, acts in an autocrine and paracrine manner to suppress the tumor suppressor proteins and to increase nuclear β-catenin accumulation and epithelial–mesenchymal transition potentially participating in tumor progression (46,47).

GH AND IMMUNE SYSTEM

Although the majority of data on the relation between GH and the immune system are from animal studies, it seems that GH may pose immunomodulatory actions. Immune cells express receptors for growth hormone, and respond to GH stimulation (48). The GHR is expressed by several lymphocyte subpopulations. GH stimulates *in vitro* T and B-cell proliferation and immunoglobulin synthesis, enhances human myeloid progenitor cell maturation, and modulates *in vivo* Th1/Th2 (8) and humoral immune responses (49). It has been shown that GH can induce de novo T cell production and enhance CD4 recovery in HIV+ patients. Another study with possible clinical relevance showed that sustained GH expression reduced prodromal disease symptoms and eliminated progression to overt diabetes in mouse model of type 1 diabetes, a T-cell–mediated autoimmune disease. GH altered the cytokine environment, triggered anti-inflammatory macrophage (M2) polarization, maintained activity of the suppressor T-cell population, and limited Th17 cell plasticity (49). JAK/STAT signaling, the principal mediator of GHR activation, is well-known to be involved in the modulation of the immune system, so it is tempting to assume that GH may have a role too, but clear data in humans are needed.

Growth Hormone Signaling in Humans

GROWTH RECEPTOR ACTIVATION

GH receptor signaling is a separate and prolific research field by itself (50), so this section will focus on recent data obtained in human models.

GHR have been identified in many tissues including fat, lymphocytes, liver, muscle, heart, kidney, brain, and pancreas (51,52). Activation of receptor-associated Janus kinase (JAK) 2 is the critical step in initiating GH signaling. One GH molecule binds to two GHR molecules that exist as preformed homodimers. Following GH binding, the intracellular domains of the GHR dimer undergo rotation, which brings 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 (51,53). 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. 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 been characterized in the IGF-I gene promoter region, which mediates GH-stimulated IGF-I gene activation (54). Attenuation of JAK2-associated GH signaling is mediated by a family of cytokine-inducible suppressors of cytokine signaling (SOCS) (55). 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 up regulation of SOCS-2 (56).

GH SIGNALING

Data on GHR signaling derive mainly from rodent models and experimental cell lines, although GH-induced activation of the JAK2/STAT5b and the MAPK pathways have been recorded in cultured human fibroblasts from healthy human subjects (57). STAT5b in human subjects is critical for GH-induced IGF-I expression and growth promotion as demonstrated by the identification of mutations in the STAT5b gene of patients presenting with severe GH insensitivity in the presence of normal GHR (58). 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 (59). In muscle and fat biopsies significant tyrosine phosphorylation of STAT5b was recorded after GH exposure at 30-60 minutes. There was no evidence of GH-induced activation of PI 3-kinase, Akt/PKB, or MAPK in either tissue (59).

GH AND INSULIN SIGNALING

There is animal and *in vitro* evidence to suggest that insulin and GH share post-receptor signaling pathways (60). Convergence has been reported at the levels of STAT5 and SOCS3 (61) 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 (62,63). Studies in rodent models suggest that the insulin-antagonistic effects of GH in adipose and skeletal muscle involve suppression of insulin-stimulated PI3-kinase activity (60,64). 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 (65). The infusion of GH induced a sustained increase in FFA levels and subsequently insulin resistance as assessed by the euglycemic clamp technique, as expected, but was not associated with any changes in the insulin-stimulated increase in either IRS-1 associated PI3-kinase or PKB/Akt activity. It was subsequently assessed that insulin had no impact on GH-induced STAT5b activation or SOCS3 mRNA expression (66).

INSULIN-LIKE GROWTH FACTOR-I

Physiology of IGF-I

GH acts both directly through its own receptor and indirectly through the induced production of IGF-I. GH stimulates synthesis of IGF-I in the liver and many other GH target tissues (Figure 6); about 75% of circulating IGF-I is liver-derived. IGF-I is a 70 amino-acid peptide, found in the circulation, 99% bound to transport proteins.

Following the initial discovery of IGF-I, it was thought, that GH governs somatic growth only by IGF-I produced by the liver (67). However, in the 1980s this hypothesis was changed by the identification of IGF-I production in numerous tissues (Figure 6). IGF-I is known as a global and tissue-specific growth factor as well as an endocrine factor. In some tissues IGF-I acts as a potent inhibitor of cellular apoptosis.

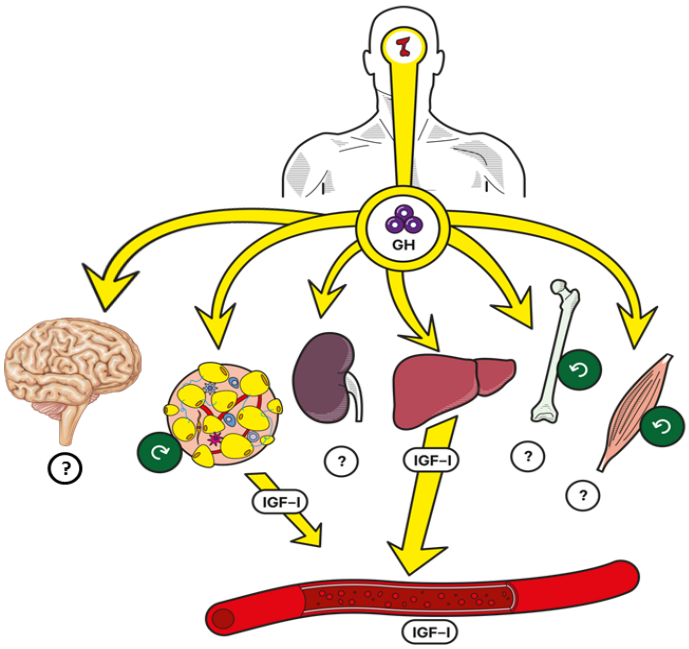

Figure 6. GH is produced in the pituitary gland. In the periphery, GH acts directly and indirectly through stimulation of IGF-I production. In the circulation, the liver is the most important source of IGF-I (75%) but other tissues (e.g. brain, adipose tissue, kidney, bone, and muscles) may contribute. Under GH stimulation the muscle, adipose tissue, and bone have been shown to secrete IGF-I that has a paracrine/autocrine effect.

Interestingly, insulin and IGF-I 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-I gene is a member of the insulin gene family and the IGF-I receptor is structurally similar to the insulin receptor in its tetrametric structure, with 2 alpha and 2 beta subunits (68). The alpha subunit binds IGF-I, IGF-II, and insulin; however, the subunit has a higher affinity towards IGF-I compared to IGF-II and insulin. Although insulin and IGF-I share many similarities, during evolution, the functionality of the two molecules has become more divergent, where insulin plays a more metabolic role and IGF-I plays a role in cell growth.

The IGF-I 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, iodothyronines, platelet-derived growth factor, and fibroblast growth factor. Following the binding of the IGF-I molecule, the receptor undergoes a conformational change, which activates tyrosine kinase, leading to auto-phosphorylation of tyrosine. 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-I (69,70).

IGF-I is bound almost 100% to a family of binding proteins (IGFBP) in the circulation. The IGFBP family comprises six binding proteins (IGFBP 1-6) with a high affinity towards IGF-I and II. 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-I due to their high affinity. 75% of IGF-I is bound to IGFBP-3. Interestingly, similar to IGF-I, IGFBP-3 production is also regulated by GH. In the plasma, IGFBP-3 is bound to a protein called acid labile subunit (ALS), which stabilizes the "IGFBP3-IGF-I" complex, prolonging its half-life to approximately 16 hours (71). IGFBP-1, on the other hand, is present in lower concentration in plasma than IGFBP-2 and 3. However, due to lower affinity for IGF-I, IGFBP-1 is usually in an unsaturated state and changing plasma concentrations of IGFBP-1 becomes important in determining the unbound fraction of IGF-I. A recently new discovered player in the regulation of IGF-I bioavailability is the pregnancy-associated plasma protein-A2 (PAPP-A2) that cleaves IGFBP3 and 5 and releases IGF-I. Homozygous mutations in PAPP-A2 result in growth failure with elevated total but low free IGF-I (72). Low IGF-I bioavailability impairs growth and glucose metabolism in a mouse model of human PAPP-A2 deficiency and treatment with recombinant human IGF-I in PAPP-A2 deficient patients improves growth and bone mass and ameliorates glucose metabolism (72,73).

Effects of IGF-I

Studies on hypophysectomized animals overexpressing IGF-I demonstrate the independent anabolic effects of IGF-I (74). IGF-I 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-I gene in mice produce a perinatal mortality of 80% with the surviving animal showing significant growth retardation compared to controls (75). Human IGF-I deficiency can be either due to GH deficiency, GHR inactivation, or IGF-I gene mutation. Interestingly, infants with congenital GH deficiency and GHR mutations present with only minor growth retardation, whereas the rare patient with IGF-I deficiency, secondary to a homozygous partial deletion of the IGF-I gene, presents with severe pre and postnatal growth failure, mental retardation, sensorineural deafness, and microcephaly (76-78). The differences in the clinical presentation are most likely due to the fact that some degree of IGF-I production is present in patients with GH deficiency, GHR, and GHRH defects. More detailed studies on transgenic mice have clearly demonstrated this fact with selective deletion of IGF-I gene expression only in the liver, showing low serum IGF-I concentrations with only 6-8% postnatal growth retardation. In contrast, animals with total IGF-I deletion or only peripherally produced IGF-I deletion showed marked growth retardation (79).

Both elevated and reduced levels of serum IGF-I are associated with excess mortality in human adults (80). In addition, it is well recognized in many species including worms, flies, rodents, and primates that a reciprocal relationship exists between longevity and activation of the insulin/IGF axis (80). The underlying mechanisms are subject to continued scrutiny and are likely to be complex. In this regard, it is noteworthy that calorie restriction is associated with increased longevity and reduced insulin/IGF activity in many species (81) albeit GH levels are increased by calorie restriction and fasting (82).

In the context of GH and IGF-I physiology it can be concluded that 1) during childhood and adolescence the combined actions of GH and IGF-I in the presence of sufficient nutrition promote longitudinal growth and somatic maturation, 2) continued excess IGF-I activity in adulthood increases the risk for cardiovascular and neoplastic diseases and hence reduces longevity, 3) calorie restriction, which suppresses IGF-I activity and stimulates GH secretion, may promote longevity in human adults (82).

METABOLIC EFFECTS OF GROWTH HORMONE

Nutritional status dictates GH effects. In the state of feast and sufficient nutrient intake where insulin is increased in the liver and IGF-I production is stimulated, GH promotes protein anabolism. Whereas, in the state with decreased nutrient intake and during sleep and exercise, the direct effect of GH are more predominant and this is mainly characterized by stimulation of lipolysis.

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 (83). Fasting hypoglycemia and pronounced sensitivity to insulin were distinct features of hypophysectomized animals. These symptoms were readily corrected by the 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 hypophysectomized dogs. Furthermore glycemic control deteriorates following exposure to a single supraphysiological dose of human GH in hypophysectomized adults with type 1 diabetes mellitus (84). Somewhat surprisingly, only modest effects of GH on glucose metabolism were recorded in the first metabolic balance studies involving adult hypopituitary patients (85,86).

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, whereas the impact of GH replacement on this feature seems to depend on the duration and the dose (87).

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.

STUDIES IN NORMAL ADULTS

More than fifty 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, which was paralleled by increased uptake and oxidation of FFA (88). This pattern was opposite to that of insulin, and GH in the same model abrogated the metabolic actions of insulin.

Administration of a GH bolus in the post absorptive state stimulates lipolysis following a lag time of 2-3 hours (89). Plasma levels of glucose and insulin, on the other hand, change very little. This is associated with small reductions in muscular glucose uptake and oxidation, which could reflect substrate competition between glucose and fatty acids (i.e., the glucose/fatty acid cycle) (Figure 7). 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. However, GH excess reduces intrahepatic lipid content suggesting that GH-induced insulin resistance is not associated with hepatic lipid accumulation (90). Apart from enhanced glucose/fatty acid cycling, it has been shown that GH induced insulin resistance is accompanied by reduced muscle glycogen synthase activity (91) and diminished glucose dependent glucose disposal (92). Bak et al. also demonstrated insulin binding and insulin receptor kinase activity from muscle biopsies to be unaffected by GH (91).

Undoubtedly, a causal link exists between GH-induced lipolysis and insulin resistance (93). Acute GH exposure in healthy individuals downregulates important suppressors of lipolysis, the G0/G1 switch gene (G0S2) and fat specific protein 27 (FSP27), in

addition to regulating the suppressor of the insulin signaling, phosphatase and tensin homolog (PTEN) (94).

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 (95). There is evidence to suggest that this hyper-metabolic state ultimately leads to beta cell exhaustion and overt diabetes mellitus (96), but it is also demonstrated that the abnormalities are completely reversed after successful surgery (95). Conversely, it has been shown that only two weeks of the administration of GH in supraphysiological doses induces comparable acromegaloid, and reversible abnormalities in substrate metabolism and insulin sensitivity (97).

Interaction of Glucose and Lipid Metabolism

Relatively few studies have scrutinized the exact modes of action of GH on glucose metabolism. There is no evidence of a GH effect on insulin binding to the receptor (91,98), 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. (99). According to his hypothesis (the glucose/fatty acid cycle), oxidation of FFA initiates an upstream, chain-reaction-like inhibition of glycolytic enzymes, which ultimately inhibits glucose uptake (Figure 7).

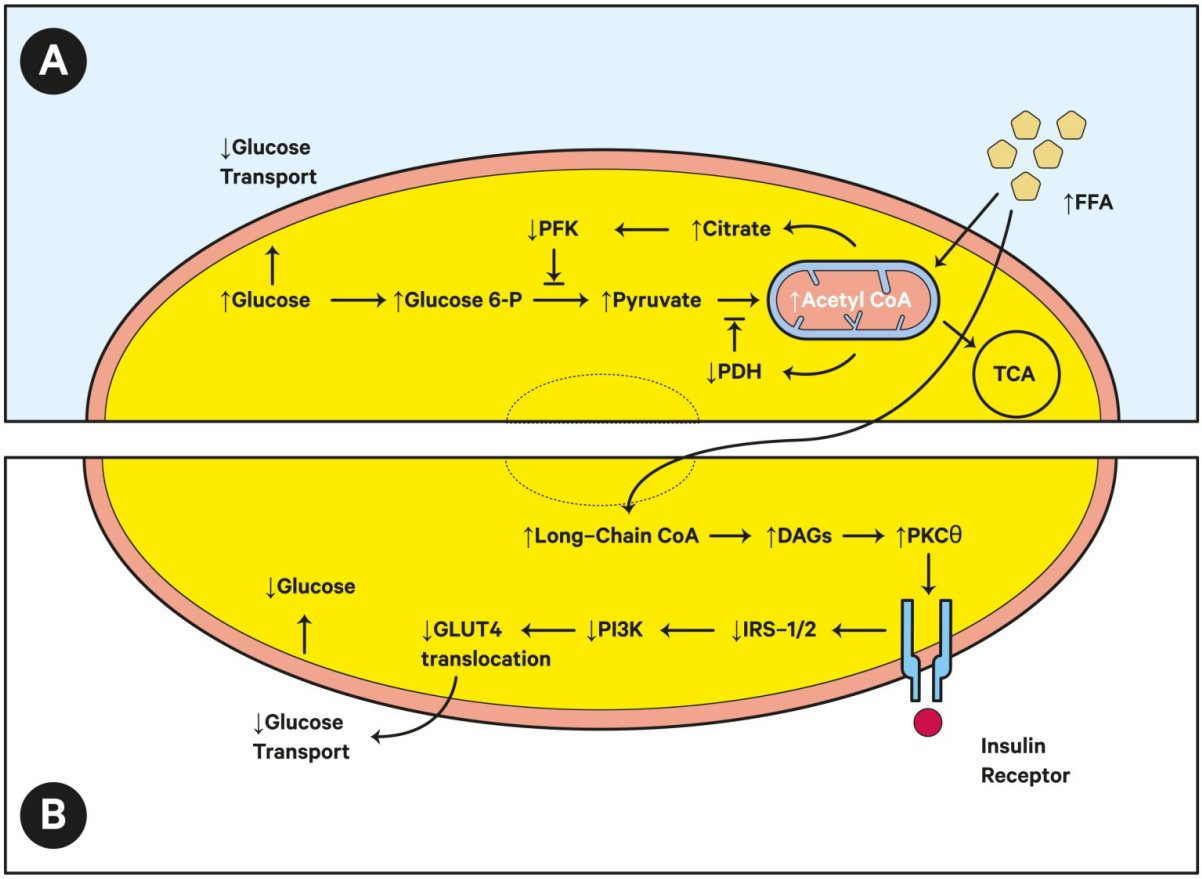

Figure 7. The glucose fatty-acid cycle.

Randle proposed in 1963 that increased FFA compete with and displace glucose utilization leading to a decreased glucose uptake. The hypothesis stated that an increase in fatty acid oxidation in muscle and fat results in higher acetyl CoA in mitochondria leading to inactivation of two rate-limiting enzymes of glycolysis (i.e., phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) complex). A subsequent increase in intracellular glucose-6-phosphate (glucose 6-P) results in high intracellular glucose concentrations and decreased glucose uptake by muscle and fat.

However, in contrast to the proposed hypothesis by Randle, studies using MR spectroscopy have shown reductions in intramyocellular glucose 6-P and glucose concentrations and have led to an alternative hypothesis. The new hypothesis proposes that a transient increase of intracellular diacylglycerol (DAG) activates theta isoform of protein kinase C (PKCθ) that causes increased serine phosphorylation of IRS-1/2 and consecutively decrease PI3K activation and glucose-transport activity leading to decrease intracellular glucose concentrations

When considering the pronounced lipolytic effects of GH the Randle hypothesis remains an appealing model to explain the insulin-antagonistic effects of GH. 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 (100). We have also shown that GH-induced insulin resistance is associated with suppressed pyruvate dehydrogenase activity in skeletal muscle (101). It has, however, also been reported that GH-induced insulin resistance precedes the increase in circulating levels of fatty acids and forearm uptake of lipid intermediates (102). This early effect of GH on muscular glucose uptake could reflect intramyocytic FFA release and oxidation and thus be compatible with the Randle hypothesis. According to the Randle hypothesis the fatty acid-induced insulin resistance will result in elevated intracellular levels of both glucose and glucose-6-phosphate (Figure 7). By contrast, muscle biopsies from GH deficient adults after GH treatment have revealed increased glucose but low-normal glucose-6-phosphate levels (103). 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 (104). 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 considered essential for glucose transportation into skeletal muscle via translocation of glucose transporter activity (GLUT 4). A more recent study showed that GH infusion does not impact insulin-stimulated PI-3 kinase activity (65).

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 favorable changes in body composition. There is little doubt that these effects of GH are dose-dependent and may be minimized or avoided if an appropriately low replacement dose is used. Still, the pharmacokinetics of subcutaneous (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 described by Rabinowitz & Zierler (105). The problem arises 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. Administration of GH in the evening probably remains the best compromise between effects and side effects (106), but it is far from physiological.

Long-acting GH analogues have been developed to improve adherence and compliance. The clinical experience is limited now but seem not to impact adversely the glucose metabolism compared with daily GH (107). However, long-term surveillance data are required to consolidate its safety profile (108).

Effects of GH on Muscle Mass and Function

The anabolic nature of 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. 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, and direct calculation of muscle area or volume by computerized tomography (CT) and magnetic resonance imaging.

EFFECT 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 (109,110). Indirect evidence of an increase in muscle cell number following GH treatment was also presented (110).

These early clinical studies were paralleled by experimental studies in rodent models. GH administration in hypophysectomized rats increased not only muscle mass, but also muscle cell number (i.e., muscle DNA content) (110). 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 (111). Another important observation of that period was made by Goldberg, who studied protein turnover in skeletal muscle of hypophysectomized 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 (112).

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. Systemic GH administration for 7 days in normal adults increased whole body protein synthesis without affecting proteolysis (113), and similar data were subsequently obtained in GH deficient adults (114). Systemically infused GH for 8 hours in normal adults lead to an acute stimulation of forearm (muscle) protein synthesis without any effects on whole body protein synthesis (115). By contrast in a design that also included co-administration of somatostatin to suppress insulin, an acute stimulatory effect of GH on whole body protein synthesis was observed, but no

stimulatory effect on leg protein synthesis (116), Finally, infusion of GH into the brachial artery was accompanied by a local increase in forearm muscle protein synthesis (117).

Based on these studies it seems that the nitrogen retaining properties of GH predominantly involve stimulation of protein synthesis without affecting (lowering) proteolysis. Theoretically, the protein anabolic effects of GH could be either direct or mediated through IGF-I, insulin, or lipid intermediates. GHR are present in skeletal muscle (52), which combined with Fryburg's intra-arterial GH studies, makes a direct GH effect conceivable. An alternative interpretation 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 (118) whereas IGF-I in combination with an amino acid infusion increase protein synthesis (119). Moreover, intra-arterial IGF-I in combination with systemic amino acid infusion increased protein synthesis (120). 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-administration of GH (121). The degree to which mobilization 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 GHR in skeletal muscle. This does not rule out a significant role of IGF-I being produced either systematically or locally.

An interesting discovery has been that infusion of GH and IGF-I into the brachial artery increases forearm blood flow several fold (117,122). This effect appears to be mediated through stimulation of endothelial nitric oxide release leading to local vasodilatation (123,124). Thus, it 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 speculate 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 resting energy expenditure (24). Moreover, it is plausible that the reduction in total peripheral resistance seen after GH administration in adult growth hormone deficiency is mediated by nitric oxide (124).

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 healthy 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 (24). This concept has been recently reviewed (125) 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 yr) undergoing a resistance exercise program for 12 weeks showed a GH induced increase in lean body mass (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 (126). In a similar study in older men (67 yr) GH also increased LBM and whole body protein synthesis, without significantly amplifying the effects of exercise on muscle protein synthesis or muscle strength (127). An increase in LBM but unaltered muscle strength following 10 weeks of GH administration plus resistance exercise training was also recorded (128). A more recent study of 52 older men (70-85 yr) 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 (129). A meta-analysis of studies administering GH to healthy adult subjects demonstrate that it increases lean body mass and reduces fat mass without improving muscle strength or aerobic exercise capacity (130).

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 (131,132).

CONCLUSIONS

GH/IGF-I axis is specifically regulated and is involved in a multitude of processes during all aspects of life from intrauterine growth, to childhood and puberty, adulthood, and lastly elderly periods. GH actions directly or via its principal metabolite, IGF-I have a wide range of physiological roles being a metabolic active hormone in adulthood. Nutritional status of an organism dictates the effects of GH, either an impairment of insulin action (fasted state) or promoting protein anabolism (feed state). As our knowledge of the GH normal physiology increases, our ability to understand and specifically target the GH/IGF-I pathway for a diverse range of therapeutic purposes should also increase.

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